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(54) **INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS**

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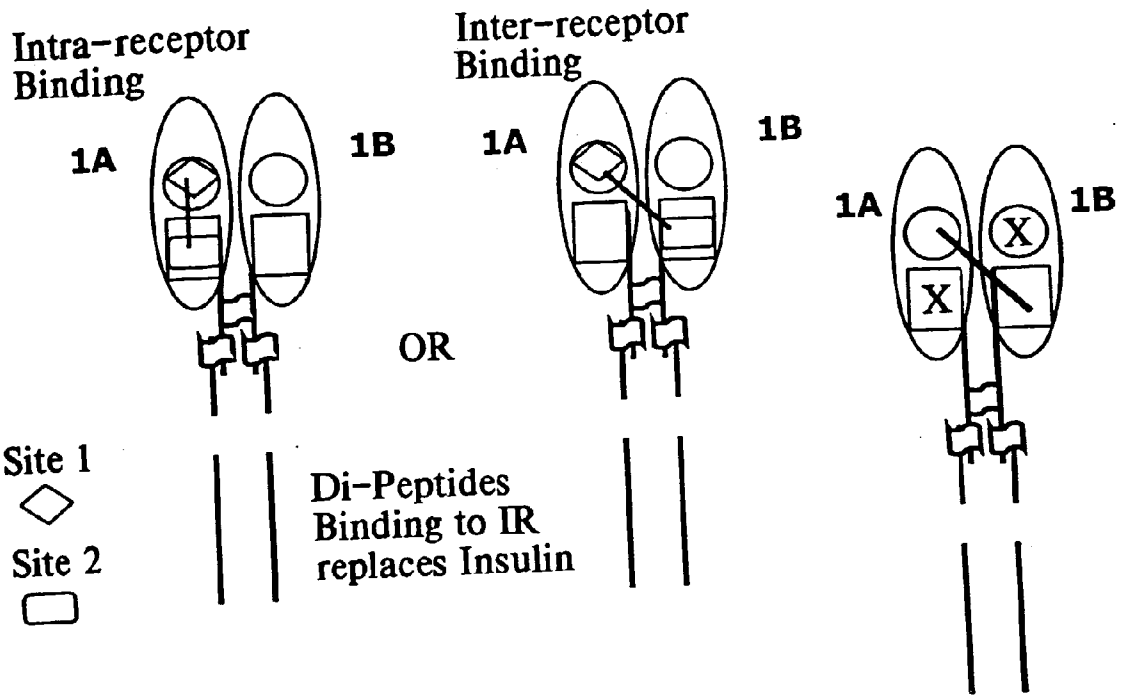
(57) **ABSTRACT**

Peptide sequences capable of binding to insulin and/or insulin-like growth factor receptors with either agonist or antagonist activity and identified from various peptide libraries are disclosed. This invention also identifies at least two different binding sites, which are present on insulin and insulin-like growth factor receptors, and which selectively bind the peptides of this invention. As agonists, certain of the peptides of this invention may be useful for development as therapeutics to supplement or replace endogenous peptide hormones. The antagonists may also be developed as therapeutics.



| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX | -- | -- | -- | -- | -- |
| R40-3-40B2-IR | IRDMHYVWVQDRDRYINGVRQWYISDRYNPGSAFYRWFID | 40.3 | 9.0 | 2.0 | 4.5 | 0.2 |
| R40-4-40B12-IR | RMGLQALAHYRKSAGPIFLSSGSVIKGSEGDPFYAWFRLQ | 60.4 | 12.9 | 2.0 | 6.5 | 0.2 |
| R40-4-40G11-IR | MPVSLFRRVWDYRDGEHETLESHYVVPQAALDRLFYSWFS | 52.6 | 37.5 | 2.0 | 18.8 | 0.1 |

FIG. 1A

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX | -- | -- | -- | -- | -- |
| R40-3-D5-IGFR | PLYGGGIHLYYPGTMGYVPGFPRQVKVLGDADKNFYDWFM | -- | -- | -- | -- | -- |
| R40-3-A6-IGFR | YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV | -- | -- | -- | -- | -- |
| R40-X-R35-IGFR | SGCCRLLGLRWMFIVIVGWSGALVCQSAASAAGFYDWFV | -- | -- | -- | -- | -- |

FIG. 1B

| Clone Design | Sequence XXXXXXXXXXXXXXXXXXXX | Ratios over Background E-Tag -- | IGFsR -- | IR -- | Comparisons IGFR/IR -- | IR/IGFR -- |
|---|---|---|---|---|---|---|
| R20α-3-20D3-IR | IGGQGQHQDGNFYDWFVEALA | 46.3 | 36.2 | 7.0 | 5.2 | 0.2 |
| R20α-3-20F1-IR | VFWNCRSQQLDFYEWFEQAA | 49.0 | 26.0 | 2.8 | 9.3 | 0.1 |
| R20α-3-20H1-IR | RVAGAISAPGLVSNKQDGLFYSWFRE | 45.6 | 35.3 | 3.3 | 10.7 | 0.1 |
| R20α-3-20D1-IR | VLQARHGCDSVSDCFYEWFA | 50.8 | 37.5 | 3.0 | 12.5 | 0.1 |
| R20β-4-B12-IR | GAFYRWFHEALVGSERVPDV | 41.9 | 2.9 | 5.7 | 0.5 | 2.0 |
| R20β-4-H3-IR | HEAFYDWFSALVDGGYELMG | 13.9 | 5.8 | 2.4 | 2.4 | 0.4 |
| R20β-4-D10-2-IR | RIGGGWARSEGFYEWFVREL | 21.5 | 7.3 | 2.9 | 2.5 | 0.4 |
| R20β-4-C8-IR | LPAGGA?GFA?RGFYEWFES | 44.9 | 31.1 | 9.6 | 3.2 | 0.3 |
| R20β-4-E7-IR | GHSWALVRHVDRLFYEWFDL | 45.0 | 18.8 | 5.9 | 3.2 | 0.3 |
| R20β-4-E7-2-IR | LGTSAGQGVGHRAFYQWFQS | 45.0 | 18.8 | 5.9 | 3.2 | 0.3 |
| R20β-4-G3-IR | RGGGTFYEWFESALRKHGAG | 38.6 | 7.5 | 2.0 | 3.8 | 0.3 |
| R20β-4-H6-IR | NSSGQQVVGLTFYSWFASQV | 14.8 | 7.6 | 2.0 | 3.8 | 0.3 |
| R20β-4-G11-IR | FYGWFSRQLSLTPRDDWGLP | 39.4 | 7.5 | 1.9 | 3.9 | 0.3 |
| R20β-4-G8-IR | RMFYEWFWSQMGAGPTEGSA | 41.2 | 15.1 | 3.4 | 4.4 | 0.2 |
| R20β-4-H9-IR | IGGQGQHQDGNFYDWFVEALA | 43.1 | 8.8 | 2.0 | 4.4 | 0.2 |
| R20β-4-H8-IR | RDKPTDQEEQNWSFYEWFRH | 47.9 | 43.7 | 9.3 | 4.7 | 0.2 |
| R20β-4-B8-IR | WSALLSVMDTGFYAWFDDAV | 44.0 | 40.1 | 8.4 | 4.8 | 0.2 |
| R20β-4-E2-IR | SRDQTNFTFNSAGFYGWFER | 16.3 | 13.9 | 2.4 | 5.8 | 0.2 |
| R20β-4-F4-IR | GVGTLTMSSDAFYTWFV | 15.3 | 5.9 | 1.0 | 5.9 | 0.2 |
| R20β-4-A8-IR | IGGSFVEFYGWFNDQV | 43.3 | 36.0 | 6.0 | 6.0 | 0.2 |
| R20β-4-C4-IR | DIGSDGHGRRWDSFYRWFEM | 17.3 | 26.8 | 4.3 | 6.2 | 0.2 |
| R20β-4-D7-IR | VLQARHGCDSVSDCFYEWFA | 44.8 | 36.2 | 5.6 | 6.5 | 0.2 |
| R20β-4-D2-IR | DPERMQSDVGFYEWFRAAVG | 31.2 | 29.4 | 2.9 | 10.1 | 0.1 |

FIG. 1C

| Clone Design | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| | XXXXXXXXXXXXXXXXXXXX | -- | -- | -- | -- | -- |
| R20-4-B9-IGFR | DPERMQSDVGFYEWFRAAVG | 40.1 | 16.6 | -- | -- | -- |
| R20-4-F8-IGFR | DIGSDGHGRRWDSFYRWFEM | 39.2 | 13.9 | -- | -- | -- |
| R20-4-G12-IGFR | PFYQWFLDQSVGGSRGGGLR | 36.7 | 8.0 | -- | -- | -- |
| R20-4-D10-IGFR | AVAPLSVRGRDSGFYSWFSS | 40.2 | 4.1 | -- | -- | -- |

FIG. 1D

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| Design | XXXXXXXXXXNFYDWFVXXXX | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| | | -- | -- | -- | -- | -- |
| A6S-3-E12-IR | GRVDWLQRNANFYDWFVAELG | 26.2 | 1.3 | 8.0 | 0.2 | 6.2 |
| A6S-2-C1-IR | RMYFSTGAPQNFYDWFVQEWD | 41.2 | 1.3 | 7.0 | 0.2 | 5.4 |
| A6S-1-A7-IR | HHTQGLQVQRNFYDWFVNELR | 47.2 | 2.3 | 11.1 | 0.2 | 4.8 |
| A6S-2-C8-IR | MHRMQHDGTSNFYDWFVLQWA | 44.9 | 1.5 | 5.5 | 0.3 | 3.7 |
| A6S-3-E10-IR | AMHVVAQGGPNFYDWFVRELR | 46.9 | 1.6 | 5.0 | 0.3 | 3.1 |
| A6S-2-D5-IR | AIQMNGNLAFNFYDWFVRELT | 31.9 | 1.2 | 3.7 | 0.3 | 3.1 |
| A6S-1-B2-IR | TDRKSVQEPRNFYDWFVWAAR | 31.6 | 1.8 | 5.3 | 0.3 | 2.9 |
| A6S-1-A4-IR | PHGHRGFAQSNFYDWFVTQEE | 43.3 | 3.6 | 9.2 | 0.4 | 2.6 |
| A6S-4-G3-IR | RLASASVPGQNFYDWFVDQLL | 31.3 | 2.3 | 5.1 | 0.5 | 2.2 |
| A6S-4-H8-IR | RQSEFSTLNSNFYDWFVRELE | 11.5 | 1.7 | 3.6 | 0.5 | 2.1 |
| A6S-3-E11-IR | GQAQLSIRDVNFYDWFVQQLV | 26.3 | 2.3 | 4.4 | 0.5 | 1.9 |
| A6S-1-A1-IR | MSEPAVGVNGNFYDWFVAQLF | 36.9 | 3.7 | 6.5 | 0.6 | 1.8 |
| A6S-2-C9-IR | VGTGRARLDRNFYDWFVGQYS | 43.6 | 1.3 | 2.3 | 0.6 | 1.8 |
| A6S-2-C4-IR | SREAVQKRNANFYDWFVQQLS | 34.5 | 5.6 | 9.6 | 0.6 | 1.7 |
| A6S-4-H10-IR | LAQFAGSRNQNFYDWFVEQLG | 39.2 | 4.4 | 6.9 | 0.6 | 1.6 |
| A6S-4-G7-IR | GQEYFDQMGLNFYDWFVRELD | 19.1 | 1.4 | 2.2 | 0.6 | 1.6 |
| A6S-4-H2-IR | RQPSQPPHGSNFYDWFVEAIN | 25.5 | 2.6 | 3.9 | 0.7 | 1.5 |
| A6S-2-C3-IR | LMQSLGSGSTNFYDWFVQQMV | 31.1 | 1.6 | 2.4 | 0.7 | 1.5 |
| A6S-2-C11-IR | DQQRSACDGTNFYDWFVCQLS | 20.9 | 3.3 | 4.6 | 0.7 | 1.4 |
| A6S-3-F3-IR | LDGTKACQRVNFYDWFVCQTE | 37.1 | 3.0 | 4.2 | 0.7 | 1.4 |
| A6S-3-E5-IR | PEARRTVVHSNFYDWFVAQLS | 31.6 | 2.5 | 3.5 | 0.7 | 1.4 |
| A6S-1-B7-IR | PWMLSVGIQDNFYDWFVGLDS | 49.2 | 1.6 | 2.3 | 0.7 | 1.4 |
| A6S-3-E7-IR | ASHQRGGSSDNFYDWFVAQMR | 37.2 | 5.0 | 6.3 | 0.8 | 1.3 |
| A6S-4-G6-IR | TLEREGEFSGNFYDWFVEQLH | 16.8 | 3.1 | 4.0 | 0.8 | 1.3 |
| A6S-2-C2-IR | DRQSIGSVHGDFYDWFVSALG | 29.7 | 2.4 | 3.1 | 0.8 | 1.3 |
| A6S-3-F1-IR | DWDKLGSLSENFYDWFVDQLA | 29.7 | 2.3 | 3.0 | 0.8 | 1.3 |
| A6S-2-C5-IR | VRVVLNQSGRNFYDWFVIQLE | 42.9 | 6.1 | 7.0 | 0.9 | 1.1 |
| A6S-3-E4-IR | MASWQSRTPDNFYDWFVRELS | 20.9 | 2.1 | 2.3 | 0.9 | 1.1 |

FIG. 1E-1

| Clone Design | Sequence XXXXXXXXXXXNFYDWFVXXXX | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| A6S-3-E9-IR | TTCHPRGEDCNFYDWFVLQLR | 36.6 | 9.0 | 8.9 | 1.0 | 1.0 |
| A6S-3-E1-IR | VRGNDSVLRANFYDWFVDQLS | 36.7 | 6.8 | 6.9 | 1.0 | 1.0 |
| A6S-4-H12-IR | TPRSQVRSDHNFYDWFVYQLA | 46.3 | 6.1 | 5.8 | 1.1 | 1.0 |
| A6S-2-D3-IR | ESLTGSRPDRNFYDWFVQQTS | 37.0 | 5.3 | 5.1 | 1.0 | 1.0 |
| A6S-3-E8-IR | PQSLTEVRTGNFYDWFVVQLH | 42.7 | 5.2 | 5.1 | 1.0 | 1.0 |
| A6S-1-A12-IR | DVGMGRVKETNFYDWFVRQLI | 39.7 | 2.1 | 2.1 | 1.0 | 1.0 |
| A6S-4-H3-IR | GADDIRSLNTNFYDWFVNQLS | 18.6 | 3.1 | 2.9 | 1.1 | 0.9 |
| A6S-3-F7-IR | GVSIQAGYKTNFYDWFVEAVR | 46.2 | 2.3 | 2.1 | 1.1 | 0.9 |
| A6S-2-D8-IR | VGEHRQMSVGNFYDWFVMQIA | 31.2 | 2.0 | 1.7 | 1.2 | 0.9 |
| A6S-3-F10-IR | GSSLGRSGPGNFYDWFVDQLE | 39.0 | 5.9 | 4.5 | 1.3 | 0.8 |
| A6S-4-G11-IR | HRQQDVVRQGNFYDWFVQALE | 44.8 | 4.3 | 3.3 | 1.3 | 0.8 |
| A6S-2-D2-IR | QDTFLTAREGNFYDWFIRALE | 33.5 | 3.6 | 2.7 | 1.3 | 0.8 |
| A6S-4-G8-IR | EAIMREEGQANFYDWFVRQLE | 11.1 | 2.5 | 1.9 | 1.3 | 0.8 |
| A6S-4-H6-IR | VCDVSTGGGTNFYDWFVCQVG | 22.4 | 2.4 | 1.9 | 1.3 | 0.8 |
| A6S-2-D10-IR | PQPRSASTPLNFYDWFVQATG | 41.3 | 2.1 | 1.7 | 1.2 | 0.8 |
| A6S-3-F4-IR | GVSRGSGGDPNFYDWFVMQLR | 37.0 | 13.5 | 9.9 | 1.4 | 0.7 |
| A6S-4-G9-IR | GPGRHDSSRGNFYDWFVEQLA | 36.2 | 11.8 | 7.8 | 1.5 | 0.7 |
| A6S-3-F5-IR | ERFALEVQGSNFYDWFVRQVI | 48.1 | 7.2 | 4.8 | 1.5 | 0.7 |
| A6S-4-H1-IR | NLKSSATVGGNFYDWFVEQL | 18.3 | 3.6 | 2.6 | 1.4 | 0.7 |
| A6S-3-F6-IR | MEGPPAGGPLNFYDWFVAQVD | 18.7 | 2.9 | 1.9 | 1.5 | 0.7 |
| A6S-3-F11-IR | RLDVAGHRGGNFYDWFVKQLH | 33.8 | 2.0 | 1.4 | 1.4 | 0.7 |
| A6S-2-C6-IR | PWSDHEALNQNFYDWFVSQVL | 46.7 | 19.2 | 12.1 | 1.6 | 0.6 |
| A6S-4-G4-IR | EDRLGNGESTNFYDWFVRQLA | 36.9 | 18.2 | 10.7 | 1.7 | 0.6 |
| A6S-4-G12-IR | GKLVASTLDDNFYDWFVRQLS | 32.8 | 12.8 | 7.9 | 1.6 | 0.6 |
| A6S-2-D7-IR | SGPVVQTQNGNFYDWFVHQLR | 33.2 | 12.0 | 7.1 | 1.7 | 0.6 |
| A6S-4-G10-IR | VDRAGPAGSDNFYDWFVAQLD | 33.9 | 10.8 | 6.8 | 1.6 | 0.6 |
| A6S-3-F9-IR | SLGRNDRPDENFYDWFVSQVQ | 44.3 | 9.6 | 5.7 | 1.7 | 0.6 |
| A6S-3-F2-IR | RVMATANAPMNFYDWFVVQLQ | 23.2 | 4.3 | 2.5 | 1.7 | 0.6 |

FIG. 1E-2

| Clone Design | Sequence XXXXXXXXXXXNFYDWFVXXXX | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| A6S-4-G1-IR | NGVERAGTGDNFYDWFVAQLH | 36.2 | 31.8 | 15.7 | 2.0 | 0.5 |
| A6S-1-A3-IR | PFAGKGDKTGNFYDWFVSLTG | 39.9 | 12.6 | 6.0 | 2.1 | 0.5 |
| A6S-3-F12-IR | GMPQEYMDQVNFYDWFVAQVD | 41.4 | 7.4 | 4.0 | 1.9 | 0.5 |
| A6S-4-G2-IR | MGTPAVGDGANFYDWFVRQLG | 26.7 | 7.0 | 3.5 | 2.0 | 0.5 |
| A6S-1-B1-IR | SKCKAWYGANNFYDWFVWQVD | 30.6 | 3.7 | 1.9 | 1.9 | 0.5 |
| A6S-2-D11-IR | EAASLGSQDRNFYDWFVRQVV | 48.4 | 37.4 | 13.5 | 2.8 | 0.4 |
| A6S-2-D1-IR | VERSASSQDGNFYDWFVVQIR | 37.8 | 30.6 | 12.0 | 2.6 | 0.4 |
| A6S-3-E2-IR | TSEVQRRSQDNFYDWFVAQVA | 33.1 | 24.7 | 9.8 | 2.5 | 0.4 |

FIG. 1E-3

| Clone Design | Sequence XXXXXXXXXXNFYDWFVXXXX | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| A6S-4-E4-IGFR | ERSAAGFREGNFYDWFVAQVN | 27 | 32 | -- | -- | -- |
| A6S-2-D2-IGFR | RAERGSMRDSNFYDWFVQQLP | 36 | 30 | -- | -- | -- |
| A6S-2-F2-IGFR | LAMSVASRPANFYDWFVAQIV | 35 | 30 | -- | -- | -- |
| A6S-4-F3-IGFR | HNSSSPMRTGNFYDWFVQELR | 26 | 30 | -- | -- | -- |
| A6S-4-G4-IGFR | SALSGPVQPINFYDWFVTGM | 26 | 30 | -- | -- | -- |
| A6S-4-G3-IGFR | GAQAIREIHHNFYDWFVAQVT | 21 | 29 | -- | -- | -- |
| A6S-2-H2-IGFR | RGQRESDSGTNFYDWFVGAIR | 40 | 28 | -- | -- | -- |
| A6S-2-E3-IGFR | VQEGLSGMEGNFYDWFVDQLF | 36 | 28 | -- | -- | -- |
| A6S-4-C6-IGFR | RLDRSSTSGVNFYDWFVAQVG | 25 | 28 | -- | -- | -- |
| A6S-4-F5-IGFR | GSQHSGREPHNFYDWFVAQVG | 24 | 28 | -- | -- | -- |
| A6S-4-H3-IGFR | GRGDQRHETTNFYDWFVRELQ | 20 | 28 | -- | -- | -- |
| A6S-4-H4-IGFR | PRMVEKPSEDNFYDWFVTQLS | 20 | 28 | -- | -- | -- |
| A6S-2-H1-IGFR | RVGIQVDPHTNFYDWFVIQLT | 42 | 27 | -- | -- | -- |
| A6S-4-E6-IGFR | RSSGGLLSQGNFYDWFVSQLE | 24 | 26 | -- | -- | -- |
| A6S-4-B6-IGFR | SDARQAGLQENFYDWFVSQVR | 23 | 26 | -- | -- | -- |
| A6S-4-D2-IGFR | PPYRSSRLGENFYDWFVMQVR | 19 | 26 | -- | -- | -- |
| A6S-4-G5-IGFR | QEVTRTRDDKNFYDWFVSQIF | 18 | 26 | -- | -- | -- |
| A6S-2-A3-IGFR | SRAPYGSTAGNFYDWFVQAVS | 37 | 25 | -- | -- | -- |
| A6S-4-E2-IGFR | ?DGQSVSSKGNFYDWFVQQMT | 25 | 25 | -- | -- | -- |
| A6S-4-G6-IGFR | RLMGGIAEPQNFYDWFVREVA | 20 | 25 | -- | -- | -- |
| A6S-4-G2-IGFR | SAGHHMPRESNFYDWFVDQVV | 25 | 24 | -- | -- | -- |
| A6S-4-D6-IGFR | LGAAETWDGINFYDWFVKQVS | 22 | 24 | -- | -- | -- |
| A6S-4-F4-IGFR | VGHSGVPPYPNFYDWFVMQVS | 22 | 24 | -- | -- | -- |
| A6S-4-C3-IGFR | VTMLDKGAQDNFYDWFVREVA | 21 | 24 | -- | -- | -- |
| A6S-4-H5-IGFR | HHSPGNEHGYNFYDWFVLQVA | 19 | 24 | -- | -- | -- |
| A6S-4-H6-IGFR | GSIAQLIMRANFYDWFVEQTN | 18 | 24 | -- | -- | -- |
| A6S-4-F6-IGFR | LKGSSQPLSVNFYDWFVQQIK | 17 | 24 | -- | -- | -- |
| A6S-3-H1-IGFR | PASNKNSLAENFYDWFVQQTR | 30 | 23 | -- | -- | -- |

FIG. 1F-1

| Ratios over Background | | Comparisons | | | | |
|---|---|---|---|---|---|---|
| Clone | Sequence | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | XXXXXXXXXXNFYDWFVXXXX | -- | -- | -- | -- | -- |
| A6S-4-A6-IGFR | HVEHMAVGDGNFYDWFVVQLR | 21 | 23 | -- | -- | -- |
| A6S-4-E3-IGFR | RGMTGMVGRGNFYDWFVGQLR | 21 | 23 | -- | -- | -- |
| A6S-4-D3-IGFR | GLRSEQGNRLNFYDWFVAQIA | 20 | 23 | -- | -- | -- |
| A6S-3-E10-IGFR | RVREKLPRPENFYDWFVNQIH | 23 | 22 | -- | -- | -- |
| A6S-4-D1-IGFR | SNPSRQDASVNFYDWFVREVA | 22 | 22 | -- | -- | -- |
| A6S-4-B2-IGFR | QSVDLSRPDSNFYDWFVEVLS | 21 | 22 | -- | -- | -- |
| A6S-4-A2-IGFR | IGGQGQHQDGNFYDWFVEALA | 20 | 22 | -- | -- | -- |
| A6S-4-A5-IGFR | VEVQRHIRKDNFYDWFVKQID | 19 | 22 | -- | -- | -- |
| A6S-4-C1-IGFR | CWARPCGDAANFYDWFVQQAS | 16 | 22 | -- | -- | -- |
| A6S-4-B1-IGFR | RHERGKEGPGNFYDWFVSQVV | 19 | 21 | -- | -- | -- |
| A6S-4-B4-IGFR | ERSPRPALASNFYDWFVQQVV | 19 | 21 | -- | -- | -- |
| A6S-4-D4-IGFR | IARMRETFQPNFYDWFVDQLA | 18 | 21 | -- | -- | -- |
| A6S-3-F8-IGFR | GRGQGLKRPDNFYDWFVAAAK | 25 | 20 | -- | -- | -- |
| A6S-3-H9-IGFR | YSIEVQDWNENFYDWFVSQLG | 23 | 20 | -- | -- | -- |
| A6S-3-G2-IGFR | TWMWEERKQDNFYDWFVGQLK | 21 | 20 | -- | -- | -- |
| A6S-4-H2-IGFR | VTFTSAVFHENFYDWFVRQVS | 19 | 20 | -- | -- | -- |
| A6S-4-A3-IGFR | LAINDLVTHKNFYDWFVDQLR | 18 | 20 | -- | -- | -- |
| A6S-3-G10-IGFR | GAVGLAEAGPNFYDWFVSQVQ | 24 | 19 | -- | -- | -- |
| A6S-3-E5-IGFR | RYRGERHDGRNFYDWFVEQVN | 21 | 19 | -- | -- | -- |
| A6S-3-H2-IGFR | QGAEGRLSEGNFYDWFVQAVS | 21 | 19 | -- | -- | -- |
| A6S-3-G3-IGFR | PRLHMGSDMGDFYDWFVVQIA | 21 | 18 | -- | -- | -- |
| A6S-4-H1-IGFR | IVAGARHSEVNFYDWFVIQVR | 18 | 18 | -- | -- | -- |
| A6S-4-G1-IGFR | AELVGAGVRGNFYDWFVDQLV | 16 | 16 | -- | -- | -- |
| A6S-4-A1-IGFR | DSSRLWLGERNFYDWFVAQIS | 17 | 12 | -- | -- | -- |
| A6S-2-F1-IGFR | VGQVGRYVRSNFYDWFVQQAM | 30 | 8 | -- | -- | -- |
| A6S-2-G1-IGFR | RPQLVESGSKNFYDWFVQVVR | 30 | 8 | -- | -- | -- |
| A6S-1-C5-IGFR | RIHNQTERGGNFYDWFVHQLV | 27 | 7 | -- | -- | -- |
| A6S-2-B2-IGFR | EMYGDTSERVNFYDWFVSALQ | 30 | 5 | -- | -- | -- |

FIG. 1F-2

| Ratios over Background Clone Design | Sequence XXXXXXXXXXNFYDWFVXXXX | Comparisons E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| A6S-1-D5-IGFR | RVGSGMEDLGNFYDWFVRQAQ | 25 | 5 | -- | -- | -- |
| A6S-1-A2-IGFR | KDPVTVSQGRNFYDWFVVQIQ | 20 | 5 | -- | -- | -- |
| A6S-3-E6-IGFR | DARDHGVWVMSNFYDWFVAQVS | 20 | 5 | -- | -- | -- |
| A6S-1-G3-IGFR | VATVHVGGGMNFYDWFVAQVG | 19 | 5 | -- | -- | -- |
| A6S-3-G4-IGFR | CADPGACSSLNFYDWFVQMRG | 21 | 4 | -- | -- | -- |
| A6S-3-H8-IGFR | NPTSVQQYGVNFYDWFVNVLS | 20 | 4 | -- | -- | -- |
| A6S-3-E3-IGFR | RPSLPEVRPGNFYDWFVQSVR | 19 | 4 | -- | -- | -- |
| A6S-3-D9-IGFR | SLQGADFQQGNFYDWFVSELA | 17 | 4 | -- | -- | -- |
| A6S-2-A1-IGFR | LSSRGRVTMRNFYDWFVAQVV | 31 | 3 | -- | -- | -- |
| A6S-1-H4-IGFR | HKSWTTMSPLNFYDWFVAQVE | 18 | 3 | -- | -- | -- |
| A6S-3-C1-IGFR | RPVIGGGGTRNFYDWFVAQMI | 17 | 3 | -- | -- | -- |
| A6S-3-B10-IGFR | YDQDPPYWGLNFYDWFVREVA | 16 | 3 | -- | -- | -- |

FIG. 1F-3

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Parental/Design | YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV | 19.0 | .4.0 | -- | -- | -- |
| A6L-3-D1-IR | QRGMLVRGRISHGAGKIAYEPPDCLGQKACAVNFYDWFV | 22.6 | 19.8 | 26.5 | 0.7 | 1.3 |
| A6L-4-H7-IR | QRGMLLLGRISDDAGKVASEPSARRGQKVFAFNFYDWFV | 37.5 | 3.5 | 4.2 | 0.8 | 1.2 |
| A6L-4-H4-IR | YRGILVLGRISEGAGKVASEPAARIGQKVFADFYDWFV | 38.5 | 21.1 | 25.8 | 0.8 | 1.2 |
| A6L-4-E4-IR | QRGMLALGRISDGAGKVASEPPAGIGQKVFAFNFYDWFV | 38.1 | 5.4 | 6.0 | 0.9 | 1.1 |
| A6L-4-G7-IR | FRGRLVLGHFSDGAGKVGSEPAARIGQKVFDVNFYDWFV | 38.6 | 16.2 | 18.5 | 0.9 | 1.1 |
| A6L-3-C3-IR | YRGMLVLGRISDGAGKVASEPPARIGQEVFADNFYDWFV | 34.7 | 21.8 | 23.1 | 0.9 | 1.1 |
| A6L-3-B6-IR | YRGMLVLGRISDGAGEVASEPPARIGQEVFALNFYDWFV | 33.1 | 27.8 | 30.3 | 0.9 | 1.1 |
| A6L-4-G11-IR | VPWYAGSGSSSDGAGKVASEPPARIDQKVFAVNFYDWFV | 27.6 | 2.0 | 2.0 | 1.0 | 1.0 |
| A6L-4-G12-IR | YRGQLVLGRISYGAGKVGCDPPARIGQKDWAVNFYDWFV | 32.0 | 2.3 | 2.3 | 1.0 | 1.0 |
| A6L-3-A10-IR | QRGLLVLGRFSDGAGNVASEPPAGIGQEVFPVNFYDWFV | 21.1 | 2.4 | 2.4 | 1.0 | 1.0 |
| A6L-4-E12-IR | QRGMLVLGRISDGAGKVAAEPPDCLGQKVCAVNFYDWFV | 3.1 | 2.4 | 2.4 | 1.0 | 1.0 |
| A6L-4-E10-IR | QRGMRVLGRISDGAGKVASELPPRIGQKDFAVNFYDWFV | 30.1 | 3.8 | 3.8 | 1.0 | 1.0 |
| A6L-4-G8-IR | QRGMLVLGSISDGAGKVAYEAPARIGQTVFAVNFYDWFV | 37.9 | 4.7 | 4.7 | 1.0 | 1.0 |
| A6L-3-C12-IR | QPWCAGSGRIYDGACKVASEPPAHIGQEVFAVNFYDWFV | 29.5 | 5.7 | 5.7 | 1.0 | 1.0 |
| A6L-4-H11-IR | QRGMLVLDRISDGAGKVASGPPARIGQNVLAVNFYDWFV | 35.4 | 9.6 | 9.6 | 1.0 | 1.0 |
| A6L-4-F10-IR | YRGMLVVGRISDGTGKVASQPPARIGQKVFAVNFYDWFV | 31.6 | 10.5 | 10.5 | 1.0 | 1.0 |
| A6L-4-E9-IR | YRGMLVLGRISDGAGKVASVPPAHIGQKVFAFNFYDWFV | 39.8 | 12.9 | 12.9 | 1.0 | 1.0 |
| A6L-4-H8-IR | QHGMLVLGRVSVGAGKVPSEPQARIGHKVFDVNFYDWFV | 38.2 | 14.6 | 14.6 | 1.0 | 1.0 |
| A6L-3-A11-IR | YSGYAGSGSFSDGAGKVASEPPARISQEVLADNFYDWFV | 29.0 | 17.5 | 17.5 | 1.0 | 1.0 |
| A6L-4-F9-IR | YRGMLVLGRISDGAGKVASEPPARIGQKVSAVNFYDWFV | 35.7 | 18.4 | 18.4 | 1.0 | 1.0 |
| A6L-4-G2-IR | YHGKLDLGRISVGVGKVASEPPARIGQKVFADNFYDWFV | 29.5 | 21.4 | 20.7 | 1.0 | 1.0 |
| A6L-4-E8-IR | YRGQAGSGVGSLTVAGKVASDPPARIGQKVFADNFYDWFV | 28.7 | 21.6 | 21.6 | 1.0 | 1.0 |
| A6L-4-H10-IR | HRGMLVLGRISEGAGNVDPEPPARIGQNVFAGNFYDWFV | 30.0 | 22.1 | 22.1 | 1.0 | 1.0 |
| A6L-4-G9-IR | QRGMPVLGRISDGAGKVGSEPPARIARKVFPVNFYDWFI | 37.1 | 22.6 | 22.6 | 1.0 | 1.0 |
| A6L-4-F7-IR | QGGLLVTGRISDGAGKVASEPPGGIGQKVFAGNFYDWFV | 28.6 | 23.6 | 24.4 | 1.0 | 1.0 |
| A6L-4-E11-IR | YPWYGGSGTYLDGAGKVASEPPARIDQQVFAGNFYDWFV | 38.4 | 26.5 | 26.5 | 1.0 | 1.0 |

FIG. 1G-1

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| **Parental/Design** | **YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV** | **19.0** | **.4.0** | -- | -- | -- |
| A6L-4-H9-IR | YRAMLVLRRISDVAGIVDSEPPTRIGQKVFAGNFYDWFV | 37.5 | 27.3 | 27.3 | 1.0 | 1.0 |
| A6L-4-E1-IR | YRGMLVLGRISQGAGNVASEPSSRIGQKVFAGNFYDWFI | 35.4 | 32.6 | 31.4 | 1.0 | 1.0 |
| A6L-3-A5-IR | YRGMLVLGRISDGAGKVDYEPPARIGQKVFAGNFYDWFV | 38.3 | 34.6 | 35.5 | 1.0 | 1.0 |
| A6L-4-G4-IR | YRGMLGLGGISAGAGIVASEPPARVGQKVFAGNFYDWFV | 30.4 | 17.7 | 15.2 | 1.2 | 0.9 |
| A6L-4-H2-IR | YRGILFQGRIPDGAGKVASEPPTRIGERVFAVNFYDWFV | 36.1 | 4.2 | 3.6 | 1.1 | 0.9 |
| A6L-4-E6-IR | QGGMPVLGRISDGAGKVAFEPPARIGQKVFAGNFYDWFV | 28.6 | 24.1 | 22.7 | 1.1 | 0.9 |
| A6L-4-H5-IR | YRGMLVLGRIQDGAGKVASEPPARIGQKVFTGNFYDWFV | 37.2 | 24.6 | 23.1 | 1.1 | 0.9 |
| A6L-4-H3-IR | QRGMLVLGGVSDGAGKVASDPPASIGQNVFAVNFYDWFV | 37.1 | 9.1 | 7.2 | 1.3 | 0.8 |
| A6L-4-E5-IR | YPGMLILDRISDGASKVVSEPPASIGQKVFAVNFYDWFV | 42.1 | 30.6 | 24.4 | 1.3 | 0.8 |
| A6L-3-C5-IR | YRGMLVLDRISDGAGKVASEQPARIGQEVYAVNFYDWFV | 42.2 | 21.9 | 17.5 | 1.2 | 0.8 |
| A6L-4-G6-IR | YRGMLDLGRISGGVGKVASESPARIGQKVYAVNFYDWFV | 29.8 | 4.3 | 2.8 | 1.5 | 0.7 |
| A6L-3-D4-IR | QRGMMVLGRISDGAGEVASEKVFAVNFYDWFV | 39.9 | 12.4 | 8.4 | 1.5 | 0.7 |
| A6L-3-A7-IR | QRGMLVLGRVSDGAGKVDSAPPARIGQKVFAGNFYDWFV | 31.0 | 21.2 | 14.0 | 1.5 | 0.7 |
| A6L-3-A6-IR | QRGMLVLGRMSDGAGKVAFEPPARIGQRGFAGNFYDWFV | 25.5 | 12.3 | 8.8 | 1.4 | 0.7 |
| A6L-4-E7-IR | QRGTLVLGRISDGAGKAASEPPARIGQNVFAVNFYDWFV | 38.4 | 12.5 | 7.1 | 1.7 | 0.6 |
| A6L-3-C6-IR | QRGMLVLDRISDGAGKVAAEPPARIGQKVFALNFYDWFI | 28.8 | 10.9 | 6.7 | 1.6 | 0.6 |
| A6L-4-F5-IR | QRGMLVLGRISDGAGEVASEPPARIGEKVYAVNFYDWFV | 33.8 | 6.3 | 4.1 | 1.5 | 0.6 |
| A6L-3-B7-IR | QRGILVRGRISDGAGKVGSEPPARSGEKVFAVNFYDWFI | 27.6 | 9.4 | 5.0 | 1.9 | 0.5 |
| A6L-4-F4-IR | QLGMVVLGRISDGSGKAASEPAARISQKVFAVNFYDWFV | 38.9 | 17.6 | 9.4 | 1.9 | 0.5 |
| A6L-4-E3-IR | QRGMLVLGRISDGDGKVASEPPARIGQRVFAVNFYDWFV | 38.0 | 6.9 | 3.8 | 1.8 | 0.5 |
| A6L-0-E6-IR | YRGMLVLGRSSDGAGKVAFERPARIGQTVFAVNFYDWFV | 31.0 | 31.0 | 1.8 | 17.0 | 0.1 |
| A6L-0-E4-IR | YRGMLVLGRISDGAG#VASEPPARIGRKVFAVNFYDWFV | 26.0 | 16.0 | 1.3 | 13.0 | 0.1 |
| A6L-0-H3-IR | YRGMLVLGRISGGAGKAASERPARIGQKVSAVNFYDWFV | 27.0 | 26.0 | 2.0 | 13.0 | 0.1 |

FIG. 1G-2

| Clone Parental/Design | Sequence | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV | 19 | 4 | -- | -- | -- |
| A6L-4-F8-IGFR | YRGMMVQGRISDGAGKVASVSPVRIGQKVIAVNFYDWFV | 26 | 28 | -- | -- | -- |
| A6L-2-G9-IGFR | YRGRLGLGRISDVAGKVACDPSARIGQKVLPVNFYDWFV | 39 | 22 | -- | -- | -- |
| A6L-4-E7-IGFR | YRGMLVLGRISDGAGRVASEPQARIGQKVFAVNFYDWFV | 23 | 22 | -- | -- | -- |
| A6L-4-G10-IGFR | QGGMLVPGRISDGAGKVASQPPARIGPKGFAGNFYDWFV | 19 | 22 | -- | -- | -- |
| A6L-2-E9-IGFR | YRGMRVLGRISDGAGKVASEPPTHIGQKVFPVNFYDWFV | 38 | 21 | -- | -- | -- |
| A6L-2-D6-IGFR | YRGMLVLGRISDGAGKVGSEPAARIGQKVFALNFYDWFV | 34 | 21 | -- | -- | -- |
| A6L-3-H12-IGFR | YRGQGMVLGRISDGAGKVASEPPGRIGQKVFPVNFYDWFV | 24 | 21 | -- | -- | -- |
| A6L-4-A7-IGFR | YRGMLGLGRITGGAGKVASEPPDRIGQHVFVDNFYDWFV | 20 | 20 | -- | -- | -- |
| A6L-4-B8-IGFR | DGMLVLGRISDGAGNVASEAPARIGQKVFAVNFYDWFV | 20 | 19 | -- | -- | -- |
| A6L-4-G7-IGFR | YRGMRVRGRISDGAGKAASDPRARIGQTVLDVNFYDWFV | 19 | 19 | -- | -- | -- |
| A6L-2-D9-IGFR | YRGMWVLGRISYGAGKVAYEPPARMGQKGFAVNFYDWFV | 38 | 18 | -- | -- | -- |
| A6L-4-F7-IGFR | YRGMLVGGRIAGGAGIVASEPPARIGQKVFAVNFYDWFV | 18 | 18 | -- | -- | -- |
| A6L-4-E12-IGFR | YRGLLGLGGISDGAGKVASEPPARNGQKVFAVNFYDWFV | 15 | 13 | -- | -- | -- |
| A6L-4-H7-IGFR | YRGMLGLGRISAGAGKVASGAPARIGQEDFAVNFYDWFV | 14 | 13 | -- | -- | -- |
| A6L-4-H12-IGFR | YRGMLALGRISEGAGKVASEPPARIGQNVFAVNFYDWFV | 13 | 12 | -- | -- | -- |
| A6L-2-A4-IGFR | YRGMLVLGRISDGAGKVASEPPARIGQKVLAVNFYDWFV | 17 | 4 | -- | -- | -- |
| A6L-3-D10-IGFR | YPGMLVPGRISDGAGEGATDPPPRIGQKVFAFNFYDWFV | 16 | 4 | -- | -- | -- |
| A6L-2-F6-IGFR | YRGMLVPGRISDGAGKVAYEPPARIGQKIFAVNFYDWFV | 15 | 4 | -- | -- | -- |
| A6L-2-B11-IGFR | YRGVLVLGRVSDGVGKVASEPPAHRGQRVFGVNFYDWFV | 26 | 3 | -- | -- | -- |
| A6L-1-B7-IGFR | YRRMLVLGRISDGAANVASGPPDRIGQKVFAGNFYDWFV | 23 | 3 | -- | -- | -- |
| A6L-1-D8-IGFR | YRRMLALGRFSDVTGDVASEPPAHIGQKVVAVNFYDWFV | 23 | 3 | -- | -- | -- |
| A6L-0-A11-IGFR | YRGMVVRGRIFDGPGKVASEPRARIGQKVFAVNFYDWFV | 19 | 3 | -- | -- | -- |
| A6L-3-B7-IGFR | YRGMLILGRISDGAGKVASEPPARVGQDVVAVNFYDWFV | 9 | 3 | -- | -- | -- |
| A6L-1-G7-IGFR | YPGRLVGGRISDGVGKVASEPPGRIGQKVFAVNFYDWFV | 20 | 2 | -- | -- | -- |
| A6L-1-B9-IGFR | QRGLLVLGRIFDGAGKVASDPPARIGQKDFADNFYDWFV | 18 | 2 | -- | -- | -- |
| A6L-1-C9-IGFR | YRGMLVLGRISDGAGKVAFEPPARIGQNVFAVNFYDWFV | 18 | 2 | -- | -- | -- |
| A6L-0-G10-IGFR | YRCMPVLGRISDGAG#VASDRPARIGQKVFAVNFYDWFV | 18 | 2 | -- | -- | -- |
| A6L-1-G8-IGFR | YRGRLVLGRISDGAGKVAAEPPASMDSKVFAGNFYDWFV | 15 | 2 | -- | -- | -- |

FIG. 1H

| Clone Design | Sequence GFREGNFYDWFVAQVT | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| E4Dα-1-B8-IR | GFREGQRWYWFVAQVT | 40.7 | 1.0 | 12.3 | 0.1 | 12.3 |
| E4Dα-3-E5-IR | GFREGYFYDWFLAQVT | 39.6 | 2.0 | 1.5 | 1.3 | 0.8 |
| E4Dα-1-A1-IR | GFREGDFYEWFVAQVT | 48.7 | 44.9 | 31.4 | 1.4 | 0.7 |
| E4Dα-2-D9-IR | GFREGQFYEWFAAQVT | 22.9 | 3.3 | 2.4 | 1.4 | 0.7 |
| E4Dα-1-B3-IR | GFREGTFYDWFVAQVT | 41.8 | 38.6 | 26.5 | 1.5 | 0.7 |
| E4Dα-1-A6-IR | GFREGNFYDWFEAQVT | 56.3 | 51.2 | 32.6 | 1.6 | 0.6 |
| E4Dα-1-A10-IR | GFREGAFYDWFEAQVT | 48.9 | 42.2 | 26.5 | 1.6 | 0.6 |
| E4Dα-1-A8-IR | GFREGAFYDWFVAQVT | 46.9 | 41.5 | 26.2 | 1.6 | 0.6 |
| E4Dα-1-B1-IR | GFREGKFYQWFEAQVT | 44.1 | 31.1 | 19.7 | 1.6 | 0.6 |
| E4Dα-2-C9-IR | GFREGDFYDWFQAQVT | 34.0 | 8.1 | 4.8 | 1.7 | 0.6 |
| E4Dα-1-A3-IR | GFREGTFYEWFVAQVT | 45.3 | 40.3 | 22.5 | 1.8 | 0.6 |
| E4Dα-1-A9-IR | GFREGNFYDWFVAQVT | 46.9 | 41.0 | 22.5 | 1.8 | 0.5 |
| E4Dα-3-F3-IR | GFREGQFYEWFLAQVT | 37.2 | 14.1 | 8.0 | 1.8 | 0.6 |
| E4Dα-2-D3-IR | GFREGQFYDWFLAQVT | 35.1 | 16.3 | 8.7 | 1.9 | 0.5 |
| E4Dα-2-D6-IR | GFREGEFYDWFQAQVT | 33.2 | 5.6 | 2.8 | 2.0 | 0.5 |
| E4Dα-3-F10-IR | GFREGQFYDWFRAQVT | 27.8 | 4.5 | 2.3 | 2.0 | 0.5 |
| E4Dα-2-D5-IR | GFREGYFYEWFQAQVT | 43.8 | 23.8 | 11.4 | 2.1 | 0.5 |
| E4Dα-3-F4-IR | GFREGDFYQWFEAQVT | 25.9 | 7.6 | 3.7 | 2.1 | 0.5 |
| E4Dα-3-E3-IR | GFREGSFYGWFQAQVT | 34.6 | 4.0 | 1.9 | 2.1 | 0.5 |
| E4Dα-3-F8-IR | GFREGSFYAWFQAQVT | 20.9 | 16.0 | 7.4 | 2.2 | 0.5 |
| E4Dα-2-C1-IR | GFREGQFYDWFVAQVT | 43.1 | 11.6 | 5.0 | 2.3 | 0.4 |
| E4Dα-1-B4-IR | GFREGIFYEWFVAQVT | 45.3 | 6.6 | 2.9 | 2.3 | 0.4 |

FIG. 1I-1

| Clone Design | Sequence GFREGNFYDWFVAQVT | Ratios over Background E-Tag -- | IGFsR -- | IR -- | Comparisons IGFR/IR -- | IR/IGFR -- |
|---|---|---|---|---|---|---|
| E4Dα-4-H5-IR | GFREGSFYEWFQAQVT | 47.2 | 36.0 | 14.7 | 2.4 | 0.4 |
| E4Dα-1-B12-IR | GFREGNFYDWFAAQVT | 47.6 | 33.4 | 13.8 | 2.4 | 0.4 |
| E4Dα-4-G2-IR | GFREGSFYDWFVAQVT | 23.4 | 20.4 | 8.6 | 2.4 | 0.4 |
| E4Dα-3-F9-IR | GFREGDFYDWFVAQVT | 36.2 | 15.6 | 6.3 | 2.5 | 0.4 |
| E4Dα-4-G6-IR | GFREGDFYQWFVAQVT | 26.0 | 4.9 | 2.0 | 2.5 | 0.4 |
| E4Dα-4-H9-IR | GFREGGFYDWFVAQVT | 47.8 | 24.8 | 9.5 | 2.6 | 0.4 |
| E4Dα-2-C10-IR | GFREGDFYGWFQAQVT | 42.4 | 23.2 | 9.0 | 2.6 | 0.4 |
| E4Dα-1-B2-IR | GFREGVFYDWFVAQVT | 39.4 | 18.7 | 7.2 | 2.6 | 0.4 |
| E4Dα-3-F12-IR | GFREGGFYEWFQAQVT | 38.9 | 16.6 | 5.6 | 3.0 | 0.3 |
| E4Dα-2-D11-IR | GFREGSFYDWFQAQVT | 40.2 | 11.1 | 3.3 | 3.4 | 0.3 |
| E4Dα-4-H2-IR | GFREGNFYEWFQAQVT | 37.8 | 33.9 | 8.2 | 4.1 | 0.2 |
| E4Dβ-4-A12-IR | GFREGKFYDWFLAQVT | 41.1 | 8.3 | 28.7 | 0.3 | 3.5 |
| E4Dβ-4-A10-IR | GFREGEFYEWFVAQVT | 5.8 | 1.2 | 2.4 | 0.5 | 2.0 |
| E4Dβ-4-E10-IR | GFREGRFYDWFVAQVT | 9.6 | 1.2 | 2.2 | 0.5 | 1.8 |
| E4Dβ-4-B11-IR | GFREGTFYDWFVAQVT | 36.1 | 15.2 | 26.9 | 0.6 | 1.8 |
| E4Dβ-4-C10-IR | GFREGEFYEWFAAQVT | 27.8 | 13.3 | 23.7 | 0.6 | 1.8 |
| E4Dβ-4-E8-IR | GFREGDFYEWFEAQVT | 28.7 | 16.7 | 28.2 | 0.6 | 1.7 |
| E4Dβ-4-G7-IR | GFREGHFYDWF?AQVT | 30.9 | 14.7 | 24.7 | 0.6 | 1.7 |
| E4Dβ-4-C8-IR | GFREGEFYDWFVAQVT | 35.5 | 22.5 | 32.9 | 0.7 | 1.5 |
| E4Dβ-4-A8-IR | GFREGSFYDWFVAQVT | 31.2 | 14.5 | 22.2 | 0.7 | 1.5 |
| E4Dβ-4-A9-IR | GFREGSFYDWFGAQVT | 35.8 | 9.0 | 13.1 | 0.7 | 1.5 |
| E4Dβ-4-G11-IR | GFREGTFYDWFQAQVT | 28.9 | 9.7 | 13.6 | 0.7 | 1.4 |
| E4Dβ-4-B9-IR | GFREGNFYEWFTAQVT | 27.2 | 9.1 | 12.5 | 0.7 | 1.4 |
| E4Dβ-4-F10-IR | GFREGSFYNWFQAQVT | 7.7 | 1.5 | 2.1 | 0.7 | 1.4 |
| E4Dβ-4-D12-IR | GFREGNFYDWFVAQVT | 41.1 | 27.2 | 36.1 | 0.8 | 1.3 |
| E4Dβ-4-B8-IR | GFREGDFYDWFVAQVT | 35.9 | 27.0 | 35.2 | 0.8 | 1.3 |
| E4Dβ-4-G10-IR | GFREGAFYDWFAAQVT | 38.5 | 25.5 | 33.7 | 0.8 | 1.3 |
| E4Dβ-4-D9-IR | GFREGSFYDWFEAQVT | 34.1 | 19.3 | 25.7 | 0.8 | 1.3 |

FIG. 1I-2

| Clone Design | Sequence GFREGNFYDWFVAQVT | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| E4Dβ-4-F8-IR | GFREGSFYDWFAAQVT | 39.3 | 35.6 | 44.4 | 0.8 | 1.2 |
| E4Dβ-4-E12-IR | GFREGSFYEWFDAQVT | 40.2 | 27.8 | 33.4 | 0.8 | 1.2 |
| E4Dβ-4-H12-IR | GFREGAFYDWFEAQVT | 41.2 | 27.1 | 32.3 | 0.8 | 1.2 |
| E4Dβ-4-C9-IR | GFREGQFYDWFAAQVT | 38.0 | 22.5 | 27.6 | 0.8 | 1.2 |
| E4D□-4-H9-IR | GFREGNFYDWFAAQVT | 38.7 | 33.3 | 36.6 | 0.9 | 1.1 |
| E4D□-4-G9-IR | GFREGDFYDWFAAQVT | 10.9 | 4.9 | 5.6 | 0.9 | 1.1 |
| E4Dβ-4-F12-IR | GFREGSFYEWFEAQVT | 14.8 | 5.9 | 6.1 | 1.0 | 1.0 |
| E4Dβ-4-F9-IR | GFREGGFYDWFLAQVT | 39.3 | 31.3 | 28.3 | 1.1 | 0.9 |
| E4Dβ-4-F7-IR | GFREGGFYAWFAAQVT | 31.0 | 22.2 | 19.5 | 1.1 | 0.9 |
| E4Dβ-4-B7-IR | GFREGGFYEWF?AQVT | -- | -- | -- | -- | -- |

FIG. 1I-3

| Clone Design | Sequence GFREGNFYDWFVAQVT | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| E4D-2-E7-IGFR | GFREGDFYDWFRAQVT | 20.8 | 22.8 | -- | -- | -- |
| E4D-2-C11-IGFR | GFREGSFYDWFVAQVT | 21.5 | 22.6 | -- | -- | -- |
| E4D-2-B1-IGFR | GFREGDFYGWFQAQVT | 22.0 | 22.5 | -- | -- | -- |
| E4D-2-D10-IGFR | GFREGGFYDWFQAQVT | 20.6 | 22.1 | -- | -- | -- |
| E4D-2-A9-IGFR | GFREGDFYDWFVAQVT | 17.4 | 21.5 | -- | -- | -- |
| E4D-2-E5-IGFR | GFREGDFYDWFQAQVT | 24.2 | 21.2 | -- | -- | -- |
| E4D-2-H9-IGFR | GFREGGFYDWFVAQVT | 19.1 | 20.7 | -- | -- | -- |
| E4D-1B-C4-IGFR | GFREGDFYDWFAAQVT | 24.3 | 20.5 | -- | -- | -- |
| E4D-2-E10-IGFR | GFREGNFYDWFQAQVT | 21.0 | 20.5 | -- | -- | -- |
| E4D-2-F4-IGFR | GFREGNFYDWFLAQVT | 25.0 | 20.2 | -- | -- | -- |
| E4D-2-C10-IGFR | GFREGHFYDWFQAQVT | 22.8 | 20.1 | -- | -- | -- |
| E4D-3-D8-IGFR | GFREGQFYEWFEAQVT | 21.1 | 19.8 | -- | -- | -- |
| E4D-3-F9-IGFR | GFREGSFYEWFQAQVT | 22.6 | 19.7 | -- | -- | -- |
| E4D-1B-E5-IGFR | GFREGDFYDWFLAQVT | 24.2 | 18.8 | -- | -- | -- |
| E4D-2-F3-IGFR | GFREGHFYDWFVAQVT | 23.6 | 18.0 | -- | -- | -- |
| E4D-3-D5-IGFR | GFREGQFYEWFVAQVT | 22.2 | 18.0 | -- | -- | -- |
| E4D-3-G10-IGFR | GFREGQFYDWFAAQVT | 22.1 | 17.6 | -- | -- | -- |
| E4D-2-F6-IGFR | GFREGQFYDWFVAQVT | 24.6 | 17.5 | -- | -- | -- |
| E4D-2-F7-IGFR | GFREGDFYQWFAAQVT | 19.0 | 17.5 | -- | -- | -- |
| E4D-3-B7-IGFR | GFREGNFYDWFVAQVT | 23.0 | 16.4 | -- | -- | -- |
| E4D-1B-C12-IGFR | GFRDGSFYDWFVAQVT | 23.0 | 16.1 | -- | -- | -- |
| E4D-3-B1-IGFR | GFREGHFYEWFQAQVT | 21.6 | 16.0 | -- | -- | -- |
| E4D-2-E2-IGFR | GFREGDFYDWFSAQVT | 21.9 | 14.1 | -- | -- | -- |
| E4D-2-D1-IGFR | GFREGHFYDWFDAQVT | 24.5 | 13.2 | -- | -- | -- |
| E4D-1-D4-IGFR | GFREGYFYDWFKAQVT | 18.9 | 12.4 | -- | -- | -- |
| E4D-1B-A10-IGFR | GFREGHFYDWFEAQVT | 23.9 | 10.8 | -- | -- | -- |
| E4D-1B-A3-IGFR | GFREGDFYDWFEAQVT | 22.2 | 10.8 | -- | -- | -- |
| E4D-1-B5-IGFR | GFREGTFYDWFVAQVT | 19.0 | 10.8 | -- | -- | -- |

FIG. 1J-1

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | GFREGNFYDWFVAQVT | -- | -- | -- | -- | -- |
| E4D-1B-B8-IGFR | GFREGDYYGWFEAQVT | 23.8 | 10.7 | -- | -- | -- |
| E4D-1-G7-IGFR | GFREGDFYAWFMAQVT | 14.3 | 10.5 | -- | -- | -- |
| E4D-1B-A11-IGFR | GFREGNFYEWFLAQVT | 24.0 | 10.0 | -- | -- | -- |
| E4D-1-C3-IGFR | GFREGSFYDWFDAQVT | 15.8 | 9.3 | -- | -- | -- |
| E4D-2-H1-IGFR | GFREGNFYDQFVAQVT | 19.6 | 4.9 | -- | -- | -- |
| E4D-1-C2-IGFR | GFREGHFYEWFAAQVT | 11.5 | 4.5 | -- | -- | -- |
| E4D-1B-A12-IGFR | GFREGNFYEWFVAQVT | 18.4 | 3.5 | -- | -- | -- |
| E4D-1B-A1-IGFR | GFREGKFYDWFVAQVT | 22.5 | 2.9 | -- | -- | -- |
| E4D-2-A3-IGFR | GFREGMFDVQLLAQVT | 22.7 | 2.1 | -- | -- | -- |

FIG. 1J-2

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | XXXXXXFHENFYDWFVRQVSXXXXXX | -- | -- | -- | -- | -- |
| Parental | VTFTSAVFHENFYDWFVRQVS | 29.8 | 17.5 | 16.3 | 1.1 | 0.9 |
| H2CA-4-F11-IR | TYKARFLHENFYDWFNRQVSQYFGRV | 37.7 | 2.2 | 18.1 | 0.1 | 8.2 |
| H2CA-4-E10-IR | QRLSLHEQFYDWFVGQVSPLGAGG | 31.2 | 4.4 | 18.8 | 0.2 | 4.3 |
| H2CA-4-G3-IR | GGGKVNFHEDFYGWFVQQFSGVGSDR | 36.1 | 13.4 | 25.7 | 0.5 | 1.9 |
| H2CA-3-A11-IR | LVGDAPFHEDFYDWFARQVFGCCQEQ | 35.6 | 12.1 | 22.0 | 0.5 | 1.8 |
| H2CA-4-F8-IR | TGAEVSFHENFYDWFDRQYSSWLDRD | 36.0 | 21.1 | 33.5 | 0.6 | 1.6 |
| H2CA-4-G4-IR | QPHSSRLHESFYDWFDRQVPWYALDR | 37.1 | 23.3 | 34.3 | 0.7 | 1.5 |
| H2CA-4-F4-IR | SRALAAVHEQFYDWFVRQVSGLDWGY | 39.8 | 25.0 | 35.6 | 0.7 | 1.4 |
| H2CA-4-H10-IR | QPKDGTLHENFYDWFVRQVSSSGWVG | 33.5 | 5.1 | 6.6 | 0.8 | 1.3 |
| H2CA-4-F1-IR | RGRLIQLHEDFYDWFLRQVSGMGGGS | 36.1 | 19.6 | 25.1 | 0.8 | 1.3 |
| H2CA-3-D5-IR | QRGAPKSDENFYDWFVRQVLRFGEND | 39.3 | 24.3 | 31.9 | 0.8 | 1.3 |
| H2CA-4-E11-IR | AARTSLFHEDFYEWFDRQVRQEGMWG | 8.2 | 2.6 | 3.2 | 0.8 | 1.2 |
| H2CA-3-B6-IR | GTSNHSLHENFYDWFVRQLSSVQSSG | 35.9 | 9.9 | 12.1 | 0.8 | 1.2 |
| H2CA-3-A9-IR | VSHVHLFHENFYDWFVRQLAAEGFSG | 37.3 | 30.1 | 36.2 | 0.8 | 1.2 |
| H2CA-4-H5-IR | GRQDSGLHEHFYDWFSRQVQGEVALG | 38.6 | 35.4 | 37.3 | 1.0 | 1.1 |
| H2CA-3-C9-IR | SNDERQFHETFYDWFVRQVSADGADR | 29.3 | 5.1 | 5.6 | 0.9 | 1.1 |
| H2CA-3-A10-IR | LSTEQRFHEKFYDWFVHQVSTSGGGT | 37.2 | 16.9 | 19.1 | 0.9 | 1.1 |
| H2CA-3-A3-IR | SLSREQFHENFYDWFARQVSELEGVV | 29.2 | 28.6 | 32.2 | 0.9 | 1.1 |
| H2CA-4-G8-IR | IPGRRSLHENFYDWFVRQVSPGGGSA | 32.4 | 29.1 | 31.6 | 0.9 | 1.1 |
| H2CA-4-G9-IR | TQKAQSLDEKFYDWFVRQVSGGGLTG | 36.1 | 34.4 | 36.4 | 0.9 | 1.1 |
| H2CA-4-G10-IR | VSQLSDFHENFYGWFARQIAGQAEWT | 34.2 | 35.5 | 37.7 | 0.9 | 1.1 |
| H2CA-4-H7-IR | NGTSQALHQNFYDWFAQQISGSEPGP | 37.0 | 36.0 | 40.0 | 0.9 | 1.1 |
| H2CA-4-F9-IR | VGQSVTFHGDFYDWFDRQLSGSQEFG | 37.5 | 36.7 | 39.5 | 0.9 | 1.1 |
| H2CA-4-F7-IR | TIDHHPLHEQFYDWFARQVSDLESLG | 37.7 | 37.6 | 39.9 | 0.9 | 1.1 |
| H2CA-3-D10-IR | PNVGYAFHENFYDWFIRQVSIEEKAG | 18.7 | 3.6 | 3.5 | 1.0 | 1.0 |
| H2CA-3-B1-IR | SRGSGVFHESFYNWFDRQVSEWIQFG | 26.5 | 21.4 | 21.5 | 1.0 | 1.0 |
| H2CA-3-A5-IR | QPVSGSVHERFYDWFVRQVSGSAGGG | 32.9 | 22.9 | 22.4 | 1.0 | 1.0 |
| H2CA-4-F10-IR | ASQLPPVYENFYEWFDRQVSLDAQRE | 26.6 | 27.7 | 28.5 | 1.0 | 1.0 |

FIG. 1K-1

| Clone Design | Sequence XXXXXXFHENFYDWFVRQVSXXXXXX | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| H2CA-3-D9-IR | VSGRGAFHENFYDWFVRQVFRDEQDT | 36.6 | 30.6 | 30.9 | 1.0 | 1.0 |
| H2CA-3-C2-IR | ARPPPTVHENFYDWFVRQVSETWRQD | 38.3 | 30.7 | 31.0 | 1.0 | 1.0 |
| H2CA-4-G1-IR | QGGDRLFHERFYDWFDRLVSSDSTGE | 34.1 | 30.7 | 30.4 | 1.0 | 1.0 |
| H2CA-4-E2-IR | QHIAAGLHENFYDWFIRQVSGVNVPA | 33.9 | 31.0 | 31.8 | 1.0 | 1.0 |
| H2CA-4-H9-IR | QPNDGLLHENFYDWFVRQVSNAVDGG | 38.9 | 31.1 | 31.4 | 1.0 | 1.0 |
| H2CA-3-D2-IR | PVEFTVYHDNFYDWFARQVSDGLGQF | 33.0 | 31.1 | 29.8 | 1.0 | 1.0 |
| H2CA-3-B3-IR | FCVQASIHENFYDWFVRQVAENQVFS | 35.3 | 31.4 | 30.0 | 1.0 | 1.0 |
| H2CA-4-G11-IR | GRPRGSFHENFYDWFARQVSGDGAGT | 37.9 | 31.9 | 31.0 | 1.0 | 1.0 |
| H2CA-4-F2-IR | IVGASLCHESFYDWFACQVTNLQSQG | 38.1 | 32.0 | 31.9 | 1.0 | 1.0 |
| H2CA-3-C5-IR | IGLRQMFHENFYDWFAREVSKEAGDG | 36.9 | 32.3 | 31.6 | 1.0 | 1.0 |
| H2CA-3-B2-IR | LGGAIEGHGNFYDWFVRQVSLDVGGE | 36.6 | 32.7 | 32.5 | 1.0 | 1.0 |
| H2CA-3-B11-IR | LNALQQLHENFYDWFGRQVSATPPGG | 35.5 | 32.8 | 33.3 | 1.0 | 1.0 |
| H2CA-4-G2-IR | VGNCDTFPENFYDWFACQVSELGGMN | 35.9 | 33.0 | 33.4 | 1.0 | 1.0 |
| H2CA-3-A4-IR | FSQDGNFHENFYDWFDRQLSLVGAGT | 33.3 | 33.0 | 32.9 | 1.0 | 1.0 |
| H2CA-4-H3-IR | PAGNRALHESFYDWFVRQVSEFQLGA | 39.5 | 33.7 | 33.7 | 1.0 | 1.0 |
| H2CA-4-G5-IR | DRLRARFNENFYDWFDRQVSGQGSMP | 35.3 | 34.0 | 35.6 | 1.0 | 1.0 |
| H2CA-4-E8-IR | VLGVAQFHDKFYDWFARQVSQLESAG | 35.7 | 34.7 | 34.9 | 1.0 | 1.0 |
| H2CA-4-G6-IR | GVVGGAFHEQFYDWFDRQVSAAFKGD | 36.2 | 35.0 | 33.5 | 1.0 | 1.0 |
| H2CA-3-B7-IR | DESEMRLHEQFYDWFARLVSLEGGSA | 37.6 | 36.5 | 35.3 | 1.0 | 1.0 |
| H2CA-3-B4-IR | EGGGVAIHENFYDWFDRQVSLQGWSD | 39.8 | 36.5 | 35.1 | 1.0 | 1.0 |
| H2CA-3-C7-IR | SRIVSRFHENFYDWFVRQVSGDAPVQ | 40.2 | 36.7 | 35.9 | 1.0 | 1.0 |
| H2CA-4-E5-IR | IPAGAQLHENFYDWFARQVSGEDGGA | 37.3 | 37.0 | 36.3 | 1.0 | 1.0 |
| H2CA-4-E7-IR | GSSAAGFDEQFYDWFDRQVSEAFRDG | 39.7 | 37.6 | 37.6 | 1.0 | 1.0 |
| H2CA-3-B9-IR | RLALRTFHQDFYDWFVRQVAAEDTDP | 39.4 | 37.7 | 37.6 | 1.0 | 1.0 |
| H2CA-4-F5-IR | QGSFAVLHENFYDWFARQVSGVEGLA | 38.8 | 38.0 | 37.8 | 1.0 | 1.0 |
| H2CA-3-B10-IR | QGNMSALHENFYDWFVRQVSEADRVD | 41.9 | 38.9 | 38.0 | 1.0 | 1.0 |
| H2CA-3-A12-IR | VAYPALLHEQFYDWFVRQVSAVAGTT | 37.8 | 7.3 | 6.3 | 1.2 | 0.9 |
| H2CA-3-A8-IR | PDTINSQHKNFYDWFVRQVSGVGTSS | 36.8 | 22.5 | 19.2 | 1.2 | 0.9 |

FIG. 1K-2

| Clone Design | Sequence XXXXXXFHENFYDWFVRQVSXXXXXX | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| H2CA-3-D12-IR | SEDVDSRHENFYDWFVRQVSGIGLQD | 36.8 | 34.1 | 29.6 | 1.2 | 0.9 |
| H2CA-3-B5-IR | PAPADAFDHNFYDWFARQLSATTTIQ | 38.8 | 35.2 | 30.5 | 1.2 | 0.9 |
| H2CA-4-E1-IR | MVQRISIHENFYDWFVRQISGSAVPP | 29.8 | 12.5 | 11.3 | 1.1 | 0.9 |
| H2CA-3-D3-IR | GNVRGQFHGQFYDWFARQVSGSEGDA | 33.1 | 29.9 | 27.5 | 1.1 | 0.9 |
| H2CA-4-E3-IR | PDAEKQFHETFYGWFVRQISEDSANS | 33.3 | 32.3 | 30.2 | 1.1 | 0.9 |
| H2CA-4-E12-IR | FGRGVHCDENFYDWFVCQVSGALLEG | 36.0 | 32.4 | 29.4 | 1.1 | 0.9 |
| H2CA-3-A6-IR | ETPLTELHEQFYDWFVRQVSGFPGGV | 34.0 | 33.1 | 30.6 | 1.1 | 0.9 |
| H2CA-4-E9-IR | QHRGPHFHEDFYDWFVRQVSSAVPSD | 38.8 | 33.7 | 29.7 | 1.1 | 0.9 |
| H2CA-4-F3-IR | RQDPGLFHDNFYDWFDRLVSAWDGQE | 41.0 | 34.2 | 32.0 | 1.1 | 0.9 |
| H2CA-4-H6-IR | QAAVGVCNKDFYAWFACQVREDFAKA | 37.1 | 34.5 | 30.8 | 1.1 | 0.9 |
| H2CA-4-H2-IR | RNWNLQFNENFYDWFDRQVSALRGGG | 41.8 | 35.3 | 32.8 | 1.1 | 0.9 |
| H2CA-3-D4-IR | RSEQYRFHENFYEWFDRQVSRMGLLG | 38.7 | 35.5 | 32.3 | 1.1 | 0.9 |
| H2CA-3-D1-IR | GAGGRDFDEDFYDWFVRQVSGQVTSG | 34.5 | 35.5 | 31.3 | 1.1 | 0.9 |
| H2CA-3-C1-IR | SPEGNLVHDQFYDWFVRQLSSTSAGT | 39.9 | 36.1 | 32.9 | 1.1 | 0.9 |
| H2CA-3-D8-IR | QGGLGDFDEDFYDWFARQVSRRDRAD | 37.8 | 36.7 | 33.1 | 1.1 | 0.9 |
| H2CA-4-H4-IR | LSQGVGFQENFYEWFERQVSGWDGRD | 38.5 | 37.0 | 33.7 | 1.1 | 0.9 |
| H2CA-4-F6-IR | VFERSRCHDNFYDWFFCQVSGQADGG | 38.7 | 37.5 | 35.2 | 1.1 | 0.9 |
| H2CA-4-E4-IR | LLASRAFHENFYDWFARQVSGTQPPG | 38.6 | 38.0 | 34.7 | 1.1 | 0.9 |
| H2CA-3-C11-IR | VPDAQIFHESFYDWFVRQASAGGPAD | 40.3 | 38.3 | 36.1 | 1.1 | 0.9 |
| H2CA-3-C4-IR | ANQMGRFHDNFYDWFDRQVSRYERGT | 41.9 | 38.4 | 35.0 | 1.1 | 0.9 |
| H2CA-4-E6-IR | PSRKDGLHQSFYDWFARQVQDMEGRA | 39.3 | 38.8 | 35.8 | 1.1 | 0.9 |
| H2CA-3-D7-IR | QAVTRRFHENFYDWFARQVSEEGGWS | 42.5 | 39.2 | 35.5 | 1.1 | 0.9 |
| H2CA-3-A7-IR | GYAVGQYQANFYDWFVRQVDGMSNGG | 35.3 | 15.2 | 11.6 | 1.3 | 0.8 |
| H2CA-4-G12-IR | GHQRDLLHESFYDWFVRQVSEAEGGG | 37.6 | 19.4 | 15.1 | 1.3 | 0.8 |
| H2CA-3-D6-IR | DRPSSFIHENFYEWFARQVSQSGSSG | 39.4 | 36.2 | 27.6 | 1.3 | 0.8 |
| H2CA-4-H12-IR | ERTAETLHEQFYDWFVRQVSAMDGES | 40.0 | 38.4 | 29.3 | 1.3 | 0.8 |
| H2CA-3-D11-IR | LTSQLLSHEDFYDWFVRQVSGVGGSG | 38.1 | 32.9 | 27.2 | 1.2 | 0.8 |
| H2CA-3-C12-IR | PDRSDRLDDNFYDWFVRQVSQVINED | 38.5 | 38.4 | 31.7 | 1.2 | 0.8 |

FIG. 1K-3

| Clone Design | Sequence XXXXXXXFHENFYDWFVRQVSXXXXXX | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| H2CA-4-G7-IR | RAGGVGLHDNFYDWFVRQVSGGDSGP | 35.9 | 34.7 | 23.7 | 1.5 | 0.7 |
| H2CA-3-C6-IR | ADCYVQLHENFYDWFRRQVCNLQEGM | 38.7 | 37.6 | 28.2 | 1.3 | 0.7 |
| H2CA-3-B8-IR | RQGHAGFHDNFYDWFVRQVSGSTPQV | 37.8 | 19.6 | 9.9 | 2.0 | 0.5 |

FIG. 1K-4

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | XXXXXXFHENFYDWFVRQVSXXXXXX | -- | -- | -- | -- | -- |
| Parental | VTFTSAVFHENFYDWFVRQVS | 29.8 | 17.5 | 16.3 | 1.1 | 0.9 |
| H2CA-4-G9-IGFR | GIISQSCPESFYDWFAGQVSDPWWCW | 8.6 | 9.5 | 0.6 | 16.0 | 0.1 |
| H2CA-4-H6-IGFR | VGRASGFPENFYDWFGRQLSLQSGEQ | 4.9 | 10.5 | 0.7 | 14.6 | 0.1 |
| H2CA-4-F-IGFR5 | VGYQGQGDENFYDWFIRQVSGRLGVQ | 5.5 | 9.7 | 0.8 | 12.3 | 0.1 |
| H2CA-4-H8-IGFR | SACQFDCHENFYDWFARQVSGGAAYG | 5.6 | 9.2 | 1.0 | 9.4 | 0.1 |
| H2CA-4-F11-IGFR | SAAQLFFQESFYDWFLRQVAESSQPN | 3.5 | 6.8 | 1.0 | 6.7 | 0.1 |
| H2CA-4-F6-IGFR | AVRATRFDEAFYDWFVRQISDGQGNK | 3.9 | 7.3 | 1.1 | 6.4 | 0.2 |
| H2CA-4-F10-IGFR | VNQSGSIHENFYDWFERQVSHQRGVR | 4.9 | 5.7 | 1.0 | 5.9 | 0.2 |
| H2CA-1-A3-IGFR | APDPSDFQEIFYDWFVRQVSRMPGGG | 7.7 | 3.8 | 0.8 | 5.1 | 0.2 |
| H2CA-3-C8-IGFR | SSCDGAGHESFYEWFVRQVSGCRSV | 15.1 | 5.6 | 1.2 | 4.8 | 0.2 |
| H2CA-2-B9-IGFR | RAGSSDFHEDFYEWFVRQVSLSLKGK | 9.3 | 7.0 | 1.7 | 4.2 | 0.2 |
| H2CA-4-H4-IGFR | QAVQPGFHEEFYDWFVRQVSTGVGGG | 3.9 | 4.1 | 1.0 | 4.2 | 0.2 |
| H2CA-4-F7-IGFR | SSIGGGFHENFYDWFSRQLSQSPPLK | 1.5 | 3.2 | 0.8 | 4.1 | 0.2 |
| H2CA-3-D6-IGFR | QSPVGSSHEDFYDWFFRQVAQSGAHQ | 8.3 | 9.0 | 2.2 | 4.0 | 0.3 |
| H2CA-3-D8-IGFR | NYRRQVFNGNFYDWFDRQVFSLVTPG | 10.9 | 7.2 | 1.8 | 4.0 | 0.3 |
| H2CA-4-G11-IGFR | TLDGGSFEEQFYDWFVRQLSYRTNPD | 10.8 | 9.5 | 2.5 | 3.9 | 0.3 |
| H2CA-4-F1-IGFR | FYVQQWGHENFYDWFDRQVSQSGGAG | 5.8 | 3.5 | 0.9 | 3.8 | 0.3 |
| H2CA-3-D7-IGFR | LRRQAPVEENFYDWFVRQVSGDRVGG | 13.3 | 3.0 | 0.8 | 3.7 | 0.3 |
| H2CA-1-A7-IGFR | RCGRELYHSTFYDWFDRQVAGRTCPS | 8.0 | 2.2 | 0.6 | 3.7 | 0.3 |
| H2CA-2-B4-IGFR | CCLLCRFQQNFYDWFVCQGISRLRPL | 3.5 | 4.1 | 1.1 | 3.6 | 0.3 |
| H2CA-2-B3-IGFR | PPLASDLDVQFYGWFVQQVSPPGRGG | 7.7 | 3.8 | 1.0 | 3.6 | 0.3 |
| H2CA-2-B2-IGFR | GAPVDQLHEDFYDWFVRQVSQAATG | 4.1 | 3.4 | 1.0 | 3.5 | 0.3 |
| H2CA-3-D4-IGFR | RSASGSLPEQFYDWFVRQVSLSGTDK | 17.6 | 13.8 | 4.1 | 3.4 | 0.3 |
| H2CA-4-F2-IGFR | SRVTTVFHENFYDWFVRQLSDSAISG | 9.3 | 12.8 | 4.2 | 3.0 | 0.3 |
| H2CA-3-D11-IGFR | DERGGKFREDFYDWFVRQVSESRFGQ | 12.2 | 6.9 | 2.3 | 3.0 | 0.3 |
| H2CA-4-H9-IGFR | RGAVAGFHDQFYDWFDRQVSRVHKFG | 8.7 | 5.6 | 1.9 | 3.0 | 0.3 |
| H2CA-2-B11-IGFR | AICDAGFHEHFYDWFALQVSDCGRQS | 11.9 | 4.6 | 1.6 | 3.0 | 0.3 |
| H2CA-3-E8-IGFR | LGYQEPFQQNFYDWFVRQVSGAENAG | 13.2 | 6.3 | 2.2 | 2.9 | 0.3 |

FIG. 1L-1

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | XXXXXXFHENFYDWFVRQVSXXXXXX | -- | -- | -- | -- | -- |
| H2CA-3-E6-IGFR | WRGHGTFHEDFYDWFVRQVSGSGSST | 15.7 | 8.7 | 3.1 | 2.8 | 0.4 |
| H2CA-4-F4-IGFR | GGRVGVLHENFYDWFDRQVSLRGADG | 11.5 | 7.4 | 3.0 | 2.5 | 0.4 |
| H2CA-3-D10-IGFR | CNLTAGFHEQFYHWFAIQVCGDAENA | 9.4 | 6.8 | 2.9 | 2.3 | 0.4 |
| H2CA-3-E1-IGFR | ERGEDMFHENFYDWFVRQISGRQGGG | 12.5 | 6.4 | 2.8 | 2.3 | 0.4 |
| H2CA-2-B6-IGFR | TNQGVGFYDSFYGWFVRQIQYGVDSG | 18.0 | 6.2 | 2.7 | 2.3 | 0.4 |
| H2CA-3-E11-IGFR | HLADGQFHEKFYDWFERQISSRCNDC | 4.7 | 2.2 | 1.0 | 2.2 | 0.5 |
| H2CA-4-H2-IGFR | QTFGKSLHENFYDWFVRQVSREEGGD | 9.8 | 9.9 | 4.8 | 2.1 | 0.5 |
| H2CA-3-C11-IGFR | FRTLAAQHDSFYDWFDRQVSGAAGER | 9.3 | 3.3 | 1.6 | 2.1 | 0.5 |
| H2CA-2-B8-IGFR | SASTHQFHENFYDWFVRQVSGAQKIL | 14.6 | 7.9 | 3.9 | 2.0 | 0.5 |

FIG. 1L-2

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | XXXXXXFHXXFYXWFXXXXXX | -- | -- | -- | -- | -- |
| Parental | VTFTSAVFHENFYDWFVRQVS | 29.8 | 17.5 | 16.3 | 1.1 | 0.9 |
| H2CBα-3-B12-IR | QSDSGTVHDRFYGWFRDT*A | 26.0 | 1.3 | 20.4 | 0.1 | 16.0 |
| H2CBα-3-D2-IR | WTDVDGFHSGFYRWFQNQWER | 20.6 | 1.7 | 12.1 | 0.1 | 7.0 |
| H2CBα-3-D12-IR | VASGHVLHGQFYRWFVDQFAL | 24.6 | 2.1 | 14.0 | 0.1 | 6.7 |
| H2CBα-3-H5-IR | QARVGNVHQQFYEWFREVMQG | 16.7 | 2.4 | 15.1 | 0.2 | 6.3 |
| H2CBα-3-B6-IR | VGDFCVSHDCFYGWFLRESMQ | 31.4 | 2.5 | 13.9 | 0.2 | 5.6 |
| H2CBα-3-G11-IR | SGSRPVFHEQFYEWFVDQLG | 22.7 | 1.4 | 6.4 | 0.2 | 4.7 |
| H2CBα-3-A6-IR | QFSAGAFHGDFYGWFRALYNG | 25.9 | 1.7 | 7.1 | 0.2 | 4.3 |
| H2CBα-3-B1-IR | SRFDERLHHQFYEWFRVLNEP | 33.4 | 6.0 | 25.5 | 0.2 | 4.3 |
| H2CBα-3-F8-IR | DSVNSDLHRAFYGWFAEQWRA | 23.0 | 4.8 | 19.8 | 0.2 | 4.1 |
| H2CBα-3-E11-IR | GSVDREIHGPFYSWFSEQLWG | 14.0 | 2.2 | 8.5 | 0.3 | 4.0 |
| H2CBα-3-G4-IR | SAKTPVLHDGFYMWFEAQSES | 24.9 | 2.2 | 6.9 | 0.3 | 3.2 |
| H2CBα-3-D3-IR | LVVGRRFHQSFYDWFVAAAGG | 23.6 | 2.6 | 8.0 | 0.3 | 3.1 |
| H2CBα-3-C1-IR | IMWPCTFQDPFYCWFQTEQGR | 27.0 | 5.6 | 16.4 | 0.3 | 2.9 |
| H2CBα-3-C3-IR | VVGPLDIHERFYGWFHQQGGA | 23.3 | 1.1 | 3.1 | 0.4 | 2.8 |
| H2CBα-3-G3-IR | VVPKAGFHEAFYEWFRRQDRD | 23.7 | 6.7 | 17.6 | 0.4 | 2.6 |
| H2CBα-3-E4-IR | QSFVTSVHTRFYAWFASALEM | 28.8 | 8.3 | 21.9 | 0.4 | 2.6 |
| H2CBα-3-G5-IR | SRGLGLYHSGFYGWFERQFNQ | 26.7 | 7.0 | 17.2 | 0.4 | 2.5 |
| H2CBα-3-B11-IR | GADTGAVHRRFYLWFEQLSGG | 28.0 | 8.6 | 19.4 | 0.4 | 2.3 |
| H2CBα-3-A1-IR | PGNRPTFHAEFYRWFREAQGS | 31.3 | 11.3 | 24.9 | 0.5 | 2.2 |
| H2CBα-3-H1-IR | VAVAWGLHESFYAWFENQFSD | 27.2 | 10.6 | 23.9 | 0.4 | 2.2 |
| H2CBα-3-F12-IR | GFNTGTFHDQFYYWFWEAAGG | 21.1 | 6.1 | 12.7 | 0.5 | 2.1 |
| H2CBα-3-H7-IR | GDGLTAFHQGFYEWFDIQMYG | 21.0 | 9.7 | 19.1 | 0.5 | 2.0 |
| H2CBα-3-C12-IR | VGVNRQFHTRFYAWFDEQLGG | 26.0 | 12.7 | 24.7 | 0.5 | 1.9 |

FIG. 1M-1

| Clone Design | Sequence XXXXXXFHXXFYXWFXXXXXX | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| H2CBα-3-D11-IR | GPRGQRLHDAFYSWFDALRVN | 27.8 | 13.0 | 24.8 | 0.5 | 1.9 |
| H2CBα-3-H12-IR | LGTLAVFHELFYGWFERQLGG | 27.4 | 7.2 | 12.4 | 0.6 | 1.7 |
| H2CBα-3-A10-IR | LGGYCGFNCQFYRWFDNLADR | 27.1 | 13.2 | 22.3 | 0.6 | 1.7 |
| H2CBα-3-A5-IR | FSGWADYQSGFYQWFAEELAN | 28.3 | 16.1 | 28.1 | 0.6 | 1.7 |
| H2CBα-3-C4-IR | WGPFSVFDESFYRWFAQASDD | 30.7 | 17.2 | 29.2 | 0.6 | 1.7 |
| H2CBα-3-B8-IR | PRNEGLVHGLFYDWFQRALSG | 25.6 | 11.3 | 18.6 | 0.6 | 1.6 |
| H2CBα-3-H11-IR | DEGGAPLDVMFYRWFEQAVRG | 28.8 | 14.0 | 22.4 | 0.6 | 1.6 |
| H2CBα-3-E10-IR | QSGNRGSHGAFYSWFRDVLAN | 27.7 | 14.3 | 23.0 | 0.6 | 1.6 |
| H2CBα-3-C2-IR | MRQRDGFNSSFYGWFAAALGE | 28.4 | 17.0 | 26.7 | 0.6 | 1.6 |
| H2CBα-3-F6-IR | SEERKKVHSQFYSWFDRQLLG | 27.3 | 14.5 | 21.8 | 0.7 | 1.5 |
| H2CBα-3-D4-IR | PSPNAPFHGGFYDWFDWVQGS | 29.0 | 18.9 | 27.1 | 0.7 | 1.4 |
| H2CBα-3-A7-IR | FHRPGSFNTNFYQWFDDQMNQ | 29.1 | 19.4 | 26.9 | 0.7 | 1.4 |
| H2CBα-3-H4-IR | SDDSSTLNGRFYTWFHMQLLD | 27.2 | 20.1 | 27.9 | 0.7 | 1.4 |
| H2CBα-3-B7-IR | QRGGGGFHEGFYSWFRSQSLL | 28.6 | 18.0 | 23.6 | 0.8 | 1.3 |
| H2CBα-3-F9-IR | SGSRPVFHEQFYEWFVDQLGL | 26.1 | 19.1 | 24.3 | 0.8 | 1.3 |
| H2CBα-3-H6-IR | GGSSQAFHGAFYEWFSAQLRG | 24.8 | 21.6 | 27.3 | 0.8 | 1.3 |
| H2CBα-3-F5-IR | AFVSERVNQRFYDWFRDQMRS | 29.4 | 22.0 | 27.8 | 0.8 | 1.3 |
| H2CBα-3-A2-IR | VRHPTRFHDEFYRWFTEQLTT | 30.7 | 22.5 | 29.1 | 0.8 | 1.3 |
| H2CBα-3-F3-IR | ARLLNIFDRGFYNWFQRQLDE | 16.3 | 6.7 | 9.0 | 0.7 | 1.3 |
| H2CBα-3-G6-IR | PSLSSNLHESFYRWFDQLVST | 24.9 | 21.0 | 24.4 | 0.9 | 1.2 |
| H2CBα-3-G7-IR | FAFGLGFHQGFYDWFAHQLEG | 24.4 | 18.7 | 23.0 | 0.8 | 1.2 |
| H2CBα-3-C5-IR | VSATVMLHREFYDWFGLQLLD | 26.4 | 21.2 | 25.4 | 0.8 | 1.2 |
| H2CBα-3-G1-IR | GGVSGVLHDRFYSWFERQLAG | 26.9 | 21.5 | 26.3 | 0.8 | 1.2 |
| H2CBα-3-E3-IR | GLGIASFHEGFYSWFTAQLGA | 24.2 | 17.2 | 19.3 | 0.9 | 1.1 |

FIG. 1M-2

| Clone Design | Sequence XXXXXXFHXXFYXWFXXXXXX | Ratios over Background E-Tag -- | IGFsR -- | IR -- | Comparisons IGFR/IR -- | IR/IGFR -- |
|---|---|---|---|---|---|---|
| H2CBα-3-A9-IR | RVDAAALNAGFYEWFRGVIQG | 30.5 | 21.7 | 24.1 | 0.9 | 1.1 |
| H2CBα-3-C11-IR | GGAGRSFHDAFYEWFERQMAG | 26.4 | 21.8 | 23.2 | 0.9 | 1.1 |
| H2CBα-3-B4-IR | EGARQGFHARFYSWFAQQLAL | 30.9 | 22.0 | 24.3 | 0.9 | 1.1 |
| H2CBα-3-F11-IR | VLLPGVVHGGFYDWFSRQLSS | 24.5 | 22.5 | 23.9 | 0.9 | 1.1 |
| H2CBα-3-G10-IR | GALSDRYNNVFYDWFREQLLG | 28.3 | 23.6 | 27.1 | 0.9 | 1.1 |
| H2CBα-3-D7-IR | PDSFMSLHQRFYSWFQAQVGT | 31.4 | 23.6 | 25.3 | 0.9 | 1.1 |
| H2CBα-3-E2-IR | RVYKANFHNEFYGWFREQLLG | 26.8 | 24.0 | 25.7 | 0.9 | 1.1 |
| H2CBα-3-B5-IR | HSGMRDVHARFYSWFSEQLSG | 28.7 | 25.0 | 26.4 | 0.9 | 1.1 |
| H2CBα-3-C7-IR | ARLLERFQDPFYEWFETLMGD | 30.0 | 25.2 | 28.7 | 0.9 | 1.1 |
| H2CBα-3-G9-IR | RNSSGNFHDKFYNWFEAQLKG | 27.8 | 25.2 | 26.7 | 0.9 | 1.1 |
| H2CBα-3-A12-IR | GSMSPVFNDQFYGWFRDLVDE | 28.0 | 26.4 | 28.7 | 0.9 | 1.1 |
| H2CBα-3-C9-IR | SCTGRQFDGCFYAWFEDQLVG | 32.1 | 28.7 | 31.9 | 0.9 | 1.1 |
| H2CBα-3-B10-IR | GIAVQSLHDSFYRWFDNALGS | 33.5 | 30.8 | 33.2 | 0.9 | 1.1 |
| H2CBα-3-E1-IR | IGPPGSLHRGFYDWFAEQVEA | 31.7 | 30.5 | 29.0 | 1.1 | 1.0 |
| H2CBα-3-G12-IR | GAAGISFHRGFYDWFAAQVRD | 29.1 | 31.4 | 29.8 | 1.1 | 1.0 |
| H2CBα-3-F7-IR | GVDVTDFHKDFYSWFQRQLNG | 23.2 | 20.7 | 20.3 | 1.0 | 1.0 |
| H2CBα-3-G8-IR | WAGRAGIHGGFYEWFNRQLRG | 22.8 | 20.9 | 20.4 | 1.0 | 1.0 |
| H2CBα-3-C6-IR | LGQLAAFHLGFYEWFSEAVAA | 26.7 | 21.2 | 22.0 | 1.0 | 1.0 |
| H2CBα-3-H9-IR | VHSVSRLNVGFYQWFQDQLSG | 23.4 | 22.5 | 22.0 | 1.0 | 1.0 |
| H2CBα-3-H8-IR | LGLMAIFDRGFYGWFEQQLSG | 23.5 | 23.4 | 23.2 | 1.0 | 1.0 |
| H2CBα-3-F2-IR | VARGSSLHDDFYEWFASQLRT | 25.5 | 24.3 | 25.2 | 1.0 | 1.0 |
| H2CBα-3-D5-IR | LGYIGALNTQFYSWFADLVGS | 26.7 | 24.5 | 25.6 | 1.0 | 1.0 |
| H2CBα-3-D10-IR | EDSRLRLHEGFYGWFRKQLGD | 26.8 | 24.9 | 24.9 | 1.0 | 1.0 |
| H2CBα-3-F10-IR | GRDNMKFHSGFYDWFTQQLAG | 25.7 | 25.6 | 26.1 | 1.0 | 1.0 |

FIG. 1M-3

| Clone Design | Sequence XXXXXXFHXXFYXWFXXXXXX | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| H2CBα-3-D6-IR | AGVMGGFHQEFYLWFERALSN | 27.9 | 26.0 | 25.8 | 1.0 | 1.0 |
| H2CBα-3-H3-IR | AGHVGQVYDGFYGWFREQLGA | 27.0 | 26.9 | 26.2 | 1.0 | 1.0 |
| H2CBα-3-F4-IR | FVQNIGFDYDFYGWFVREVEK | 31.2 | 27.2 | 27.7 | 1.0 | 1.0 |
| H2CBα-3-E9-IR | PVGIGGLHRAFYQWFQSQVDA | 31.6 | 27.7 | 28.2 | 1.0 | 1.0 |
| H2CBα-3-H10-IR | GSRQEADHQAFYDWFNLVLGV | 26.9 | 27.9 | 28.8 | 1.0 | 1.0 |
| H2CBα-3-G2-IR | AGGRKPFHDDFYGWFRDQLAE | 29.1 | 28.1 | 28.8 | 1.0 | 1.0 |
| H2CBα-3-B2-IR | DLASHGFHDAFYNWFSVQLNS | 29.4 | 28.1 | 28.2 | 1.0 | 1.0 |
| H2CBα-3-E8-IR | GSNGGGVHGQFYAWFVEALSG | 31.5 | 28.4 | 29.1 | 1.0 | 1.0 |
| H2CBα-3-E5-IR | RGRASTFHDGFYGWFSQQLRF | 33.0 | 28.7 | 28.9 | 1.0 | 1.0 |
| H2CBα-3-E6-IR | SPARRVSHHDFYGWFAKQLES | 29.6 | 29.0 | 28.1 | 1.0 | 1.0 |
| H2CBα-3-E7-IR | SSDVGAFHSAFYDWFKAQLSG | 30.4 | 30.2 | 30.2 | 1.0 | 1.0 |
| H2CBα-3-C8-IR | PTVHRAFDDLFYGWFAKQVED | 31.9 | 31.2 | 31.5 | 1.0 | 1.0 |
| H2CBα-3-A4-IR | SSNTVGLDERFYAWFVDQLGA | 32.2 | 31.9 | 32.6 | 1.0 | 1.0 |
| H2CBα-3-D1-IR | PGAAEGFHSAFYDWFAQAVSG | 32.9 | 32.5 | 31.5 | 1.0 | 1.0 |
| H2CBα-3-B9-IR | MRSEASFHVEFYSWFEEQLRS | 33.2 | 33.8 | 33.3 | 1.0 | 1.0 |
| H2CBα-3-D8-IR | VSRYGGQQDGFYHWFSDLLKG | 26.3 | 20.2 | 19.1 | 1.1 | 0.9 |
| H2CBα-3-F1-IR | RPSSGGLHYGFYHWFRVQEEM | 28.8 | 28.0 | 26.4 | 1.1 | 0.9 |
| H2CBα-3-A11-IR | SNIEEHFHMQFYRWFSDALGN | 20.5 | 21.5 | 17.7 | 1.2 | 0.8 |
| H2CBα-3-A3-IR | ANDCLGLHAGFYGWFACQLGG | 30.4 | 29.6 | 21.8 | 1.4 | 0.7 |

FIG. 1M-4

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | XXXXXXFHXXFYXWFXXXXXX | -- | -- | -- | -- | -- |
| H2CBβ-3-E8-IR | TGHRLGLDEQFYWWFRDALSG | 15.9 | 1.9 | 11.8 | 0.2 | 6.1 |
| H2CBβ-4-F8-IR | VLTSNTLHQRFYSWFAAARRE | 13.4 | 0.8 | 2.6 | 0.3 | 3.4 |
| H2CBβ-3-C4-IR | CVAQGGFQSSFYCWFAGLDID | 21.1 | 1.3 | 4.0 | 0.3 | 3.1 |
| H2CBβ-3-D5-IR | NGQSSRFHTAFYDWFAAQLSG | 14.0 | 3.3 | 10.2 | 0.3 | 3.1 |
| H2CBβ-3-E6-IR | SVPRGTVHDAFYQWFREVALG | 5.7 | 0.7 | 2.1 | 0.3 | 3.1 |
| H2CBβ-4-G12-IR | GARGSTFHDQFYEWFWVQLGD | 6.8 | 1.8 | 5.4 | 0.3 | 3.1 |
| H2CBβ-4-F4-IR | PPGMNGFHTSFYSWFVDQLGD | 17.9 | 1.9 | 5.6 | 0.3 | 3.0 |
| H2CBβ-4-F11-IR | AVGTLGYHSGFYRWFERQLGG | 15.0 | 1.7 | 4.8 | 0.3 | 2.9 |
| H2CBβ-3-E5-IR | ELQARGVHRNFYRWFEAQVSG | 17.0 | 1.8 | 5.0 | 0.4 | 2.8 |
| H2CBβ-4-F2-IR | HRVARAFHEQFYDWFEKAVSG | 15.9 | 1.3 | 3.4 | 0.4 | 2.6 |
| H2CBβ-4-G4-IR | GAMEPDYHRSFYQWFAAALGE | 8.7 | 1.4 | 3.5 | 0.4 | 2.6 |
| H2CBβ-3-C8-IR | CPDRQSVDDRFYNWFADALAS | 4.9 | 1.4 | 3.2 | 0.4 | 2.3 |
| H2CBβ-4-F10-IR | GGAQISFHERFYQWFLQEAAG | 10.2 | 1.0 | 2.4 | 0.4 | 2.3 |
| H2CBβ-4-H4-IR | HKRGIVQHGAFYAWFDSLLSG | 20.8 | 4.2 | 9.5 | 0.4 | 2.3 |
| H2CBβ-4-G6-IR | QASDNRSDGQFYLWFEKLLSS | 14.5 | 5.6 | 8.5 | 0.7 | 1.5 |
| H2CBβ-4-H1-IR | DRGRMGVDEGFYNWFARQMQE | 17.0 | 10.1 | 13.2 | 0.8 | 1.3 |

FIG. 1M-5

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| **Design** | **XXXXXXFHXXFYXWFXXXXXX** | -- | -- | -- | -- | -- |
| **Parental** | **VTFTSAVFHENFYDWFVRQVS** | **29.8** | **17.5** | **16.3** | **1.1** | **0.9** |
| H2CB-3-D2-IGFR | TASQECFDDGFYGWFRAWRCT | 22.9 | 18.6 | 11.8 | 1.6 | 0.6 |
| H2CB-3-C12-IGFR | SLDWRWSEEPFYRWFQRALAG | 17.3 | 19.6 | 13.0 | 1.5 | 0.7 |
| H2CB-3-B11-IGFR | CMSLSDCHRKFYGWFKSQGGE | 24.6 | 17.1 | 11.9 | 1.4 | 0.7 |
| H2CB-4-E2-IGFR | LALCRRSPGSFYGWFQAAVGC | 22.4 | 21.0 | 16.5 | 1.3 | 0.8 |
| H2CB-3-A5-IGFR | PRSATMSDGGFYWWFASQLGL | 28.8 | 26.1 | 22.6 | 1.2 | 0.9 |
| H2CB-4-G12-IGFR | LRRSSVFHDPFYE*ISRLVGG | 23.7 | 23.8 | 19.4 | 1.2 | 0.8 |
| H2CB-3-B2-IGFR | ARLQQQFHGGFYEWFRAQVSP | 23.0 | 19.9 | 16.4 | 1.2 | 0.8 |
| H2CB-3-D1-IGFR | AQLDNLCHEPFYSWFCAVTRE | 21.5 | 19.5 | 15.7 | 1.2 | 0.8 |
| H2CB-3-B6-IGFR | WTCDTAFHQDFYQWFCDKLGV | 16.3 | 4.5 | 3.7 | 1.2 | 0.8 |
| H2CB-4-F7-IGFR | GKEGFGLDRDFYWWFREQLGP | 22.0 | 19.0 | 18.0 | 1.1 | 0.9 |
| H2CB-4-G8-IGFR | GRAPSSFDCDFYCWFRNQVQS | 20.2 | 18.6 | 16.5 | 1.1 | 0.9 |
| H2CB-3-D4-IGFR | DVEAETQHRLFYAWFLSQLGS | 21.9 | 18.3 | 16.9 | 1.1 | 0.9 |
| H2CB-3-D5-IGFR | ISVTAVFHDGFYGWFNEQVSK | 21.4 | 17.9 | 16.4 | 1.1 | 0.9 |
| H2CB-4-E6-IGFR | NSEHGRLDVDFYGWFARVIQQ | 19.6 | 15.8 | 14.8 | 1.1 | 0.9 |
| H2CB-3-C2-IGFR | GPLGDGCQDGFYGWFMCQVST | 18.8 | 12.2 | 10.8 | 1.1 | 0.9 |
| H2CB-3-A6-IGFR | KRSAYNFHDPFYDWFRMQLSG | 26.8 | 29.0 | 28.1 | 1.0 | 1.0 |
| H2CB-4-H12-IGFR | ASEPGGYLDPFYGWFREQLRA | 23.9 | 28.3 | 28.1 | 1.0 | 1.0 |
| H2CB-3-B10-IGFR | NRGDGGVHSGFYNWFRLQLSG | 27.1 | 27.5 | 27.3 | 1.0 | 1.0 |
| H2CB-4-F11-IGFR | ASKGSSLHNDFYGWFAQQLAR | 25.5 | 25.5 | 24.6 | 1.0 | 1.0 |
| H2CB-4-G11-IGFR | ANVSMWIQVGFYDWFDAQLRQ | 25.3 | 25.4 | 25.3 | 1.0 | 1.0 |
| H2CB-4-E12-IGFR | RTSPGSLHDPFYDWFQQQLGG | 27.8 | 24.9 | 24.7 | 1.0 | 1.0 |
| H2CB-4-G10-IGFR | PGVMSSFHGGFYSWFREQLNG | 25.1 | 24.6 | 24.2 | 1.0 | 1.0 |
| H2CB-3-B9-IGFR | CLANSEDHDSFYGWFCQALGG | 25.6 | 23.3 | 23.7 | 1.0 | 1.0 |
| H2CB-3-B7-IGFR | GGSMGGMHGSFYEWFALQLRS | 24.0 | 23.2 | 23.5 | 1.0 | 1.0 |
| H2CB-4-H4-IGFR | RPQGGSIHAGFYQWFRDAVAG | 23.5 | 23.1 | 23.8 | 1.0 | 1.0 |

FIG. 1N-1

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | XXXXXXFHXXFYXWFXXXXXX | -- | -- | -- | -- | -- |
| H2CB-4-H10-IGFR | GALSSLFDAAFYDWFNRQLEG | 21.9 | 22.4 | 23.3 | 1.0 | 1.0 |
| H2CB-4-H5-IGFR | KVDLRGFHDGFYGWFARQLAG | 22.3 | 22.3 | 21.6 | 1.0 | 1.0 |
| H2CB-4-G7-IGFR | CSGLQRCHDSFYSWFESVVRE | 23.1 | 21.6 | 20.6 | 1.0 | 1.0 |
| H2CB-4-F4-IGFR | DSLGISFHEGFYDWFRRQLDM | 21.3 | 20.9 | 21.3 | 1.0 | 1.0 |
| H2CB-3-D8-IGFR | SGVFNGTFYDWFRIQLGE | 20.0 | 20.5 | 21.6 | 1.0 | 1.0 |
| H2CB-4-E4-IGFR | GYREMRSDLGFYQWFRDQLGL | 21.6 | 20.5 | 21.2 | 1.0 | 1.0 |
| H2CB-4-E5-IGFR | SVFMQHDHVGFYAWFRSLMEE | 22.0 | 19.9 | 20.9 | 1.0 | 1.0 |
| H2CB-4-E8-IGFR | FRHITEVDRSFYGWFVEQLRG | 21.1 | 19.7 | 20.7 | 1.0 | 1.1 |
| H2CB-3-D12-IGFR | WAGGSDVDGSFYDWFQRLLAS | 26.6 | 17.3 | 16.8 | 1.0 | 1.0 |
| H2CB-4-G9-IGFR | GLQNVSFHSGFYEWFARQVSQ | 21.6 | 14.5 | 15.2 | 1.0 | 1.1 |
| H2CB-3-C8-IGFR | SRVSDPYHVGFYQWFEEVVRG | 20.8 | 13.4 | 13.9 | 1.0 | 1.0 |
| H2CB-3-A12-IGFR | MGGATFFHTGFYDWFAAQLQH | 28.6 | 27.5 | 29.2 | 0.9 | 1.1 |
| H2CB-3-B12-IGFR | RPASRPFHSGFYQWFADQLSH | 27.8 | 25.2 | 27.1 | 0.9 | 1.1 |
| H2CB-3-A9-IGFR | GLAPGNFHEDFYRWFQEQTLG | 27.7 | 24.3 | 25.7 | 0.9 | 1.1 |
| H2CB-3-A3-IGFR | TAAISDFNSLFYGWFEQLLSS | 26.9 | 24.1 | 26.5 | 0.9 | 1.1 |
| H2CB-3-B4-IGFR | LDEDLPQHAGFYGWFAEALGV | 25.8 | 23.8 | 25.3 | 0.9 | 1.1 |
| H2CB-4-E7-IGFR | ASHKSAFDDNFYRWFSMQLRD | 24.6 | 21.6 | 24.0 | 0.9 | 1.1 |
| H2CB-4-G6-IGFR | HTGAGDLHGAFYNWFLEQLGG | 22.4 | 21.1 | 23.0 | 0.9 | 1.1 |
| H2CB-4-E9-IGFR | RRGRDGFHGGFYDWFAAQLSD | 24.3 | 20.7 | 22.0 | 0.9 | 1.1 |
| H2CB-4-H2-IGFR | GNFREAFHADFYSWFERQLQS | 21.6 | 20.2 | 21.9 | 0.9 | 1.1 |
| H2CB-3-A10-IGFR | RDTLPAFHQHFYQWFEKQVSA | 24.3 | 19.9 | 21.5 | 0.9 | 1.1 |
| H2CB-3-C4-IGFR | ERETAAFGQAFYQWFRDQIAG | 23.1 | 19.2 | 22.0 | 0.9 | 1.1 |
| H2CB-3-B5-IGFR | WGEGGGFYDWFYDQLGWEPSH | 24.2 | 18.8 | 20.7 | 0.9 | 1.1 |
| H2CB-4-G4-IGFR | SLVAADLHEGFYGWFRSQLGG | 21.7 | 18.7 | 21.2 | 0.9 | 1.1 |
| H2CB-3-D9-IGFR | TSEVGDFHAEFYSWFEIQLGR | 24.4 | 18.6 | 20.0 | 0.9 | 1.1 |
| H2CB-3-C3-IGFR | TGADGLLHARFYAWFEEQLRE | 20.3 | 18.4 | 21.1 | 0.9 | 1.1 |
| H2CB-3-D3-IGFR | RRSDSSLHRSFYDWFSVQLLN | 22.5 | 18.3 | 21.3 | 0.9 | 1.2 |
| H2CB-4-F2-IGFR | SESKYLLHSGFYGWFEAQLRG | 18.0 | 16.8 | 18.3 | 0.9 | 1.1 |

FIG. 1N-2

| Clone Design | Sequence XXXXXXFHXXFYXWFXXXXXX | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| H2CB-4-H1-IGFR | HGVIRADHTGFYGWFSKQLSD | 18.3 | 15.3 | 16.5 | 0.9 | 1.1 |
| H2CB-4-F9-IGFR | LINA.VFRRGFYAWFEEQVSK | 22.9 | 14.4 | 15.3 | 0.9 | 1.1 |
| H2CB-4-E10-IGFR | LQRYIGFHDPFYDWFSRALSG | 26.1 | 20.1 | 24.5 | 0.8 | 1.2 |
| H2CB-4-F8-IGFR | MRTAELFHVGFYDWFDAQLMD | 21.5 | 14.8 | 19.0 | 0.8 | 1.3 |
| H2CB-3-A8-IGFR | WAPPDALHGQFYRWFQRQLDQ | 20.7 | 14.7 | 18.2 | 0.8 | 1.2 |
| H2CB-4-F1-IGFR | AVHAATFHDDFYRWFEQVVGS | 22.2 | 14.6 | 18.8 | 0.8 | 1.3 |
| H2CB-3-C6-IGFR | FDAVHGFDGGFYGWFKRELQR | 15.7 | 7.8 | 10.2 | 0.8 | 1.3 |
| H2CB-4-E11-IGFR | QAGGMEFHGAFYNWFLQQLSG | 26.1 | 17.6 | 24.1 | 0.7 | 1.4 |
| H2CB-3-D6-IGFR | GRSVSRMNAEFYQWFGHQLAA | 21.6 | 13.0 | 18.8 | 0.7 | 1.5 |
| H2CB-4-F3-IGFR | AAVNSLFHDEFYLWFQDQLDG | 17.3 | 11.1 | 16.4 | 0.7 | 1.5 |
| **H2CB-3-A4-IGFR** | QLGMDWFHADFYEWFLAQLPS | 27.4 | 11.0 | 14.8 | 0.7 | 1.3 |
| H2CB-3-B1-IGFR | RLAGSGIHEGFYGWFVDQLLA | 20.0 | 11.0 | 15.2 | 0.7 | 1.4 |
| H2CB-3-C5-IGFR | GREIGGVHDGFYDWFRQQSEQ | 19.9 | 10.5 | 15.6 | 0.7 | 1.5 |
| H2CB-4-F6-IGFR | VRSEQRFDSSFYQWFNDLLMS | 18.6 | 10.1 | 14.6 | 0.7 | 1.4 |
| H2CB-3-B8-IGFR | QSPYGFFHDGFYRWFLQQTGM | 20.7 | 6.9 | 9.5 | 0.7 | 1.4 |
| H2CB-3-C7-IGFR | FQCGAAFHVDFYRWFTCQEQF | 16.2 | 1.8 | 2.5 | 0.7 | 1.4 |
| H2CB-4-H7-IGFR | GAFGSEFHEQFYRWFEDALSF | 21.8 | 14.1 | 22.7 | 0.6 | 1.6 |
| H2CB-4-F5-IGFR | EHTSYQIHRQFYEWFDRALGR | 12.9 | 4.0 | 7.2 | 0.6 | 1.8 |
| H2CB-4-G1-IGFR | SGTAADLHSRFYGWFALQARE | 20.4 | 10.3 | 19.7 | 0.5 | 1.9 |
| H2CB-3-D11-IGFR | EGFGVLFHGQFYRWFQLQLDG | 24.1 | 8.8 | 18.6 | 0.5 | 2.1 |
| H2CB-3-D7-IGFR | QQSAGHPHSSFYLWFSELLGA | 22.1 | 6.5 | 13.6 | 0.5 | 2.1 |
| H2CB-3-C10-IGFR | YLQRAGFHRSFYGWFDQALRD | 21.7 | 5.1 | 10.4 | 0.5 | 2.0 |
| H2CB-4-E3-IGFR | MWLWATLHSDFYSWFEQVVSG | 20.3 | 4.6 | 8.9 | 0.5 | 1.9 |
| H2CB-3-C1-IGFR | GANALGFKDRFYEWFAAQLWD | 22.3 | 6.7 | 15.7 | 0.4 | 2.3 |
| H2CB-4-G2-IGFR | GSGLYVFHWGFYDWFEQQMGG | 19.9 | 3.3 | 10.7 | 0.3 | 3.3 |
| H2CB-3-A11-IGFR | LDKGWGFDLQFYRWFEAATRA | 23.9 | 2.5 | 7.7 | 0.3 | 3.1 |
| H2CB-4-G5-IGFR | QRSAVEFHADFYDWFLRLLTP | 19.3 | 2.5 | 7.9 | 0.3 | 3.1 |
| H2CB-4-F12-IGFR | DQRMGSFHGEFYRWFEETLLS | 16.7 | 1.7 | 5.4 | 0.3 | 3.1 |

FIG. 1N-3

| Clone Design | Sequence $X_n$-FyxWF-$X_n$ | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| 20E2A-3-B11-IR | GRFYGWFQDAIDQLMPWGFDP | 24.6 | 1.4 | 23.6 | 0.1 | 16.8 |
| 20E2Bβ-3-E3-IR | IQGWEPFYGWFDDVVAQMFEE | 23.0 | 0.9 | 15.3 | 0.1 | 16.3 |
| rB6-3-F6-IR | RYGRWGLAQQFYDWFDR | 40.9 | 1.0 | 13.3 | 0.1 | 13.3 |
| rB6-4-F9-IR | RGRLGSLSTQFYNWFAE | 34.1 | 1.0 | 12.6 | 0.1 | 12.6 |
| 20E2Bα-3-A8-IR | ASAYTPFYQWFADVVSEYMQQ | 35.4 | 7.4 | 34.4 | 0.2 | 4.6 |
| A6L-4-F6-IR | PYRMEGTEKWNFYDWFVAQLQ | 28.9 | 4.1 | 18.1 | 0.2 | 4.4 |
| 20E2Bα-4-H9-IR | SAVHFQFYKWFDNLLPVPLSA | 37.8 | 9.4 | 26.7 | 0.4 | 2.9 |
| 20E2Bα-3-B1-IR | VPVNKSFYRWFQLVLGGSDDW | 41.8 | 12.9 | 36.8 | 0.4 | 2.9 |
| 20E2Bβ-4-F9-IR | QSPRASFYGWFDDVLRAAGVV | 25.9 | 4.2 | 10.1 | 0.4 | 2.4 |
| 20E2Bβ-3-E9-IR | TGFYEWFYEQLHSRMLPNPLD | 27.0 | 7.7 | 17.2 | 0.5 | 2.2 |
| 20E2Bβ-3-E10-IR | RRGVGGFYGWFSQQLQGMGVA | 22.2 | 2.6 | 5.5 | 0.5 | 2.1 |
| 20E2Bα-3-C12-IR | SSQDRRFYRWFEQAIVGGRDG | 39.0 | 6.7 | 12.0 | 0.6 | 1.8 |
| 20E2Bβ-3-C12-IR | TRGQLGFYNWFQQALSTSGMG | 20.2 | 2.2 | 3.8 | 0.6 | 1.8 |
| 20E2Bβ-3-E7-IR | CADLNAFYQWFCGVLDRGSDH | 9.2 | 1.2 | 1.9 | 0.6 | 1.6 |
| 20E2Bβ-3-E11-IR | TLIQDQFYWWFSDLLSAEPGD | 20.7 | 1.3 | 2.1 | 0.6 | 1.6 |
| 20E2Bα-3-B11-IR | IDQLDAFYRWFDGVMLGMGDP | 36.0 | 20.7 | 32.8 | 0.6 | 1.6 |
| NNKH-4-G2-IR | RGGGTFYEWFESALRKHGAG | 10.8 | 6.3 | 8.9 | 0.7 | 1.4 |
| 20E2Bα-3-A7-IR | RGLDQDFYRWFQNLVGVEYDR | 19.0 | 4.2 | 5.5 | 0.8 | 1.3 |
| 20E2Bα-4-G12-IR | MQGHRGFYGWFARVLEQDRGW | 37.0 | 22.3 | 29.5 | 0.8 | 1.3 |
| 20E2Bα-3-C11-IR | ERLHLRFYEWFDTVIGQDGSD | 37.3 | 26.8 | 34.8 | 0.8 | 1.3 |
| 20E2Bα-3-C10-IR | MHVQSDFYHWFQSLLGQGGPD | 37.7 | 24.8 | 30.5 | 0.8 | 1.2 |

FIG. 10-1

| Clone Design | Sequence $X_n$-FyxWF-$X_m$ | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| 20E2Bα-3-D7-IR | TMGTQGFYRWFQNVVKEHLSG | 35.4 | 26.9 | 31.3 | 0.9 | 1.2 |
| 20E2Bα-3-A12-IR | ITHNRGFYSWFLDVVQGGAGA | 31.7 | 22.0 | 23.3 | 0.9 | 1.1 |
| 20E2Bα-3-D10-IR | VRRDAGFYQWFADILTQLDFE | 32.7 | 27.3 | 29.1 | 0.9 | 1.1 |
| 20E2Bα-4-G7-IR | MQLQDEFYNWFRGIMLNDGQD | 34.2 | 29.0 | 30.7 | 0.9 | 1.1 |
| 20E2Bα-4-F5-IR | GIRSSGFYQWFDRVLAGVGDG | 33.8 | 32.1 | 34.0 | 0.9 | 1.1 |
| 20E2Bα-3-C9-IR | ANLNSQFYSWFASVTGEASPS | 39.4 | 33.2 | 35.5 | 0.9 | 1.1 |
| 20E2Bα-3-A4-IR | QSPRASFYGWFDDVLRAAGVV | 38.2 | 31.6 | 35.9 | 0.9 | 1.1 |
| 20E2Bα-4-E12-IR | MQRNQGFYSWFDDLVSSTVGV | 36.0 | 30.8 | 29.7 | 1.0 | 1.0 |
| 20E2Bα-4-E11-IR | ASGFDPFYAWFLEQLRVANGS | 35.1 | 31.2 | 30.7 | 1.0 | 1.0 |
| 20E2Bα-4-E8-IR | SGTPYGFYRWFQSALASATSG | 36.1 | 30.5 | 30.7 | 1.0 | 1.0 |
| 20E2Bα-4-H10-IR | QGVEGGFYEWFDRAMGDVRPW | 38.9 | 30.6 | 30.7 | 1.0 | 1.0 |
| 20E2Bα-4-F6-IR | DNMSGGFYRWFAQVVADSGGD | 34.9 | 33.2 | 32.0 | 1.0 | 1.0 |
| 20E2Bα-4-G4-IR | RGTDDTFYGWFDQLLQGWCDD | 34.1 | 33.7 | 32.2 | 1.0 | 1.0 |
| 20E2Bα-4-F8-IR | TVDHTQFYDWFSRVLGESGSA | 37.7 | 32.0 | 32.7 | 1.0 | 1.0 |
| 20E2Bα-4-G5-IR | GRQDREFYYWFELQAGGMDGD | 34.9 | 33.9 | 33.4 | 1.0 | 1.0 |
| 20E2Bα-3-B10-IR | RLLLGGFYEWFDQVLKETKEV | 38.2 | 34.9 | 33.6 | 1.0 | 1.0 |
| 20E2Bα-3-C7-IR | GVLSTGFYEWFALQLHGLAAG | 37.6 | 34.2 | 34.8 | 1.0 | 1.0 |
| 20E2Bα-3-C5-IR | PAVGQSFYGWFEAVLRGSKAG | 40.4 | 36.0 | 35.6 | 1.0 | 1.0 |
| 20E2Bα-3-B9-IR | SNGISGFYEWFAAQVQTSDFQ | 39.6 | 35.8 | 37.1 | 1.0 | 1.0 |
| A6L-4-F11-IR | LLGLSQAAYANFYDWFVSQLA | 33.1 | 4.6 | 4.6 | 1.0 | 1.0 |
| 20E2Bα-3-C2-IR | VPNSWMFYNWFAEQIEGSEGE | 44.1 | 40.0 | 38.1 | 1.0 | 1.0 |
| 20E2Bα-3-B2-IR | ARRADGFYDWFREQVSGSAVQ | 43.1 | 40.1 | 39.0 | 1.0 | 1.0 |
| 20E2Bα-4-G2-IR | GVVEGTFYEWFDRLLGGVQGD | 34.1 | 33.6 | 29.8 | 1.1 | 0.9 |
| 20E2Bα-4-H6-IR | SHLTDPFYQWFVDQLRAGVRG | 39.4 | 36.0 | 31.9 | 1.1 | 0.9 |

FIG. 10-2

| Clone Design | Sequence $X_n$-FyxWF-$X_m$ | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| 20E2Bα-4-H5-IR | RSNDDAFYRWFSNILQVDGGG | 38.7 | 35.1 | 32.3 | 1.1 | 0.9 |
| 20E2Bα-4-G3-IR | DSDGAQFYIWFEDQLRSAGWD | 35.5 | 36.1 | 32.7 | 1.1 | 0.9 |
| 20E2Bα-4-H4-IR | PGLHRAFYQWFAEAVRSANKE | 38.8 | 37.9 | 35.0 | 1.1 | 0.9 |
| 20E2Bα-3-C1-IR | SLGQGGFYDWFASQVGGADI | 43.7 | 42.1 | 39.0 | 1.1 | 0.9 |
| 20E2Bα-4-E6-IR | CGQTQSFYQWFCEVMRVESGD | 38.0 | 34.3 | 29.7 | 1.2 | 0.9 |
| H5-3-D5-IR | IVVPGDTQGVNFYDWFVKQLQ | 43.8 | 21.8 | 18.2 | 1.2 | 0.8 |
| JBA5-3-D9-IR | RDVSMGSASTNFYDWFVQQLG | 38.3 | 29.8 | 25.3 | 1.2 | 0.8 |
| 20E2Bβ-4-G6-IR | SQAGSAFYAWFDQVLRTVHSA | 22.4 | 6.2 | 1.9 | 3.3 | 0.3 |
| 20E2Bβ-4-H10-IR | SNGISGFYEWFAAQVQTSDFQ | 23.5 | 32.2 | 9.7 | 3.3 | 0.3 |
| rB6-4-G8-IR | RRDRGGLDVFFYQWFMD | -- | -- | -- | -- | -- |

FIG. 10-3

| Clone Design | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| | XXXXXXXXXXXXXXXXXXXX | -- | -- | -- | -- | -- |
| R20β-4-F8-IR | HLCVLEELFWGASLFGYCSG | 39.1 | 1.8 | 27.7 | 0.1 | 15.4 |

FIG. 2A

| Clone Design | Sequence HLCVLEELFWGASLFGYCSG | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| F815-4-H9-IR | PLCVLEELFWSTPLFGQCSY | 34.9 | 0.9 | 37.6 | <0.1 | 40.8 |
| F815-3-B1-IR | HLCVLEELFWGASLFAQCVG | 31.7 | 0.9 | 35.8 | <0.1 | 39.3 |
| F815-3-D1-IR | DLCVLEELFWGASRFGQCSG | 30.4 | 0.9 | 33.5 | <0.1 | 38.9 |
| F815-3-D4-IR | HLCVLEELFWGASLFGQCAG | 31.5 | 0.9 | 33.6 | <0.1 | 38.8 |
| F815-3-C5-IR | HLCVVEELFWGASLFGQCSG | 31.1 | 0.8 | 31.2 | <0.1 | 38.5 |
| F815-4-H3-IR | NLCDLEVLFWGASLFRQCSG | 33.7 | 1.0 | 37.2 | <0.1 | 38.4 |
| F815-3-A5-IR | PLCVLEEQFWGASLFGQCSG | 37.4 | 1.1 | 40.9 | <0.1 | 38.3 |
| F815-3-D7-IR | QLCVLEELFWGASEFGQCSG | 33.6 | 0.9 | 34.3 | <0.1 | 38.3 |
| F815-3-A1-IR | HLCELEELFWGASLFGQCSG | 29.8 | 0.9 | 34.8 | <0.1 | 38.0 |
| F815-4-H4-IR | PLCVLEELFWGESLFGQCSG | 31.1 | 0.9 | 32.7 | <0.1 | 38.0 |
| F815-3-A3-IR | HLCVLEELFWGASRFGQCSG | 32.8 | 1.0 | 39.1 | <0.1 | 37.9 |
| F815-3-B3-IR | KLCVLEELFWGASLFGQCSG | 33.7 | 1.0 | 37.5 | <0.1 | 37.5 |
| F815-3-A4-IR | YLCVLEELSWGASLFGQCSG | 32.5 | 1.0 | 36.9 | <0.1 | 37.5 |
| F815-3-D2-IR | HLCVLEELLWGASLFAQCSG | 31.9 | 0.9 | 34.1 | <0.1 | 37.4 |
| F815-3-C4-IR | QLCVLEQLFWGESLFGQCSG | 31.6 | 0.8 | 31.8 | <0.1 | 37.4 |
| F815-3-B4-IR | HLCVLEELFWGGNLFSQCSG | 33.8 | 1.0 | 36.7 | <0.1 | 37.3 |
| F815-3-C1-IR | HLCVLEELFWGASLYGQCSG | 29.0 | 0.9 | 35.0 | <0.1 | 37.3 |
| F815-4-G9-IR | SLCALEEQFWGAALFGYCSG | 36.5 | 1.0 | 38.9 | <0.1 | 37.1 |
| F815-4-G6-IR | HLCVLEEQFWGASLFDGCAG | 34.9 | 1.0 | 36.4 | <0.1 | 37.0 |
| F815-3-A8-IR | QLCVLEELFWGASLFGQCSG | 34.7 | 1.1 | 39.3 | <0.1 | 36.9 |
| F815-4-G5-IR | PLCVLEELFWGAALFGQCSG | 26.5 | 1.0 | 35.1 | <0.1 | 36.8 |
| F815-3-B5-IR | HLCVLEELFWGASLFGQCTG | 33.2 | 0.9 | 34.1 | <0.1 | 36.8 |
| F815-4-F4-IR | PLCVLEELFWGGSLFGQCSG | 28.6 | 0.8 | 30.0 | <0.1 | 36.7 |
| F815-3-A2-IR | QLCVLEELVWGASLFGQCSG | 32.5 | 1.0 | 36.6 | <0.1 | 36.6 |
| F815-3-B6-IR | HLCVVEELIWGASLFGQCSR | 31.6 | 0.9 | 32.9 | <0.1 | 36.5 |
| F815-4-H7-IR | DLCVLEELFWGASLFGQCAG | 33.7 | 1.0 | 37.6 | <0.1 | 36.4 |
| F815-4-H8-IR | QLCVLEERFWGASLFGQCSG | 35.8 | 1.0 | 37.0 | <0.1 | 36.4 |
| F815-4-G7-IR | NLCVLEELFWGAALFGQCSG | 33.7 | 1.0 | 35.8 | <0.1 | 36.3 |

FIG. 2B-1

| Clone Design | Sequence HLCVLEELFWGASLFGYCSG | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| F815-3-A6-IR | QLCVLEELFWGSSLFGQCSG | 34.6 | 1.1 | 39.0 | <0.1 | 36.2 |
| F815-3-D3-IR | DLCVVEELFWGKSLFGQCSG | 33.8 | 1.0 | 36.2 | <0.1 | 36.2 |
| F815-3-B12-IR | DLCVLEELFWGSSLFGQCSG | 33.2 | 1.0 | 35.7 | <0.1 | 36.2 |
| F815-4-G10-IR | YLCVLEEQFWGASLFRQCFG | 35.4 | 1.0 | 37.2 | <0.1 | 36.1 |
| F815-4-E3-IR | HLCVLEELLWGSSLFGQCSG | 32.4 | 1.0 | 35.0 | <0.1 | 36.1 |
| F815-4-E6-IR | PLCGLEELFWGASLFGQCSD | 33.2 | 1.0 | 34.5 | <0.1 | 36.1 |
| F815-4-F1-IR | HLCVLEELFWGSSLFAQCSG | 29.4 | 0.9 | 32.5 | <0.1 | 36.0 |
| F815-4-G8-IR | PLCAIEELFWGAALFGQCSG | 36.8 | 1.1 | 38.2 | <0.1 | 35.9 |
| F815-4-H12-IR | HLCVLEEQFWGASLFGDCSG | 30.5 | 0.9 | 31.9 | <0.1 | 35.9 |
| F815-4-G3-IR | PLCVLEELFWGAPLFGQCSD | 31.4 | 1.0 | 35.7 | <0.1 | 35.7 |
| F815-3-C2-IR | DLCGLEELFWGAALFGQCTS | 32.3 | 1.0 | 36.1 | <0.1 | 35.6 |
| F815-4-E10-IR | QLCVLEKQLWGASLFWQCSG | 35.4 | 1.0 | 36.5 | <0.1 | 35.4 |
| F815-3-A12-IR | HLCVLEELFWGASLYGQCPG | 32.1 | 1.0 | 36.3 | <0.1 | 35.3 |
| F815-3-B8-IR | HLCVLEELFWGASLFDQCSG | 33.6 | 1.0 | 35.8 | <0.1 | 35.3 |
| F815-3-B2-IR | HLCVLEELLWGASLFGQCSG | 31.0 | 1.0 | 35.3 | <0.1 | 35.3 |
| F815-3-C3-IR | PLCVLEELFWGVSLFGQCGG | 30.1 | 1.0 | 35.3 | <0.1 | 35.3 |
| F815-3-A7-IR | HLCVLEELFWGASQWGQCSG | 33.1 | 1.0 | 35.8 | <0.1 | 35.2 |
| F815-4-F9-IR | RLCVLEEQFWGGALFGQCSG | 33.4 | 1.0 | 35.7 | <0.1 | 35.2 |
| F815-3-B7-IR | QLCVLEELFWGVSLFAQCSG | 32.0 | 1.0 | 33.5 | <0.1 | 35.0 |
| F815-4-E4-IR | HLCVLEELFWGAALFGQCFG | 28.0 | 1.0 | 33.4 | <0.1 | 35.0 |
| F815-4-E12-IR | YLCVLEELFWGASQFGQCSG | 28.0 | 0.9 | 30.2 | <0.1 | 34.8 |
| F815-4-F8-IR | HLCVLEELYWGASLFGQCSG | 33.8 | 1.0 | 35.2 | <0.1 | 34.7 |
| F815-3-C7-IR | HLCVLEERFWGVSLFGQCSG | 33.9 | 1.0 | 34.7 | <0.1 | 34.7 |
| F815-4-F10-IR | PLCVLEELFWGASRFGQCSG | 32.7 | 1.0 | 34.2 | <0.1 | 34.7 |
| F815-3-D11-IR | HLCVLEDLFWGASLFDQCSG | 35.4 | 1.1 | 37.3 | <0.1 | 34.6 |
| F815-4-E7-IR | HLCDLEVLFWGASLFGQCSG | 30.3 | 0.9 | 32.2 | <0.1 | 34.6 |
| F815-3-A10-IR | QLCILEEQFWGTSLFGYCSG | 34.0 | 1.1 | 36.4 | <0.1 | 34.3 |
| F815-3-B11-IR | ALCVLEELFWGESLFGQCSG | 33.7 | 1.1 | 36.3 | <0.1 | 34.2 |

FIG. 2B-2

| Clone Design | Sequence HLCVLEELFWGASLFGYCSG | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| F815-4-F11-IR | RLCVLEERFWGAALFGQCSG | 31.8 | 1.0 | 33.7 | <0.1 | 34.2 |
| F815-3-A9-IR | PLCVLEELFWGASLFGQCSG | 31.9 | 1.0 | 35.5 | <0.1 | 34.1 |
| F815-4-G11-IR | SLCVLEELFWGGSRFGQCSG | 32.3 | 1.0 | 34.4 | <0.1 | 33.9 |
| F815-3-D8-IR | HLCLLEEQFWGASLFGYCFE | 32.3 | 1.0 | 33.3 | <0.1 | 33.7 |
| F815-4-G4-IR | HLCVLEEQFWGASLFGQCSG | 23.8 | 1.0 | 32.2 | <0.1 | 33.7 |
| F815-3-C8-IR | DLCLLEELLWGASRFGQCSG | 33.9 | 1.0 | 35.1 | <0.1 | 33.6 |
| F815-4-G12-IR | YLCVLEERFWGASLFGQCSG | 31.7 | 1.0 | 33.5 | <0.1 | 33.5 |
| F815-3-D12-IR | HLCVLEEQFWGASLFGSCSG | 33.3 | 1.0 | 34.8 | <0.1 | 33.4 |
| F815-4-F7-IR | QLCVLEEQLWGASLFGQCSG | 33.3 | 1.0 | 34.3 | <0.1 | 33.4 |
| F815-4-F2-IR | HLCVLEELF*GESLFGYCSG | 26.1 | 1.0 | 33.8 | <0.1 | 33.3 |
| F815-3-B9-IR | HLCVLEELFWGASLFGQCSG | 33.6 | 1.1 | 35.7 | <0.1 | 33.2 |
| F815-4-H2-IR | PLCVLEELFWGASHFGQCSG | 36.1 | 1.2 | 38.4 | <0.1 | 33.0 |
| F815-4-E11-IR | HLCVLEELVWGASLFGQCAG | 33.2 | 1.1 | 35.4 | <0.1 | 33.0 |
| F815-4-G1-IR | QLCVLEELIWGASLFGQCAG | 27.9 | 1.0 | 31.5 | <0.1 | 32.8 |
| F815-3-A11-IR | HLCGLEELFWGASLFGQCSG | 37.7 | 1.2 | 40.1 | <0.1 | 32.7 |
| F815-4-F6-IR | HLCVLEELVWGESLFGQCSG | 32.3 | 1.1 | 34.6 | <0.1 | 32.6 |
| F815-3-D9-IR | RLCVLEELYWGASLFGQCSG | 31.4 | 1.0 | 32.5 | <0.1 | 32.5 |
| F815-3-C11-IR | RLCILEELFWGASLFGQCSG | 33.4 | 1.1 | 35.7 | <0.1 | 31.9 |
| F815-4-G2-IR | HLCVLEELFWGATLFDQCSG | 30.2 | 1.1 | 34.3 | <0.1 | 31.4 |
| F815-3-C9-IR | HLCFLEELFWGASMFGQCSG | 29.7 | 1.0 | 31.4 | <0.1 | 31.0 |
| F815-4-H10-IR | HLCIVEELFWAAPLFGQCSG | 31.9 | 0.9 | 27.6 | <0.1 | 29.4 |
| F815-4-F3-IR | HLCVLEELWWGASLFAQCSA | 19.4 | 1.0 | 28.0 | <0.1 | 28.9 |
| F815-4-F5-IR | NLCALEELFWGASQFRYCPG | 12.3 | 0.9 | 24.8 | <0.1 | 26.8 |
| F815-4-H1-IR | RLCVLEELFWGASLFGQCSG | 6.9 | 1.0 | 15.8 | 0.1 | 16.5 |
| F815-4-E5-IR | PLCVLEELFWGASLFGQCPG | 3.5 | 1.0 | 13.6 | 0.1 | 14.0 |
| F815-4-H5-IR | NLCVLEELFWGASLFGQCSG | 5.5 | 1.0 | 13.1 | 0.1 | 13.5 |
| F815-3-C10-IR | QLCVLG#RFWGGSLCGYCSD | 3.5 | 1.1 | 5.2 | 0.2 | 4.5 |

FIG. 2B-3

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| **Parental/Design** | **HLCVLEELFWGASLFGYCSG** | **39.1** | **1.8** | **27.7** | **0.1** | **15.4** |
| F815-4-F11-IGFR | PLCFLQELFGGASLGGYCSG | 33.4 | 12.3 | 1.0 | 12.3 | 0.1 |
| F815-4-E12-IGFR | FMCGLQELVGGAALLGHCSG | 33.7 | 15.1 | 1.7 | 8.9 | 0.1 |
| F815-4-H10-IGFR | PLCFLQELFGGGSLSGYCSG | 30.1 | 8.5 | 1.0 | 8.5 | 0.1 |
| F815-4-B7-IGFR | FLCGLEELAWGVSRSGYCFG | 35.2 | 23.9 | 4.8 | 5.0 | 0.2 |
| F815-3-B5-IGFR | PLCFLAELFSGSALGGDCSR | 33.9 | 4.8 | 1.0 | 4.8 | 0.2 |
| F815-4-D12-IGFR | PLCVLQELFGGGSLGGYCSG | 33.6 | 7.0 | 1.8 | 3.9 | 0.3 |
| F815-4-C11-IGFR | QLCVLE#LFWGACLFGYCAG | 13.9 | 4.6 | 1.8 | 2.6 | 0.4 |
| F815-4-C7-IGFR | FLCGLQELSGVASLFGQCSG | 16.8 | 2.0 | 1.0 | 2.0 | 0.5 |
| F815-4-E7-IGFR | RVCVLEQLVWGASLFGA*SG | 26.9 | 3.8 | 1.9 | 2.0 | 0.5 |
| F815-4-G7-IGFR | FYCGLEELSWGAALFGYCSG | 30.4 | 9.0 | 5.0 | 1.8 | 0.6 |
| F815-4-A10-IGFR | FLCGLEELSQGAVLFGHCYG | 30.8 | 3.7 | 2.2 | 1.7 | 0.6 |
| F815-3-B3-IGFR | HLCVLVGLFWDASLFGQCSG | 7.6 | 1.0 | 2.0 | 0.5 | 2.0 |
| F815-3-G1-IGFR | QRCIRAALFWCATLLGGCAG | 20.5 | 1.0 | 2.0 | 0.5 | 2.0 |
| F815-4-G12-IGFR | HQCIPDGMSQGAALRGNCSD | 7.6 | 1.0 | 2.5 | 0.4 | 2.5 |
| F815-3-H1-IGFR | HLCVLEDELWGVSLFGYCSS | 18.4 | 1.0 | 6.8 | 0.1 | 6.8 |

FIG. 2C

| Clone Parental/Design | Sequence | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | **HLCVLEELFWGASLFGYCSG** | **39.1** | **1.8** | **27.7** | **0.1** | **15.4** |
| F820-4-B5-IR | HLCMLEEQFWGASLFSRCSG | 28.1 | 0.9 | 17.9 | <0.1 | 21.1 |
| F820-4-A2-IR | TCAFWKNGSGVRRCSVTAVV | 34.0 | 1.6 | 22.7 | 0.1 | 13.9 |
| F820-4-E2-IR | PLCGLKN.SGVRLCSSPALV | 21.3 | 0.7 | 9.0 | 0.1 | 13.4 |
| F820-4-D10-IR | PLCLQEELFWGASLFGYCSG | 34.1 | 1.0 | 12.1 | 0.1 | 12.1 |
| F820-4-H7-IR | PLCDLEELFWGASLFGDCPG | 14.2 | 0.6 | 6.5 | 0.1 | 11.6 |
| F820-4-G6-IR | DLCVLEELFWDGSLFASCSG | 14.0 | 0.5 | 6.1 | 0.1 | 11.5 |
| F820-4-C2-IR | PLCVLEEQLWGTALFGSCTG | 38.1 | 1.2 | 11.8 | 0.1 | 9.9 |
| F820-4-B4-IR | PLCLVEELLWGASLFSQCTG | 15.1 | 0.7 | 6.4 | 0.1 | 8.7 |
| F820-4-C7-IR | PLCDLEELYWGAALFGSCSG | 46.3 | 2.7 | 22.2 | 0.1 | 8.2 |
| F820-4-F10-IR | GLCFLEEQFWGTSLFRDCPG | 14.5 | 0.6 | 4.7 | 0.1 | 8.0 |
| F820-4-G5-IR | PLCVVEELFWGASLYGQCSG | 8.8 | 0.6 | 4.4 | 0.1 | 7.5 |
| F820-4-F2-IR | RLCVLEELFWGASRFRGCSG | 11.7 | 0.6 | 4.2 | 0.1 | 7.4 |
| F820-4-H8-IR | PLCVLEELHWGAALFGYCSG | 16.0 | 0.6 | 4.7 | 0.1 | 7.3 |
| F820-4-D7-IR | NLCVVEELFWGASLFPNCSG | 14.5 | 0.8 | 5.9 | 0.1 | 7.1 |
| F820-4-B2-IR | QLCVLEELFWGASMFEDCSG | 5.0 | 0.4 | 2.4 | 0.2 | 6.9 |
| F820-4-C3-IR | HLCVLEEQFWGASLFGQCSG | 37.5 | 1.1 | 7.5 | 0.2 | 6.6 |
| F820-4-H4-IR | PLCVLEEIYWGAALFGDCYG | 21.2 | 1.1 | 6.4 | 0.2 | 5.9 |
| F820-4-B10-IR | PLCVLEELFWGLSLDKNCS | 7.5 | 0.7 | 3.7 | 0.2 | 5.6 |
| F820-4-A5-IR | QLCVLEELFWGASLFSGCSG | 5.3 | 0.8 | 4.4 | 0.2 | 5.2 |
| F820-4-F6-IR | PLCDLEALFWGESLFGGCSG | 5.7 | 0.6 | 3.0 | 0.2 | 4.9 |
| F820-4-F1-IR | HLCVLEEMFWGTSHFDGCSG | 9.1 | 1.0 | 4.7 | 0.2 | 4.7 |
| F820-4-A3-IR | DLCVLEELFWGAPLFGLCSG | 5.9 | 0.8 | 3.5 | 0.2 | 4.5 |
| F820-4-D1-IR | DLCVLEELFWGVALYGGCSG | 25.7 | 2.3 | 10.5 | 0.2 | 4.5 |
| F820-4-F5-IR | QLCVLEELYWGASLFGHCSG | 3.7 | 0.6 | 2.7 | 0.2 | 4.2 |
| F820-4-F12-IR | HLCVLEDRFWGASLFGPCSG | 11.3 | 0.6 | 2.2 | 0.3 | 3.5 |
| F820-4-A11-IR | HLCGMEEMFWGVALFRNCSG | 7.6 | 0.8 | 2.7 | 0.3 | 3.5 |
| F820-4-E8-IR | PLCVLEQLYWGESLFVYCSG | 8.0 | 1.2 | 4.3 | 0.3 | 3.5 |
| F820-4-H3-IR | HLCLLEELFWGEALWGYCSG | 17.5 | 2.6 | 9.0 | 0.3 | 3.4 |

FIG. 2D-1

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Parental/Design | HLCVLEELFWGASLFGYCSG | -- | -- | -- | -- | -- |
| F820-4-A8-IR | QLCVMEELFWGASRFGQCSG | 6.4 | 0.7 | 2.4 | 0.3 | 3.4 |
| F820-4-G1-IR | HLCVLEELFWGASMFGQCSG | 3.9 | 0.6 | 1.9 | 0.3 | 3.4 |
| F820-4-F3-IR | QLCVLEEMFWGGSRFVQCSA | 9.8 | 1.3 | 3.6 | 0.4 | 2.9 |
| F820-4-D6-IR | PLCILEELFWGEALFDQCGA | 5.4 | 1.2 | 3.2 | 0.4 | 2.6 |
| F820-4-A1-IR | YLCVQEELFWGASLFGYCSV | 25.5 | 2.4 | 6.1 | 0.4 | 2.5 |
| F820-4-H2-IR | HLCALEEAFFGPSLFNSCQG | 15.9 | 1.6 | 4.1 | 0.4 | 2.5 |
| F820-4-F4-IR | HLCVLEERFWGASLFGQCSG | 6.8 | 1.9 | 4.7 | 0.4 | 2.5 |
| F820-4-B6-IR | QLCDLEELFWGASLFGYCPG | 4.1 | 0.8 | 1.9 | 0.4 | 2.4 |
| F820-4-B11-IR | HLCVLEERFWGASIWGSCSG | 22.2 | 3.1 | 7.0 | 0.4 | 2.3 |
| F820-4-H6-IR | QLCVLEELFWGGSLWGQCSR | 4.1 | 1.1 | 2.4 | 0.5 | 2.2 |
| F820-4-H9-IR | PLCVLEELFWGAAQFGQCSG | 3.1 | 0.9 | 1.9 | 0.5 | 2.1 |
| F820-4-D3-IR | QLCDLEERFWGVSLFGLCSG | 4.6 | 1.3 | 2.5 | 0.5 | 1.9 |
| F820-4-C1-IR | QLCVLEEVFWGASLFGLCTG | 13.0 | 1.1 | 2.1 | 0.5 | 1.9 |
| F820-4-D12-IR | QL.DLNTWSGLCLCSVTVRV | 10.4 | 1.2 | 2.0 | 0.6 | 1.7 |
| F820-4-B8-IR | DLCVLEESLWGKALFGYCSD | 7.2 | 2.2 | 3.4 | 0.6 | 1.5 |
| F820-4-C6-IR | HLCVLEEVFWGSSMFGDCSG | 13.9 | 2.5 | 2.8 | 0.9 | 1.1 |
| F820-4-C10-IR | HLCDLEELFWGASLFGDCQG | 5.3 | 2.6 | 2.9 | 0.9 | 1.1 |
| F820-4-D4-IR | QLCVLDALMWGGCRLGHQCG | 3.5 | 2.3 | 2.1 | 1.1 | 0.9 |
| F820-4-E1-IR | QLCVLEEKFWGTSLFGDCMG | 1.6 | 1.6 | 1.5 | 1.1 | 0.9 |
| F820-4-B3-IR | HLCVLEEVFWGAAQFGSCSG | 15.9 | 0.6 | 5.0 | 1.2 | 0.8 |
| F820-4-D2-IR | QLCVLEELFWGPSMFGYCSG | 7.8 | 3.2 | 2.5 | 1.3 | 0.8 |
| F820-4-C5-IR | HLCDLEELFWGASGFAQCYG | 21.5 | 4.0 | 2.3 | 1.8 | 0.6 |

FIG. 2D-2

| Clone Design | Sequence HLCVLEELFWGASLFGYCSG | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| A6L-3-C4-IR | DLCVLEERFWGASLFGQCSG | 36.9 | 1.0 | 40.5 | <0.1 | 42.5 |
| A6L-3-D7-IR | QLCVLEELHWGASLFGYCSG | 38.6 | 1.0 | 40.1 | <0.1 | 40.7 |
| A6L-3-A1-IR | PLCVLEEQFWGASLFGQCSG | 39.6 | 1.1 | 44.8 | <0.1 | 40.6 |
| A6L-3-C1-IR | YLCDLEERFWGASLFGQCSS | 37.3 | 1.0 | 40.3 | <0.1 | 40.3 |
| A6L-3-D5-IR | HLCLLEERFWGSSQFGFCSG | 42.9 | 1.1 | 44.4 | <0.1 | 40.2 |
| A6L-3-A4-IR | HLCVLEELFWGASQFGQCSG | 26.7 | 1.1 | 42.2 | <0.1 | 40.2 |
| A6L-3-D3-IR | HLCYLEERFWGASLFGQCSG | 34.6 | 0.9 | 36.9 | <0.1 | 39.8 |
| **A6L-3-B1-IR** | HLCVMEELFWGTSLFGQCTG | 33.9 | 1.0 | 38.7 | <0.1 | 39.3 |
| A6L-3-B5-IR | HLCVLEERFWGASLFGQCSG | 35.3 | 1.1 | 42.4 | <0.1 | 38.6 |
| A6L-3-B2-IR | HLCVLEERFWGASLFSQCSG | 38.1 | 1.1 | 42.7 | <0.1 | 37.7 |
| B6H-4-G12-IR | HLCVLEELFWGASLFGQCSG | 31.6 | 1.1 | 39.6 | <0.1 | 36.7 |
| B6C-4-H10-IR | QLCLLEELFWGAASFGQCSG | 38.5 | 1.1 | 41.1 | <0.1 | 36.5 |
| B6H-4-G8-IR | HLCVLEEMFWGASLFGQCSG | 31.7 | 1.1 | 39.7 | <0.1 | 36.2 |
| A6L-3-D6-IR | HLCDLEELFWGASLFSQCSR | 35.5 | 1.0 | 37.2 | <0.1 | 36.1 |
| B6C-4-F1-IR | QLCVLEELFWGASQFGYCSG | 32.9 | 1.1 | 38.7 | <0.1 | 35.8 |
| B6C-4-H3-IR | QLCALEEQFWGASLFSQCSG | 37.4 | 1.2 | 40.5 | <0.1 | 34.8 |
| B6H-4-E8-IR | QLCVLEELFWGASLFGYCSG | 30.2 | 1.0 | 35.7 | <0.1 | 34.3 |
| B6C-4-G1-IR | HLCVLEEWFWGDSLFGQCSR | 34.9 | 1.2 | 40.2 | <0.1 | 33.7 |
| B6H-4-E9-IR | HLCVLEERFWGASLFGQCSG | 34.4 | 1.2 | 38.8 | <0.1 | 33.2 |
| B6C-4-F5-IR | QLCELEEVFWGASLFDYCSG | 34.7 | 1.2 | 39.6 | <0.1 | 32.8 |
| B6C-4-F11-IR | HLCVLEELFWGASRFGQCSG | 34.0 | 1.2 | 37.2 | <0.1 | 31.7 |
| B6C-4-E6-IR | HLCVLEELFWGASLFGQCSA | 32.3 | 1.2 | 37.4 | <0.1 | 30.6 |
| B6C-4-E12-IR | HLCVLEELIWGASRFGQCSG | 30.9 | 1.1 | 33.3 | <0.1 | 30.2 |
| B6C-4-G10-IR | HLCVLEELFWGGSLFIQCSG | 33.0 | 1.3 | 40.3 | <0.1 | 30.1 |
| B6C-4-F8-IR | QLCVLEEQFWGASLFGNCSG | 36.4 | 1.4 | 39.8 | <0.1 | 29.3 |
| 20C-3-B5-IR | HLCVLEERFWGAALFGQCSG | 26.6 | 1.1 | 32.5 | <0.1 | 29.2 |
| B6C-4-G3-IR | HLCILEEMFWGASLFGQCGG | 34.0 | 1.4 | 38.8 | <0.1 | 28.3 |
| 20C-3-B7-IR | PLCVLEELVWGASLFVQCSG | 29.5 | 1.2 | 32.9 | <0.1 | 28.3 |

FIG. 2E-1

| Clone Design | Sequence |
|---|---|
| | **HLCVLEELFWGASLFGYCSG** |
| 20C-3-B4-IR | NLCVLEELFWGESLFGQCSG |
| 20C-3-C11-IR | HLCVLEEQFWGGSLFGYCSR |
| B6C-4-G2-IR | HLCFLEEVFWGAALFAQCSG |
| 20C-3-B8-IR | HLCDLEVLFWGSALFGQCSG |
| 20C-3-C10-IR | HLCVMEELFWGASLFGQCSG |
| 20C-3-B6-IR | HLCVLEERFWGASLFWQCSG |
| A6L-3-A3-IR | HLCVLEEQYWGESLFGYCSG |
| A6L-3-B3-IR | PLCVLEEQFWGASLFAYCSS |
| 20C-3-A5-IR | QLCVLEELFWGESLFAQCLG |
| 20C-3-B11-IR | HLCVLEELFWGQSLFGHCSD |
| 20C-3-B3-IR | HLCVLEELVWGASLFGFCSG |
| 20C-3-C12-IR | LLCVLEEQFWGASLFGQCSG |
| 20C-3-C3-IR | RLCVLEELFWGESLFGQCSG |
| 20C-3-C2-IR | HLCVLEEMFWGASLFGNCSG |
| 20C-3-A11-IR | ELCFLEELFWGASLFGQCSG |
| 20C-3-A4-IR | HLCVLEELFWGASLYGQCSS |
| 20C-3-A6-IR | HLCVLEELFWGASLFAQCPG |
| B6C-4-E4-IR | NLCVLEELFWGASEFGQCSG |
| 20C-3-A9-IR | DLCVLEEQLWGASLFRYCSG |
| B6C-3-C5-IR | HLCVLEEQFWGVALFGNCSG |
| 20C-3-B1-IR | HLCVLEVQIWGASLFGQCSG |
| 20C-3-A10-IR | HLCVLEERFWGGALFGQCTA |
| 20C-4-F1-IR | HLCDLEELFWGASLFGQCSG |
| 20C-4-E1-IR | QLCVLEELFWGTSLFAGCSG |
| 20C-3-B12-IR | QLCGLEELFWGASLFGYCSA |
| 20C-3-A8-IR | HLCVLEELFWGASLFGQCSS |
| 20C-3-A7-IR | FLCVLEELYWGASQFGQCSG |
| B6C-4-E10-IR | HLCVLEEQFWGASLFGYCSG |

| Ratios over Background | | | Comparisons | |
|---|---|---|---|---|
| E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| -- | -- | -- | -- | -- |
| 28.9 | 1.1 | 31.1 | <0.1 | 28.0 |
| 30.2 | 1.1 | 31.0 | <0.1 | 27.7 |
| 29.4 | 1.3 | 35.3 | <0.1 | 27.5 |
| 28.5 | 1.1 | 31.2 | <0.1 | 27.4 |
| 32.1 | 1.2 | 33.6 | <0.1 | 27.1 |
| 29.7 | 1.2 | 31.9 | <0.1 | 26.7 |
| 14.4 | 1.1 | 28.3 | <0.1 | 26.5 |
| 38.7 | 1.7 | 43.4 | <0.1 | 26.3 |
| 22.9 | 1.1 | 27.6 | <0.1 | 26.0 |
| 30.0 | 1.3 | 32.7 | <0.1 | 25.8 |
| 29.3 | 1.2 | 31.2 | <0.1 | 25.7 |
| 29.6 | 1.3 | 31.8 | <0.1 | 24.8 |
| 30.1 | 1.2 | 30.1 | <0.1 | 24.3 |
| 29.9 | 1.3 | 29.8 | <0.1 | 23.8 |
| 25.9 | 1.2 | 27.4 | <0.1 | 23.0 |
| 27.2 | 1.2 | 27.5 | <0.1 | 22.9 |
| 26.1 | 1.2 | 27.5 | <0.1 | 22.8 |
| 34.5 | 1.7 | 39.1 | <0.1 | 22.7 |
| 29.7 | 1.3 | 29.3 | <0.1 | 22.7 |
| 33.5 | 1.7 | 37.7 | <0.1 | 22.5 |
| 30.2 | 1.2 | 26.7 | <0.1 | 22.0 |
| 29.0 | 1.3 | 28.5 | <0.1 | 21.5 |
| 29.1 | 1.4 | 29.5 | <0.1 | 20.7 |
| 28.3 | 1.4 | 29.7 | <0.1 | 20.6 |
| 27.0 | 1.3 | 25.8 | <0.1 | 20.2 |
| 21.1 | 1.1 | 21.2 | 0.1 | 20.0 |
| 21.9 | 1.3 | 23.0 | 0.1 | 18.3 |
| 35.2 | 2.2 | 38.0 | 0.1 | 17.5 |

FIG. 2E-2

| Clone Design | Sequence HLCVLEELFWGASLFGYCSG | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | -- | -- | -- | -- | -- |
| 20C-3-A1-IR | RLCALEELFWGASLFGQCSG | 21.0 | 1.1 | 17.6 | 0.1 | 16.6 |
| 20C-3-C1-IR | HLCVLEELFWGAALFHQCSG | 30.6 | 1.4 | 21.9 | 0.1 | 16.1 |
| A6L-3-D2-IR | RLCVLEEQFWGASLFGQCSG | 7.0 | 1.1 | 14.9 | 0.1 | 14.1 |
| B6C-4-G12-IR | QLCVLEELFWGSSRLGYCSG | 31.1 | 2.5 | 33.5 | 0.1 | 13.6 |
| B6H-4-F9-IR | DLCVLEELFWGASLFGQCSG | 39.3 | 3.6 | 43.1 | 0.1 | 12.1 |
| B6C-4-E3-IR | QLCLLEEQFWGGSLFGQCSG | 34.6 | 5.3 | 40.0 | 0.1 | 7.6 |
| 20C-3-B10-IR | HLCVLEELFWGTSLFGQCSG | 29.9 | 16.9 | 31.7 | 0.5 | 1.9 |
| 20C-3-A3-IR | RLCVLEELVWGASLFDQCSR | 28.4 | 19.1 | 25.3 | 0.8 | 1.3 |

FIG. 2E-3

| Clone Design | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| | XXXXXXXXXXXXXXXXXXXX | -- | -- | -- | -- | -- |
| R20α-3-20A4-IR | EIEAEWGRVRCLVYGRCVGG | 50.2 | 1.6 | 23.1 | 0.1 | 14.4 |
| R20β-4-A7-IR | EIEAEWGRVRCLVYGRCVGG | 44.2 | 1.3 | 24.0 | 0.1 | 18.5 |
| R20β-4-D8-IR | WLDQEWAWVQCEVYGRGCPS | 44.8 | 1.4 | 24.2 | 0.1 | 17.3 |

FIG. 3A

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| Parental/Design | WLDQEWAWVQCEVYGRGCPS | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| | | 44.8 | 1.4 | 24.2 | <0.1 | 17.3 |
| D815-4-A8-IR | WLDLEWAQVQCEVYGRGCPS | 48.0 | 1.0 | 48.4 | <0.1 | 48.4 |
| D815-4-D10-IR | WLDQEWAQVQCEVFGRGCPS | 49.2 | 1.0 | 48.2 | <0.1 | 48.2 |
| D815-4-D9-IR | WLDQEWQQVQCQVYGRGCTS | 47.5 | 1.0 | 48.0 | <0.1 | 48.0 |
| D815-4-A11-IR | RLDEEWARVQCEVWGRGCRS | 47.9 | 1.0 | 48.0 | <0.1 | 48.0 |
| D815-4-E12-IR | WLEQEWAWIQCEVYGRGCPS | 49.0 | 1.0 | 47.6 | <0.1 | 47.6 |
| D815-4-B7-IR | WLEQEWAQVQCEVYGRGCPS | 45.4 | 1.0 | 47.2 | <0.1 | 47.2 |
| D815-4-D11-IR | WLDEEWEWIQCKVYGRGCPA | 49.5 | 1.0 | 47.0 | <0.1 | 47.0 |
| D815-4-D12-IR | WLEQEWAWVQCEVYGRGCQS | 48.1 | 1.0 | 46.6 | <0.1 | 46.6 |
| D815-4-F8-IR | WLDQEWAWIQCEVYGRGCPA | 47.8 | 1.0 | 46.4 | <0.1 | 46.4 |
| D815-4-A9-IR | SLDWEWAWLQCEVYGRGCPS | 47.7 | 1.0 | 45.8 | <0.1 | 45.8 |
| D815-4-E9-IR | WLEQEWEQVRCLVYGRGCPP | 47.8 | 1.0 | 45.8 | <0.1 | 45.8 |
| D815-4-B10-IR | WLDQEWAWVQCEVYGRGCPY | 49.0 | 1.0 | 45.6 | <0.1 | 45.6 |
| D815-4-H8-IR | WLDQEWAGVLCEVYGRGCPS | 49.0 | 1.0 | 45.6 | <0.1 | 45.6 |
| D815-4-E10-IR | SLDKEWEWVLCVVYGRGCPS | 47.0 | 1.0 | 45.6 | <0.1 | 45.6 |
| D815-4-D7-IR | WLEQEWAQVQCEVYGRGCRS | 44.5 | 1.0 | 45.4 | <0.1 | 45.4 |
| D815-4-G9-IR | WLEEEWAQVQCAVYGRGCSS | 44.2 | 1.0 | 44.2 | <0.1 | 44.2 |
| D815-4-G12-IR | WLDQEWALVQCEVYGRGCPS | 44.3 | 1.0 | 43.7 | <0.1 | 43.7 |
| D815-4-E11-IR | WLDQEWAWVQCEVYGRGCPS | 45.5 | 1.0 | 43.0 | <0.1 | 43.0 |
| D815-4-H7-IR | WLEQEWAWVQCEVYGRGCAS | 46.2 | 1.0 | 43.0 | <0.1 | 43.0 |
| D815-4-F12-IR | WLDQEWAWVECEVYGRRCPS | 47.2 | 1.0 | 42.6 | <0.1 | 42.6 |
| D815-4-E8-IR | WLDQEWAWVECQVYGRGCPS | 47.9 | 1.0 | 42.6 | <0.1 | 42.6 |
| D815-4-F9-IR | QLDQEWAWVLCKVYGRGCPS | 46.4 | 1.0 | 41.8 | <0.1 | 41.8 |
| D815-4-A10-IR | WLDHE*AWVQCEVYGRGCPS | 47.3 | 1.0 | 41.2 | <0.1 | 41.2 |
| D815-4-C7-IR | QLEQEWAWVRCEVYGRGCSS | 37.7 | 1.0 | 40.0 | <0.1 | 40.0 |
| D815-4-H10-IR | WLDQEWAWVQCQVYGRGCLS | 47.0 | 1.0 | 39.8 | <0.1 | 39.8 |
| D815-4-C9-IR | WLDQEWAWVRCEVYGLGCPS | 44.2 | 1.0 | 39.8 | <0.1 | 39.8 |
| D815-4-F11-IR | WLDQEWAVMKCELYGRGCPS | 40.4 | 1.0 | 39.2 | <0.1 | 39.2 |
| D815-4-H12-IR | WLEQEWAWVQCEVYGRGCLS | 45.4 | 1.0 | 38.6 | <0.1 | 38.6 |
| D815-4-A7-IR | SLDQEWAWVQCEVYGRGCLS | 37.3 | 1.0 | 37.3 | <0.1 | 37.3 |
| D815-4-H11-IR | WLDHEWAWVQCEVYGRGCTS | 2.4 | 1.0 | 37.2 | <0.1 | 37.2 |
| D815-4-F7-IR | WLDVEWAWVQCEVYGRGCPS | 32.4 | 1.0 | 34.7 | <0.1 | 34.7 |

FIG. 3B-1

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| **Parental/Design** | **WLDQEWAWVQCEVYGRGCPS** | -- | -- | -- | -- | -- |
| D815-4-G8-IR | QLDQEWARVRCEVWGRGCSS | 27.8 | 1.0 | 33.6 | <0.1 | 33.6 |
| D815-4-G7-IR | WLDLEWAQVQCKVYGRGCPS | 34.7 | 1.0 | 32.3 | <0.1 | 32.3 |
| D815-4-G11-IR | WLDEEWAWVQCQVYGRGCPS | 30.7 | 1.0 | 28.6 | <0.1 | 28.6 |
| D815-4-E7-IR | WLDQEWAWVQCEVWGRGCAF | 33.0 | 1.0 | 26.4 | <0.1 | 26.4 |
| D815-4-A12-IR | WLDREWAQVQCEVYGRGCLS | 28.4 | 1.0 | 19.0 | 0.1 | 19.0 |
| D815-4-B11-IR | WLDAEWEWVQCEVYGRGCRP | 22.1 | 1.0 | 18.8 | 0.1 | 18.8 |
| D815-4-D8-IR | SLDREWAYVQCQVYGRGCSS | 20.8 | 1.0 | 14.6 | 0.1 | 14.6 |

FIG. 3B-2

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Parental/Design | WLDQEWAWVQCEVYGRGCPS | 44.8 | 1.4 | 24.2 | 0.1 | 17.2 |
| D820-3-H2-IR | RLDLEWANIQCEVYGRGCPS | 23.9 | 1.0 | 40.0 | <0.1 | 40.0 |
| D820-3-C4-IR | WLEQEWARVQCEVYGRGCSS | 31.0 | 1.0 | 39.5 | <0.1 | 39.5 |
| D820-3-C3-IR | WLEQEWILVECEVYGRGCPT | 35.2 | 1.0 | 39.4 | <0.1 | 39.4 |
| D820-3-G6-IR | WLEQEWAQVQCEVWGRGCPS | 33.8 | 1.0 | 38.8 | <0.1 | 38.8 |
| D820-3-D2-IR | WLDQEWEWIQCEVYGRGCPL | 35.6 | 1.0 | 37.8 | <0.1 | 37.8 |
| D820-3-D3-IR | LLDEEWAQIECEIYGRGCPS | 34.8 | 1.0 | 37.7 | <0.1 | 37.7 |
| D820-3-B5-IR | ALEEEWAWVQCEVYGRGCHF | 34.1 | 1.0 | 37.1 | <0.1 | 37.1 |
| D820-3-E2-IR | C?EQEWGLVQCEVYGRGCPS | 34.4 | 1.0 | 37.0 | <0.1 | 37.0 |
| D820-3-B3-IR | WLEQEWAYVQCEVYGRGCPS | 33.6 | 1.0 | 36.7 | <0.1 | 36.7 |
| D820-3-B6-IR | WLEHEWAQVQCEVWGRGCPY | 31.2 | 1.0 | 36.6 | <0.1 | 36.6 |
| D820-3-D4-IR | WLEQEWAEVRCEVYGRGCPR | 32.0 | 1.0 | 36.2 | <0.1 | 36.2 |
| D820-3-C2-IR | ?LEQEWAWVQCEVYGRGCPS | 33.7 | 1.0 | 35.6 | <0.1 | 35.6 |
| D820-3-F6-IR | WLEQEWAGIQCKVYGRGCPS | 30.8 | 1.0 | 35.2 | <0.1 | 35.2 |
| D820-3-D5-IR | RLEQEWAQVQCEVWGRGCLP | 30.5 | 1.0 | 34.8 | <0.1 | 34.8 |
| D820-3-F5-IR | QLDHEWAGIQCEVWGRGCPS | 29.8 | 1.0 | 34.6 | <0.1 | 34.6 |
| D820-3-H3-IR | WLEQEWAQIQCEVYGAGCRS | 30.2 | 1.0 | 33.8 | <0.1 | 33.8 |
| D820-3-G2-IR | SLEQEWAWVQCVVYGRGCPI | 31.3 | 1.0 | 33.0 | <0.1 | 33.0 |
| D820-3-H6-IR | WLEQEWDQVLCEVYGRGCPY | 30.3 | 1.0 | 32.2 | <0.1 | 32.2 |
| D820-3-F3-IR | WLEQEWAQV?CEVYGRGCA? | 28.6 | 1.0 | 30.7 | <0.1 | 30.7 |
| D820-3-B4-IR | WMDQEWAWVQCEVYGRGCPS | 33.1 | 1.0 | 30.5 | <0.1 | 30.5 |
| D820-3-C5-IR | QLDQEWAWIQCEVYGRNCRT | 29.1 | 1.0 | 30.3 | <0.1 | 30.3 |
| D820-3-F4-IR | TLEQEWAQVICEVYGRGCLS | 25.9 | 1.0 | 29.5 | <0.1 | 29.5 |
| D820-3-H5-IR | RLEQEWAQVQCEVWGRGCLS | 26.3 | 1.0 | 28.6 | <0.1 | 28.6 |
| D820-3-A6-IR | WLDQEWALVQCEVYGRGCPA | 24.8 | 1.0 | 26.0 | <0.1 | 26.0 |
| D820-3-A2-IR | WLDQEWAQIQCHVWGRGCPA | 23.7 | 1.0 | 25.6 | <0.1 | 25.6 |
| D820-3-G5-IR | WLEQEWAWVQCEVYGRGCPS | 22.6 | 1.0 | 25.0 | <0.1 | 25.0 |
| D820-3-G3-IR | RLEEEWAWVQCQVYGRGCPS | 22.2 | 1.0 | 23.9 | <0.1 | 23.9 |
| D820-3-E3-IR | WLEQEWVRIQCEVYGRGCPS | 20.6 | 1.0 | 22.7 | <0.1 | 22.7 |

FIG. 3C-1

| Clone Parental/Design | Sequence WLDQEWAWVQCEVYGRGCPS | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| D820-3-E5-IR | WLEQEWTWVQCEVYGCGCPS | **44.8** | **1.4** | **24.2** | **0.1** | **17.2** |
| D820-3-D1-IR | WLEKEWAGVQCEIYGRGCPS | 25.9 | 1.0 | 22.6 | <0.1 | 22.6 |
| D820-3-E1-IR | WLEEEWAWVRCEVYGRGCQS | 27.3 | 1.0 | 22.4 | <0.1 | 22.4 |
| D820-3-F1-IR | WLEHEWAQIQCELYGRGCTY | 22.4 | 1.0 | 21.9 | <0.1 | 21.9 |
| D820-3-B2-IR | ALEEEWAWVQCEVYGRGCPS | 22.0 | 1.0 | 21.0 | <0.1 | 21.0 |
| D820-3-A3-IR | WLEQEWAQVQCEVYGRGCPS | 13.1 | 1.0 | 18.4 | 0.1 | 18.4 |
| D820-3-H4-IR | WLDDEWAQIQCEIYGRGCQS | 23.5 | 1.0 | 18.4 | 0.1 | 18.4 |
| D820-3-G1-IR | QLEEEWAGVQCEVYGRECPS | 25.6 | 1.0 | 17.5 | 0.1 | 17.5 |
| D820-3-C1-IR | WLEQEWLLVQCGVYGRGCPS | 14.5 | 1.0 | 16.3 | 0.1 | 16.3 |
| D820-3-A1-IR | WLDQEWAWIQCEVYGRGCRS | 27.8 | 1.0 | 13.9 | 0.1 | 13.9 |
| D820-3-A5-IR | WLEQEWAQVQCEVSGRGCPS | 14.7 | 1.0 | 12.8 | 0.1 | 12.8 |
| D820-3-H1-IR | W?DQEWALIQCEVYGRGCPS | 6.4 | 1.0 | 6.3 | 0.2 | 6.3 |
| D820-3-A4-IR | SLDEEWAGVLCEVYGRGCPF | 13.7 | 1.0 | 6.2 | 0.2 | 6.2 |
| D820-4-E12-IR | SVDQELEWLMCHFQGRVCPS | 6.0 | 1.0 | 4.3 | 0.2 | 4.3 |
| D820-4-B12-IR | WLEQERAWIWCEIQGSGCRA | 34.9 | 9.0 | 10.9 | 0.8 | 1.2 |
| | | 32.2 | 8.6 | 1.0 | 8.6 | 0.1 |

FIG. 3C-2

| Clone Parental/Design | Sequence WLDQEWAWVQCEVYGRGCPS | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | **44.8** | **1.4** | **24.2** | **0.1** | **17.3** |
| D820-3-D5-IGFR | WVNQALGGVQSDVQGRRCQS | 29.6 | 3.8 | 1.0 | 3.8 | 0.3 |
| D820-3-E4-IGFR | LLDHEWPWVGCEVCGRGSLS | 27.1 | 3.2 | 1.0 | 3.2 | 0.3 |
| D820-3-C5-IGFR | WLHQELAWVRGEGYPRGRRS | 25.0 | 3.1 | 1.0 | 3.1 | 0.3 |
| D820-3-F4-IGFR | WLGHDWAWIQCEVYGLGCPC | 3.9 | 2.7 | 1.0 | 2.7 | 0.4 |
| D820-3-F6-IGFR | WIDQEGVRVQCEA*GRAFPS | 26.7 | 2.6 | 1.0 | 2.6 | 0.4 |
| D820-3-G4-IGFR | WRDEEWAWVQGVVQGRGWPA | 3.8 | 2.6 | 1.0 | 2.6 | 0.4 |
| D820-3-E2-IGFR | RLGVEWSWFQRKVYGRDSTS | 15.3 | 2.6 | 1.0 | 2.6 | 0.4 |
| D820-3-G6-IGFR | WLAQGWAGVQCVVYGRGCRN | 20.3 | 2.4 | 1.0 | 2.4 | 0.4 |
| D820-4-E11-IGFR | WLEEE*AGIQCQV?GRGCPS | 12.6 | 1.0 | 3.0 | 0.3 | 3.0 |
| D820-4-H11-IGFR | WLDQEWEWVQCEVWGRGCLS | 8.1 | 1.0 | 4.6 | 0.2 | 4.6 |
| D820-4-D11-IGFR | RLEQEWALIQCEVYGRGCPS | 4.5 | 1.0 | 5.3 | 0.2 | 5.3 |
| D820-4-A8-IGFR | WLEEEWAQVQCQVYGRGCAS | 3.2 | 1.0 | 5.5 | 0.2 | 5.5 |
| D820-4-F9-IGFR | WLDLE*EWLQCEVYGRGCAT | 9.4 | 1.0 | 5.8 | 0.2 | 5.8 |
| D820-4-C8-IGFR | WLEQEWVQVRCEVYGRGCPS | 11.6 | 1.0 | 5.9 | 0.2 | 5.9 |
| D820-4-D9-IGFR | WLEEEWAQVQCEVYGRGCPS | 10.1 | 1.0 | 8.9 | 0.1 | 8.9 |
| D820-4-D7-IGFR | WLDQEWARVQCEVWGRGCTY | 34.1 | 3.5 | 33.4 | 0.1 | 9.5 |
| D820-4-H9-IGFR | YLD?EWAWVQCEVYGLGCQS | 18.4 | 1.0 | 10.1 | 0.1 | 10.1 |
| D820-4-E10-IGFR | WLDVE*AWVQCEVWGRGCPS | 26.7 | 2.6 | 27.0 | 0.1 | 10.4 |
| D820-4-E7-IGFR | WLEQEWER?QCEVYGRGCPP | 31.9 | 3.0 | 32.2 | 0.1 | 10.7 |
| D820-4-H8-IGFR | WLEEEWAQVQCEVYGRGCLS | 16.1 | 1.0 | 11.7 | 0.1 | 11.7 |
| D820-4-A11-IGFR | WLDQEWAWIQCEVYGRGCPS | 8.0 | 1.0 | 12.5 | 0.1 | 12.5 |
| D820-4-C9-IGFR | ?LEHEWAQIQCEV?GRGCQS | 19.6 | 1.0 | 14.9 | 0.1 | 14.9 |
| D820-4-E9-IGFR | WL?QEWAWIQCEVYGRGCPF | 19.3 | 1.0 | 17.3 | 0.1 | 17.3 |
| D820-4-B10-IGFR | WLD?EWAWVQCEVYGRGCPS | 19.3 | 1.0 | 21.5 | <0.1 | 21.5 |
| D820-4-F10-IGFR | GLEQGCPWVGLEVQCRGCPS | 27.8 | 1.0 | 25.7 | <0.1 | 25.7 |
| D820-4-B9-IGFR | WLEEEWAWVQCEVYGHGCPS | 31.7 | 1.0 | 26.5 | <0.1 | 26.5 |
| D820-4-G8-IGFR | WLDQEWAQIQCEVYGRGCSS | 25.6 | 1.0 | 29.3 | <0.1 | 29.3 |

FIG. 3D-1

| Clone Parental/Design | Sequence WLDQEWAWVQCEVYGRGCPS | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | **44.8** | **1.4** | **24.2** | **0.1** | **17.3** |
| D820-4-G9-IGFR | WLDQEWAQVQCEVWGRGCPS | 36.8 | 1.0 | 29.6 | <0.1 | 29.6 |
| D820-4-C10-IGFR | WLDLEWEFVQCEVYGRGCPT | 32.6 | 1.0 | 31.3 | <0.1 | 31.3 |
| D820-4-A9-IGFR | WLEQEWASVQCEVYGRGCPS | 20.4 | 1.0 | 31.4 | <0.1 | 31.4 |
| D820-4-B8-IGFR | WLDLEWEQIKCKVYGRGCPF | 31.1 | 1.0 | 32.7 | <0.1 | 32.7 |
| D820-4-F8-IGFR | WLEQEWAQIQCQIYGRGCPS | 28.3 | 1.0 | 32.9 | <0.1 | 32.9 |
| D820-4-H7-IGFR | WLEQEWALVLCEVYGHGCPA | 34.1 | 1.0 | 32.9 | <0.1 | 32.9 |
| D820-4-E8-IGFR | WLEQEWAQIQCEVWGRGCSS | 26.6 | 1.0 | 33.2 | <0.1 | 33.2 |
| D820-4-G10-IGFR | WLE?EWEWVQCEVYGRGC?S | 37.5 | 1.0 | 33.2 | <0.1 | 33.2 |
| D820-4-D10-IGFR | WLEQEWAQVQCDVYGRGCPS | 36.6 | 1.0 | 33.5 | <0.1 | 33.5 |
| D820-4-D8-IGFR | WLEQE*ARVQCEVWGRGCPS | 23.7 | 1.0 | 34.6 | <0.1 | 34.6 |
| D820-4-A10-IGFR | WL?QEWARVHCEVWGRP?QC | 29.4 | 1.0 | 35.5 | <0.1 | 35.5 |
| D820-4-B7-IGFR | PLEHEWAWVQCVVYGRGCRS | 35.4 | 1.0 | 36.9 | <0.1 | 36.9 |
| D820-4-E12-IGFR | SLE?EWAWVQCEV?GRGCP? | 37.0 | 1.0 | 37.0 | <0.1 | 37.0 |
| D820-4-H10-IGFR | WLDQEWVRVQCEVWGRGCPS | 36.8 | 1.0 | 37.1 | <0.1 | 37.1 |
| D820-4-F12-IGFR | SLDKEWAWVKCEVYGRGCPS | 36.9 | 1.0 | 37.3 | <0.1 | 37.3 |
| D820-4-F7-IGFR | LGDQEWAWVEWEV#GRGWPS | 34.4 | 1.0 | 37.5 | <0.1 | 37.5 |
| D820-4-G12-IGFR | WLEEEWAQIRCGVYGRGCPS | 30.3 | 1.0 | 37.8 | <0.1 | 37.8 |
| D820-4-D12-IGFR | WLEEE*GWVQCEVWGRGCPP | 37.2 | 1.0 | 38.6 | <0.1 | 38.6 |
| D820-4-A12-IGFR | CLDQEWA?VQCPVYGRGCPS | 30.4 | 1.0 | 39.3 | <0.1 | 39.3 |
| D820-4-C12-IGFR | QLELEWARVQCEVWDRGCPS | 37.1 | 1.0 | 39.6 | <0.1 | 39.6 |
| D820-4-A7-IGFR | RLEQEWAWIQCEVYGRGCRF | 35.4 | 1.0 | 40.8 | <0.1 | 40.8 |
| D820-4-B12-IGFR | SLEHE*AWVQCKVYGRGC?S | 36.2 | 1.0 | 41.4 | <0.1 | 41.4 |

FIG. 3D-2

| Clone Parental/Design | Sequence WLDQEWAWVQCEVYGRGCPS | Ratios over Background E-Tag | IGFsR | IR | Comparisons IGFR/IR | IR/IGFR |
|---|---|---|---|---|---|---|
| | | **44.8** | **1.4** | **24.2** | **<0.1** | **17.3** |
| B6-4-G12-IR | WLDQEWAWIQCEVYGRGCPP | 4.4 | 1.0 | 6.9 | 0.1 | 7.1 |
| B6-3-A11-IR | WLDQEWAQVRCEVYGRGCPS | 7.3 | 1.0 | 6.3 | 0.2 | 6.3 |

FIG. 3E

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | XXXXXXXXXXXXXXXXXXXX | -- | -- | -- | -- | -- |
| R20-4-C10-IGFR | PKGTRFRGDVDVWDGYSWLA | 37.8 | 3.8 | -- | -- | -- |

FIG. 4A-1

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | XXXXXXXXXXXXXXXXXXXX | -- | -- | -- | -- | -- |
| 20F-4-B7-IGFR | TPIPAGGINIASWGGYTWLS | 10.9 | 3.7 | 0.5 | 7.3 | 0.1 |
| 20F-4-E4-IGFR | HRGTVTGVWVARWPGYEWLS | 8.9 | 4.7 | 0.7 | 6.3 | 0.2 |
| 20F-4-E12-IGFR | SDVWAQPQRRNDWPGYHWLS | 9.7 | 4.7 | 0.8 | 6.0 | 0.2 |
| 20F-4-F4-IGFR | HRGTVTGVWVARWPGYEWLS | 13.9 | 10.1 | 1.8 | 5.6 | 0.2 |
| 20F-4-F7-IGFR | SDVWAQPQRRNDWPGYHWLS | 13.7 | 3.9 | 0.8 | 5.1 | 0.2 |
| 20F-4-E7-IGFR | RPHRINPQDDAVWPGYLWLG | 7.2 | 2.5 | 0.5 | 4.7 | 0.2 |
| 20F-4-F11-IGFR | HRGTVTGVWVARWPGYEWLS | 17.6 | 16.2 | 3.5 | 4.6 | 0.2 |
| 20F-4-D10-IGFR | FGRGYGGDGGGYWSGYEWLA | 9.8 | 2.4 | 0.6 | 4.1 | 0.2 |
| 20F-4-B3-IGFR | DGLVVKSGREWPGYGWLER.A | 17.3 | 14.4 | 3.6 | 4.0 | 0.2 |
| 20F-4-B12-IGFR | DGSIV.VSSSVGWPGYEWLM | 10.1 | 9.9 | 2.4 | 4.0 | 0.2 |
| 20F-3-A9-IGFR | WQQANLSNGGGRWGGYDWLM | 6.6 | 2.7 | 0.7 | 4.0 | 0.2 |
| 20F-4-G2-IGFR | FGRGYGGDGGGYWSGYEWLA | 5.1 | 1.3 | 0.5 | 2.7 | 0.4 |
| 20F-4-D11-IGFR | VNYEMDRVPPMPWGGYWWLS | 5.0 | 1.0 | 0.5 | 2.3 | 0.4 |
| 20F-4-G4-IGFR | MGGGLWVGVHIWPGYSWLSQ | 3.9 | 0.9 | 0.5 | 1.8 | 0.6 |
| 20F-4-G12-IGFR | SDVWAQPQRRNDWPGYHWLS | 3.2 | 0.9 | 0.6 | 1.5 | 0.7 |

FIG. 4A-2

| Clone Design | Sequence XXXXXXXXXXXXXXXXXXXX | Ratios over Background E-Tag -- | IGFsR -- | IR -- | Comparisons IGFR/IR -- | IR/IGFR -- |
|---|---|---|---|---|---|---|
| R20β-4-A4-IR | WPGYLFFEEALQDWRGSTED | 11.9 | 17.5 | 1.4 | 12.5 | 0.1 |
| R20β-4-F2-IR | SMFVAGSDRWPGYGVLADWL | 16.4 | 13.9 | 3.1 | 4.5 | 0.2 |
| R20β-4-E8-IR | VRGFQGGTVWPGYEWLRNAA | 41.0 | 34.9 | 3.6 | 9.7 | 0.1 |

FIG. 4B-1

| Clone Design | Sequence XXXXXXXXXXXXXXXXXXXX | Ratios over Background E-Tag -- | IGFsR -- | IR -- | Comparisons IGFR/IR -- | IR/IGFR -- |
|---|---|---|---|---|---|---|
| 20F-4-H10-IR | LDLASGDSWLGYDVLRGWLS | 10.2 | 3.1 | 2.4 | 1.3 | 0.8 |
| 20F-4-C10-IR | IHSSDGIGAWGGYAWFRDVA | 23.4 | 9.6 | 4.1 | 2.3 | 0.4 |

FIG. 4B-2

| Clone Design | Sequence XXXXXXXXXXXXXXXXXXXX | Ratios over Background E-Tag -- | IGFsR -- | IR -- | Comparisons IGFR/IR -- | IR/IGFR -- |
|---|---|---|---|---|---|---|
| R20β-4-D10-IR | LGPLLRWGSEVCGVWPDLCE | 21.5 | 1.0 | 8.0 | 0.1 | 8.0 |
| R20β-4-D9b-IR | PFGFGGRWWGIPRMWWYRNS | 32.6 | 6.8 | 15.1 | 0.5 | 2.2 |
| R20β-4-H4-IR | WWWGGRNRWWLERWGLGGER | 11.6 | 1.7 | 3.6 | 0.5 | 2.1 |
| R20β-4-A2-IR | GRVALWGPVWPRWWFMSRPV | 17.1 | 2.6 | 5.2 | 0.5 | 2.0 |

FIG. 4C

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx | -- | -- | -- | -- | -- |
| R40-3-40A2-IR | RGTRTDRLWKSGGFAIVPRWPCFSYHCLVEWITKTGSPG | 44.6 | 1.5 | 2.7 | 0.6 | 1.8 |
| R40-4-40F10-IR | GRTSMAFVPPRHLQPELAPRPVRNHAWLVGGG | 46.4 | 1.9 | 2.1 | 0.9 | 1.1 |

FIG. 4D

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | xxxxxxxxxxxxxxxxxxxx | -- | -- | -- | -- | -- |
| R20-4-F10-IGFR | CLGAGSFRAGILCLGGLPVS | 35.5 | 6.0 | -- | -- | -- |
| R20-4-F7-IGFR | GFWATACGGLQICEELGLKP | 29.1 | 4.7 | -- | -- | -- |
| R20-4-H9-IGFR | DLFCAYMAQALGLGQDLSCG | 25.7 | 3.0 | -- | -- | -- |
| R20-3-A4-IGFR | RHLLLPQIWIAS*GGWGMG | 15.6 | 2.7 | -- | -- | -- |

FIG. 4E

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | XXXXCXXXXXXXXXXXXXXXXXXXX | -- | -- | -- | -- | -- |
| 20C-3-H3-IGFR | DHRLCGTDEYLMQDLFVRGLCRLIW | 28.5 | 26.6 | 1.0 | 26.6 | <0.1 |
| 20C-3-F4-IGFR | GLLFCKQLFTLAGLQPEAGCVSSSR | 34.4 | 27.5 | 1.2 | 23.1 | <0.1 |
| 20C-4-C10-IGFR | IWIACLDELLRGQVWSSCRRRAPIG | 35.5 | 24.4 | 1.3 | 19.2 | 0.1 |
| 20C-3-G5-IGFR | DWLRCLGVILSGGLTELANTGCVQG | 29.3 | 21.1 | 1.1 | 18.7 | 0.1 |
| 20C-3-A2-IGFR | WFSFCLGGLLQAQEWSVWGRDVGCI | 33.9 | 18.3 | 1.1 | 16.9 | 0.1 |
| 20C-3-B4-IGFR | GYSWLRDVLMEKQAQLKREGSVGRQ | 39.8 | 29.1 | 1.9 | 15.2 | 0.1 |
| 20C-3-C6-IGFR | FLTRLLERLGLS*ERGEAGGPYAQA | 34.8 | 20.9 | 1.4 | 14.9 | 0.1 |
| 20C-3-E2-IGFR | FSGFCMGLERLSQVSLGYCGAGQGG | 34.8 | 28.1 | 2.0 | 14.2 | 0.1 |
| 20C-3-A3-IGFR | ISFRCQLFVLAGMHPCPVDVGGEGF | 33.7 | 14.3 | 1.2 | 12.4 | 0.1 |
| 20C-3-B1-IGFR | NTPNCSQDWGQESGFMALLLALTCK | 30.2 | 9.8 | 0.9 | 11.2 | 0.1 |
| 20C-3-F5-IGFR | LQGFCELLATVTGVTGLGCLDYQPI | 35.5 | 31.9 | 3.9 | 8.2 | 0.1 |
| 20C-4-A7-IGFR | GSSICNLLARAQIVELALCEMGVQE | 33.3 | 19.3 | 2.8 | 6.9 | 0.1 |
| 20C-4-F8-IGFR | LSFACLLSQLSGVVLPDCLLGED | 30.5 | 27.7 | 5.3 | 5.2 | 0.2 |
| 20C-4-G11-IGFR | GEHFCQLLMSLCGDDCGPVNCGGGS | 24.7 | 13.3 | 2.8 | 4.7 | 0.2 |
| 20C-3-E1-IGFR | GWFECLLASLVLQVPQGRSRASAVC | 34.0 | 5.1 | 1.6 | 3.1 | 0.3 |
| 20C-3-B6-IGFR | YRQECACSVGAVGFLCGLACLARSG | 37.3 | 32.8 | 13.7 | 2.4 | 0.4 |

FIG. 4F-1

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| Design | XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX | -- | -- | -- | -- | -- |
| 40F-4-D1-IGFR | LSCLAYSRHGIWRPSTDLGLGRSVGEGSVSTRWRGYDWFE | 4.9 | 4.6 | 0.3 | 13.1 | 0.1 |
| 40F-4-B1-IGFR | GLDHSDAVGVHLGFAWPA.ARGRWEAGGLEDTWAGYDWL | 4.1 | 3.0 | 0.2 | 13.1 | 0.1 |
| 40F-4-D10-IGFR | W.GYAWLS | 4.9 | 4.5 | 0.4 | 11.7 | 0.1 |
| 40F-3-A3-IGFR | LSCLAYSRHGIWRPSTDLGLGRSVGEGSVSTRWRGYDWFE | 2.6 | 2.0 | 0.3 | 7.9 | 0.1 |
| 40F-4-C4-IGFR | EAMAVGLQCPARFVRAAAHGDGGSWGQDHV.AWGGYWWLG | 3.8 | 2.0 | 0.5 | 4.1 | 0.2 |

FIG. 4F-2

| Clone Parental/Design | Sequence |
|---|---|
| **HLCVLEELFWGASLFGYCSG** | |
| F815-4-G11-IGFR | HFYVLVERLSGASLFGSGSA |
| F815-3-D1-IGFR | HRFVREGLLWGAYQFCYCSG |
| F815-4-C12-IGFR | FQSLLEELVWGAPLFRYGTG |
| F815-4-A11-IGFR | HLSVLEELSWGASLFGQWAG |

FIG. 4G

| Clone Parental/Design | Sequence |
|---|---|
| **HLSVLEELSWGASLFGQWAG** | |
| NNKH-4-A9-IR | NLCRLEELAWGASLFGQCAG |
| NNKH-4-H4-IR | APVSTEELRWGALLFGQWAG |
| NNKH-4-B3-IR | HLSVLEERWWRESLFGQWAG |
| NNKH-4-E1-IR | HLSVLEERWWRAALFGQWAG |
| NNKH-4-E7-IR | HLSILEEQWWRESLFGQWAG |
| NNKH-4-G3-IR | HMSVEELSWWASLFGKQAG |
| NNKH-4-B6-IR | HLSELEERWWRATLFGQWAG |
| NNKH-4-A10-IR | HLSVLEELWWRESLFGQWAG |
| NNKH-4-A5-IR | HLSLLEEQWWRESLFGQWAG |
| NNKH-4-F11-IR | HLSVLEERWWRETLFGQWAG |
| NNKH-4-C9-IR | HLSVLEEQWWRESLFGQWAG |
| NNKH-4-D12-IR | HLSVLEEQWW.ESLFGQWAG |
| NNKH-4-D10-IR | HLSVLEELWWREALFGQWAG |
| NNKH-4-E5-IR | HLSVLEERWWRATLFGEWAG |
| NNKH-2-A6-IR | HL.VLEELLWGVSLFRQWAG |
| NNKH-4-F6-IR | HLSALEEQWWRATLFGQWAG |
| NNKH-4-C7-IR | HLSVLEERWWRATLLESGQ |
| NNKH-4-F7-IR | HLSALEELWWRETLFGQWAG |
| NNKH-4-F8-IR | HLSVLEELWWRESLFGKWAG |
| NNKH-4-E9-IR | HLSVLEEAWWRESLFGHWAG |
| NNKH-4-E6-IR | HMSEQEELWWRATLFGQWAG |
| NNKH-4-B7-IR | HLSVLEERWWRETLFGEWAG |
| NNKH-2-B3-IR | HRSVLKQLSWGASLFGQWAG |

FIG. 4H

| Ratios over Background | | | Comparisons | |
|---|---|---|---|---|
| E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| **39.1** | **1.8** | **27.7** | **0.1** | **15.4** |
| 34.6 | 7.9 | 1.0 | 7.9 | 0.1 |
| 14.9 | 1.0 | 2.0 | 0.5 | 2.0 |
| 35.2 | 1.0 | 2.0 | 0.5 | 2.0 |
| 5.4 | 1.0 | 2.1 | 0.5 | 2.1 |

| Ratios over Background | | | Comparisons | |
|---|---|---|---|---|
| E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| **5.4** | **1.0** | **2.1** | **0.5** | **2.1** |
| 16.3 | 1.0 | 2.7 | 0.4 | 2.6 |
| 15.6 | 1.0 | 2.6 | 0.4 | 2.5 |
| 13.6 | 2.8 | 6.7 | 0.4 | 2.3 |
| 13.9 | 4.8 | 9.5 | 0.5 | 2.0 |
| 16.9 | 1.3 | 2.3 | 0.6 | 1.8 |
| 11.3 | 1.3 | 2.3 | 0.6 | 1.7 |
| 13.2 | 1.3 | 2.1 | 0.6 | 1.7 |
| 15.4 | 2.0 | 3.2 | 0.6 | 1.6 |
| 14.6 | 4.6 | 6.9 | 0.7 | 1.5 |
| 14.0 | 3.1 | 3.9 | 0.8 | 1.3 |
| 14.3 | 2.3 | 2.9 | 0.8 | 1.3 |
| 12.0 | 1.4 | 1.7 | 0.8 | 1.2 |
| 13.6 | 1.2 | 1.5 | 0.8 | 1.2 |
| 14.5 | 1.4 | 1.6 | 0.9 | 1.1 |
| 8.4 | 1.4 | 1.5 | 1.0 | 1.1 |
| 14.1 | 2.8 | 2.9 | 1.0 | 1.0 |
| 14.7 | 1.4 | 1.4 | 1.0 | 1.0 |
| 14.1 | 7.5 | 7.0 | 1.1 | 0.9 |
| 13.6 | 11.0 | 8.6 | 1.3 | 0.8 |
| 15.5 | 7.9 | 6.0 | 1.3 | 0.8 |
| 18.2 | 3.8 | 2.7 | 1.4 | 0.7 |
| 16.5 | 12.9 | 8.2 | 1.7 | 0.6 |
| 11.5 | 5.3 | 0.7 | 7.4 | 0.1 |

| Clone | Sequence | Ratios over Background | | | Comparisons | |
|---|---|---|---|---|---|---|
| | | E-Tag | IGFsR | IR | IGFR/IR | IR/IGFR |
| **Parental/Design** | **HLSVLEELSWGASLFGQWAG** | **5.4** | **1.0** | **2.1** | **0.5** | **2.1** |
| NNKH-2-C5-IGFR | HL*VLEELSWGASLVGQWAV | 7.3 | 0.9 | 0.7 | 1.3 | 0.8 |
| NNKH-2-D9-IGFR | HLSVLEEL*LGASMFGLWAG | 4.1 | 0.5 | 0.4 | 1.3 | 0.8 |
| NNKH-2-H12-IGFR | HLSVLKELSW*ASLFGQWAG | 5.0 | 1.3 | 1.1 | 1.2 | 0.8 |
| NNKH-2-D10-IGFR | HLSALEELSWGASLFGQWAG | 4.8 | 2.1 | 1.9 | 1.1 | 0.9 |
| NNKH-2-G9-IGFR | HLSVLAELS*GALLFGQWAG | 1.9 | 1.4 | 1.3 | 1.1 | 0.9 |
| NNKH-2-C6-IGFR | RLSVLEQLSWGASLFGPWAG | 18.2 | 1.0 | 0.9 | 1.1 | 0.9 |
| NNKH-2-C7-IGFR | HL*VLVQPSWGASLFGQWAG | 21.8 | 1.3 | 1.3 | 1.0 | 1.0 |
| NNKH-2-F11-IGFR | HQSVLEELSR*ASLFGQWAG | 6.7 | 1.3 | 1.4 | 0.9 | 1.1 |
| NNKH-2-H3-IGFR | DMSVLGGLSWGA*LFGQWSG | 4.7 | 0.7 | 0.8 | 0.9 | 1.1 |
| NNKH-2-B8-IGFR | HLSVREGQLWRASMFGRWAG | 17.5 | 3.7 | 5.2 | 0.7 | 1.4 |
| NNKH-2-B12-IGFR | QLSVLVEL*WGASLFGPWAA | 1.2 | 1.0 | 2.9 | 0.3 | 2.9 |
| NNKH-2-F9-IGFR | HLSVGEELSW*VALLGQWAR | 3.7 | 0.6 | 2.1 | 0.3 | 3.5 |

FIG. 4I

| D_Name | Clonal Name | Formula # | $K_d$(μM) HIR | $PO_4$ | Fat Cell Assay | Activity | $K_d$(μM) HIGFR | Ratio IGF/IR | Sequence |
|---|---|---|---|---|---|---|---|---|---|
| D101 | 20D3 | 1 | 0.51 | | | | 13 | 25 | KIGGQGQHQDGNFYDWFVEALAKK (ε-biotin) |
| | | | 0.27 | | | | 11 | 41 | |
| D102 | 20D1 | 1 | 1.2 | | | | 7.4 | 6.2 | KVLQARHGCDSVSDCFYEWFAKK (ε-biotin) |
| | | | 0.97 | | | | 16 | 16 | |
| D103 | B8 | 1 | 0.74 | | | | 15 | 20 | KWSALLSVMDTGFYAWFDDAVKK (ε-biotin) |
| D104 | E7 | 1 | 20 | | | | >20 | >1 | KGHSWALVRHVDRLFYEWFDLKK (ε-biotin) |
| D105 | H8 | 1 | 2.8 | | | | 12 | 4.3 | KRDKPTDQEEQNWSFYEWFRHKK (ε-biotin) |
| D106 | 20F1 | 1 | 0.97 | | | | 6.2 | 6.4 | KVFWNCRSQQLDFYEWWFEQAAKK (ε-biotin) |
| D107 | 40G11 | 1 | 1.1 | YES | | Antagonist | 9.7 | 8.8 | KLESHYVVPQAALDRLFYSWFSKK (ε-biotin) |
| D108 | 3G11 | 1 | 2.3 | | | Antagonist | 19 | 8.3 | KFYGWFSRQLSLTPRDDWGLPKK (ε-biotin) |
| D109 | 20H1 | 1 | 3.6 | | | Antagonist | 12 | 3.3 | KSAPGLVSNKQDGLFYSWFREKK (ε-biotin) |
| D110 | G3 | 1 | 0.84 | | | Antagonist | 1.4 | 1.7 | KRGGGTFYEWFESALRKHGAGKK (ε-biotin) |
| D111 | D2 | 1 | 0.62 | | | | 3.2 | 5.2 | KOPERMOSDVGFYEWFRAAVGKK (ε-biotin) |
| D112 | IGFR C1 A65-4-C1 | 1 | 0.49 | | | Neutral | 0.05* | 0.1 | DYKDCWARPCGDAANFYDWFVQQASKK (ε-biotin) |
| | | | 0.19 | | | | 0.02* | 0.1 | |
| D113 | IGFR H2 A65-4-1+2 | 1 | 0.75 | | -20 μ M | Agonist | 5.4 | 7.2 | DYKDVTFTSAVFHENFYDWFVRQVSKK (ε-biotin) |
| D114 | IGFR A6 | 1 | 8.1 | | | Neutral | >20 | >2.5 | SAKNFYDWFVKK (ε-biotin) |
| D115 | IGFR D5 | 1 | 8.1 | | | | >20 | >2.5 | ADKNFYDWFMAAKK (ε-biotin) |
| D116 | IGFR JBA5 | 9 | 4.4 cycli | | >20 μ M | Agonist | 8.1 | 1.8 | DYKDLCOSWGVRIGWLAGLCPKK (ε-biotin) |
| D117 | IGFR H2C | 1 | 0.70 | YES | -20 μ M | Agonist | 6.1 | 8.6 | FHENFYDWFVRQVSKK (ε-biotin) |
| | | | | | | | 5.1 | 8.5 | |
| D118 | 20E2 | 2 | 0.25 | YES | -20 μ M | Antagonist | 1.3 | 5.2 | DYKDFYDAIDQLVRGSARAGGTRDKK (ε-biotin) |
| D119 | 20C11 | 2 | 0.25 | YES | -20 μ M | Antagonist | 1.3 | 2.9 | KDRAFYNGLRDLVGAVYGAWDKK (ε-biotin) |
| | | | | | | | 2.5 | 0.8 | |
| D120 | E8 | 10 | 0.37 | | | Antagonist | 2.2 | 5.9 | KVRGFQGGTVWPGYEWLRNAAKK (ε-biotin) |
| D121 | F2 | 10 | 1.1 | | | Antagonist | 7.4 | 6.7 | KSMFVAGSDRWPGYGVLADWLKK (ε-biotin) |
| D122 | 20A4 (A7) | 6 | 1.2 | | | Antagonist | >20 | >17 | KEIEAEWGRVRCLVYGRCVGGKK (ε-biotin) |
| | | | 1.0 | | | | >20 | >20 | |
| D123 | D8 | 6 | 0.55 | | | Antagonist | 16 | 29 | KWLDQEWAWVQCEVYGRGCPSKK (ε-biotin) |
| | | | 1.3 | | | | >20 | >15 | |
| D124 | F8 | 4 | 0.04* | | | | 8.2 | 200 | KHLCVLEELFWGASLFGYCSGKK (ε-biotin) |
| | | | 0.09* | | | | >20 | >200 | |
| D125 | IGFR E4 | 1 | 2.6 | | | | >20 | >8 | DYKDERSAAGFRGNFYDWFVAQVNKK (ε-biotin) |
| D126 | IGFR D2C | 1 | 1.4 | | | | 18 | 13 | LGENFYDWFVMQVRKK |

FIG. 5A

| Clonal Name | D or S name | Motif | Sequence | IR-Kd | IR-IC 50 Biacore | IR-IC 50 FP-S175 | PO4 | Fat Cell Assay |
|---|---|---|---|---|---|---|---|---|
| 20-E2 | D118 | B6 | DYKDFYDAIDQLVRGSARAGGTRDK K-biotin | 250 nM | | 2.8 nM | + | ++ |
| C1 | D112 | A6 | DYKDCWARPCGDAANFYDWFVDQAS KK-biotin | 490 nM | | | - | 0 |
| D8 | D123 | C-C LOOP | KWLDQEWAWVQCEVYGRGCPSKK | 550 nM | | | 0 | - |
| E8 | D120 | GROUP 6 | KRGFQGGTVWPGYEWLRNA | 370 nM | | | - | - |
| F8 | D124 | C-C LOOP | KHLCVLEELFWGASLFGYCSGKK | 40 nM | | | - | 0 |
| H2C | D117 | A6 | FHENFYDWFVRQQVSKK | 700 nM | >5 μM | 5 nM | ++ | ++ |
| KCF9 | | | RLYYEWFWGQLEAQGRGGLS | | | | | |
| KC-G2 | | C-C-C | GLEQGCPWVGLEVQCRGCPS | | | | | |
| KCG7 | | B6 | FYCGLEELSWGAALFGYCSG | | >1 μM | | | |
| NG-C2 | | B6 | GNGDGMFYQLLSLLVGRDMH | | | | | |
| NG-G33 | | A6 | GHSQSCPESFYDWFAGQVSDPWWCW | | 2-4 μM | 4.2 nM | +++ | |
| NG-G8 | | B6 | VEGRGLFYDLLRQLLARRQNG | | >5 μM | | - | |
| NG-G9 | | B6 | RAMSFYDALVVLGLGPKK-Biotin | | | | - | |
| RP-1 | | A6 | GSRPVFHEQFYLWFVDQLGL | | 1 μM | | + | |
| RP-2 | | A6 | RSEASFHVEFYSWFEEQLRS | | 1 μM | | + | |
| RP-3 | | A6 | GREYGWFQDAIDQLMPWGFD | | >10 μM | | - | |
| RP-4 | | B6 | PPWGARFYDAIEQLVFDNL | | 5 μM | | + | |
| RP-5 | | B6 | AGVNAGFYRYFSTLLDWWDQGKK-Biotin | | 6 μM | | - | |
| RP-6 | | B6+ C-C | TFYSCLASLLTGTPQPNRGPWERCRKK-Biotin | | | | +++++ | |
| RP-7 | | A6 | AAVHEQFYDWFADQYKK | | | | | |
| RP-8 | | B6 | QSFYDYIEELLGGEWKK | | >5 μM | | + | |
| RP-8# | S287 | B6 | QSFYDYIEELLGGEWEE | | | | | |
| RP-9 | | A6 | GSLDESFYDWFERQLGKK | | | 2.9 nM | ++ | |
| RP-10 | | B6 | GSFYEALQRLVGGEQGKK | | >10 μM | | + | |
| RP-11 | | A6 | QAPSNFYDWFVREWDKK | | >10 μM | | + | |
| RP-12 | | B6 | DPFYQGLWEWLRESGKK | | | | | |
| RP-13 | | A6 | ASGFPENFYDWFGRQLSLKK | | >10 μM | | | |
| RP-14 | | A6 | SACQFDCHENFYDWFARQKK | | >10 μM | | | |
| RP-15 | | A6 | SQAGSAFYAWFDQVLRTVKK | | | | | |
| RP-16 | | B6 | VMDARDDPFYHKLSELVTKK | | | | | |
| RP-17 | | B6 | QSDAFYSGLWALIGLSDGKK | | >10 μM | | | |
| RP-18 | | B6 | LQPCSGFYDYFWQRLHLGSKK | | | | | |
| RP-19 | | A6 | LKDGFYDYFWQRLHLGSKK | | | | | |
| RP-20 | | B6 | GSASFYDAIDRLLRMRIKK | | | | | |
| RP-24 | | GROUP 6 | WPGYLFFEEALQDWRGSIED | | | | | |
| S167 | S167 | A6 | AFYDWFAKK | >20 μM | No Binding | | | |
| S173 | S173 | RB6 | LDALDRLMRYFEERPSL | 1.2 μM | | | | 0 |
| S174 | S174 | RB6 | PLAELWAYFEHSEQGRSSAH | 16 μM | | | | 0 |
| S175 | S175 | A6 | GRVDWLQRNANFYDWFVAELG | 230 nM | 2-4 μM | 0.9 nM | ++ | +++ |
| S176 | S176 | A6 | NGVERAGTGDNFYDWFVAQLH | 470 nM | | | | |

FIG. 5B

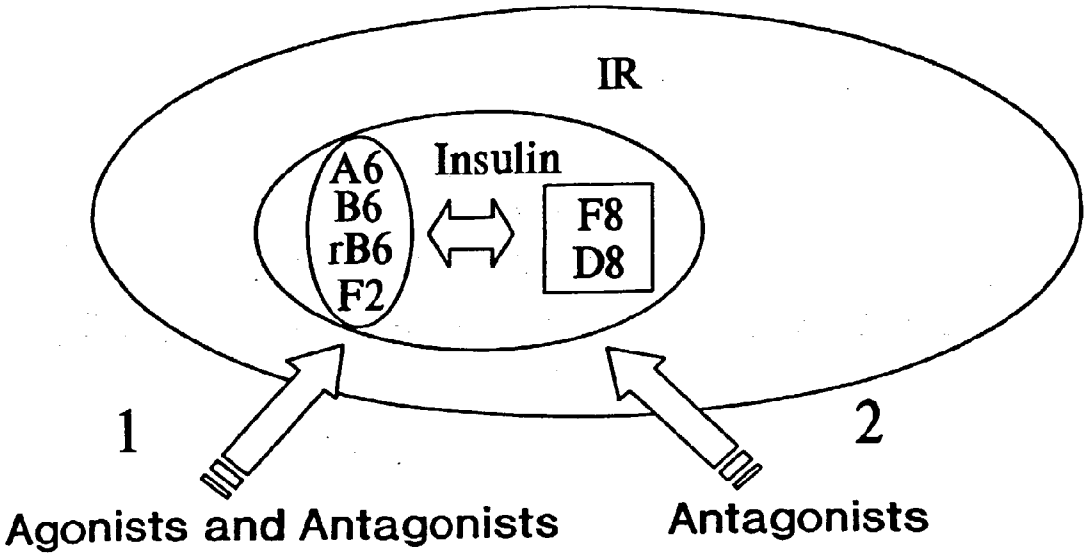

IR
Insulin
A6
B6
rB6
F2
F8
D8
1
2
Agonists and Antagonists
Antagonists

FIG. 6

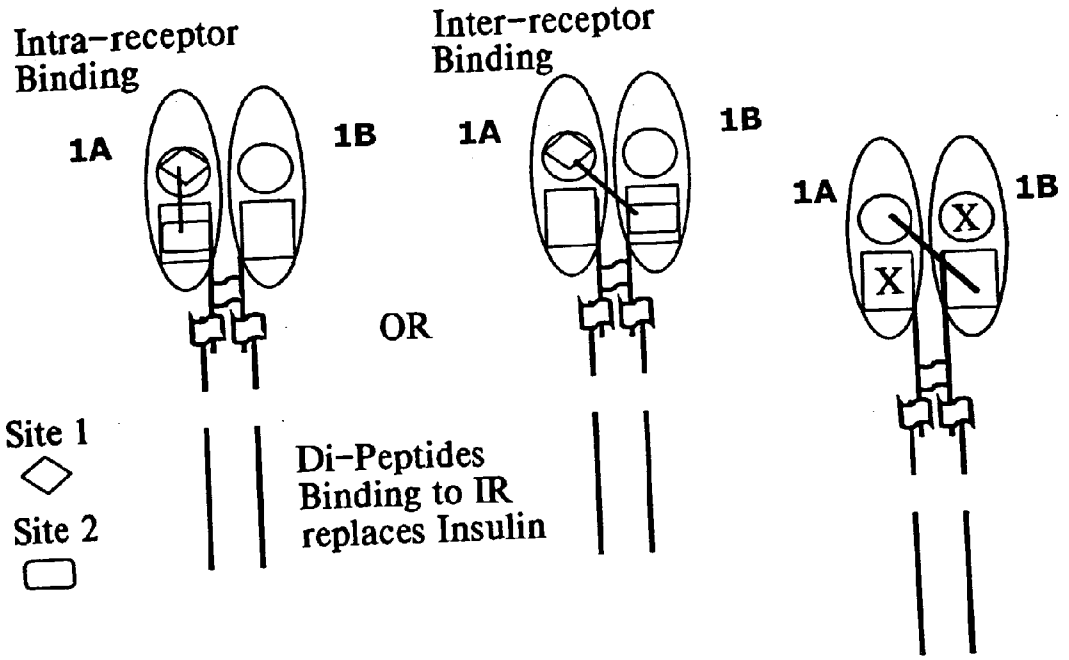

Intra-receptor
Binding
1A
1B
Inter-receptor
Binding
1A
1B
OR
1A
1B
X
X
Site 1
Site 2
Di-Peptides
Binding to IR
replaces Insulin

FIG. 7

**Group 1 (Formula 1 Motif)**

| | | Target | | |
|---|---|---|---|---|
| | | Found | IR | IGF |
| 20D3* | IGGQGQHQDGNFYDWFVEALA | 18 | + | ++++ |
| 20F1 | VFWNCRSQQLDFYEWFEQAA | 16 | + | ++++ |
| G3 | RGGGTFYEWFESALRKHGAG | 8 | + | +++ |
| 20H1 | RVAGAISAPGLVSNKQDGLFYSWFRE | 5 | + | +++ |
| 20D1* | VLQARHGCDSVSDCFYEWFA | 4 | + | ++++ |
| D2 | DPERMQSDVGFYEWFRAAVG | 3 | + | +++ |
| B8 | WSALLSVMDTGFYAWFDDAV | 2 | ++ | ++++ |
| C4 | DIGSDGHGRRWDSFYRWFEM | 2 | + | +++ |
| A8 | IGGSFVEFYGWFNDQV | 2 | + | +++ |
| E7 | GHSWALVRHVDRLFYEWFDL | 1 | ++ | +++ |
| C8 | LPAGGAQGFAVRGFYEWFES | 1 | + | +++ |
| H8 | RDKPTDQEEQNWSFYEWFRH | 1 | + | +++ |
| E2 | SRDQTNFTFNSAGFYGWFER | 1 | + | +++ |
| B12 | GAFYRWFHEALVGSERVPDV | 1 | + | +++ |
| D10-2 | RIGGGWARSEGFYEWFVREL | 1 | + | ++ |
| G8 | RMFYEWFWSQMGAGPTEGSA | 1 | + | ++ |
| H3 | HEAFYDWFSALVDGGYELMG | 1 | + | ++ |
| 3G11 | FYGWFSRQLSLTPRDDWGLP | 1 | + | ++ |
| F4 | GVGTLTMSSDAFYTWFV | 1 | + | ++ |
| E7-2 | LGTSAGQGVGHRAFYQWFQS | 1 | + | + |
| 40G11 | <--ETLESHYVVPQ----------AALDRLFYSWFS | 3 | + | ++++ |
| 40B2 | IRDMHYVWVQDRDRYINGVRQWYISDRYNPGSAFYRWFID | 2 | + | ++ |
| 40B12 | RMGLQALAHYRKSA-----GPIFLSSGSVIKGSEGDPFYAWFRLQ | 1 | + | ++ |

FIG. 8

## Group 2: Formula 6 Motif

| | | Target | | |
|---|---|---|---|---|
| | | Found | IR | IGF |
| **20A4*** | EIEAEWGRVRCLVYGRCVGG | 13 | +++ | 0 |
| **D8** | WLDQEWAWVQCEVYGRGCPS | 3 | +++ | ? |

## Group 3: Formula 2 Motif

| | | Target | | |
|---|---|---|---|---|
| | | Found | IR | IGF |
| **20E2** | *DYKD*FYDAIDQLVRGSARAGGTRD | 1 | + | ++++ |
| **20C11** | *DYKD*DRAFYNGLRDLVGAVYGAWD | 1 | + | ++++ |
| **20A12** | *DYKD*RLFYCGIQALGANLGYSGCV | 1 | + | ++++ |
| **C6** | *DYKD*FYSALWGLCGVTGCG | 1 | + | +++ |
| **A6** | RGQSDAFYSGLWALIGLSDG | 1 | + | +++ |
| **40H4** | RYFPFGGFYGNLDVLRWLRPYVASPRWGHWRPGGSLGKQPT | 1 | + | 0 |

## Group 5: Miscellaneous Motif 10

| | | Target | | |
|---|---|---|---|---|
| | | Found | IR | IGF |
| **D9-2** | PFGFGGRWWGIPRMWWYRNS | 1 | ++ | ++ |
| **H4** | WWWGGRNRWWLERWGLGGER | 1 | + | + |

FIG. 9A

## Group 4 and 6: Miscellaneous Motif 10

| | | Found | Target IR | Target IGF |
|---|---|---|---|---|
| **D10** | LGPLLRWGSEVCGVWPDLCE | 3 | ++ | 0 |
| **A2** | GRVALWGPVWPRWWFMSRPV | 1 | ++ | + |
| **F2** | SMFVAGSDRWPGYGVLADWL | 1 | ++ | ++ |
| **E8** | VRGFQGGTVWPGYEWLRNAA | 1 | ++ | ? |
| **A4** | WPGYLFFEEALQDWRGSTED | 1 | 0 | +++ |

## Group 7: Formula 4 Motif

| | | Found | Target IR | Target IGF |
|---|---|---|---|---|
| **B6** | ACSSFFVKGPEGFLQCLGSI | 1 | 0 | ++ |
| **F8** | HLCVLEELFWGASLFGYCSG | 4 | +++ | + |
| **40D6** | PERGRGLRTAMQLMRRPRDWHFPHSLFWGAPPPLSG | 1 | 0 | 0 |

FIG. 9B

FIG. 10A

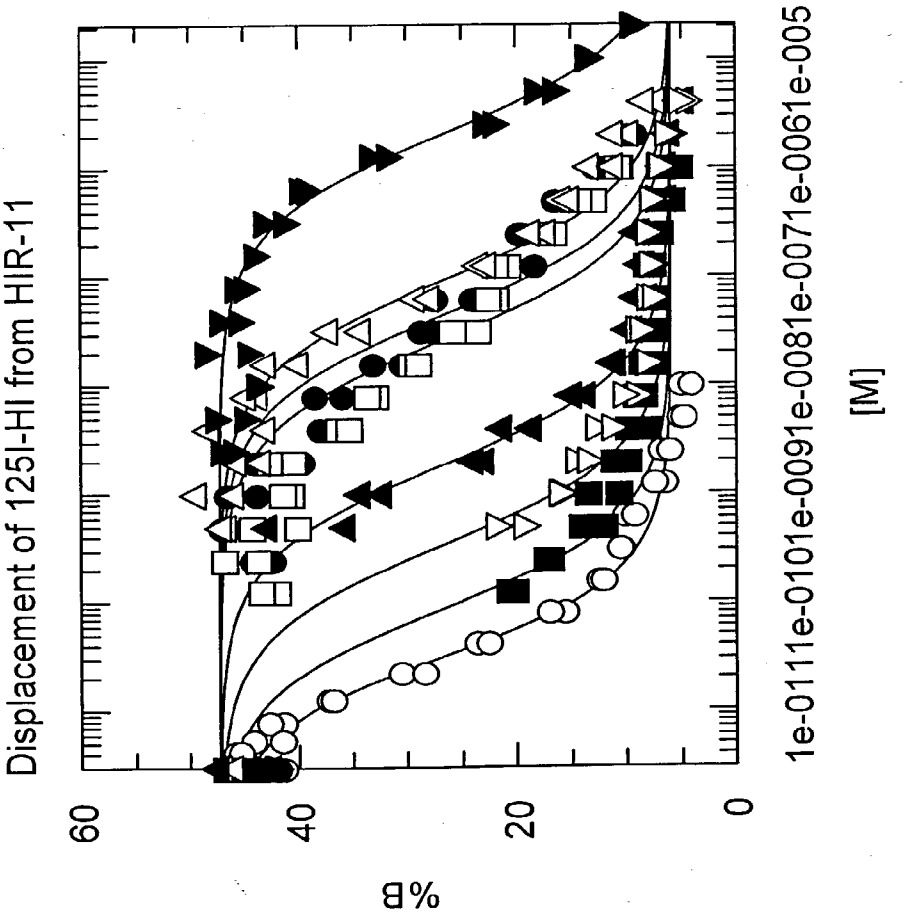



FIG. 10B

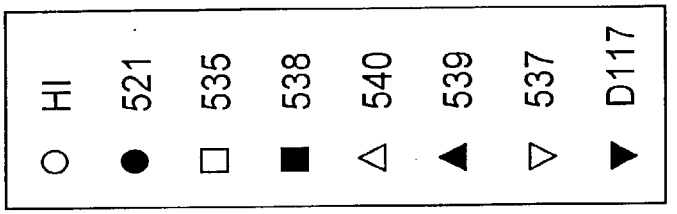



FIG. 10C

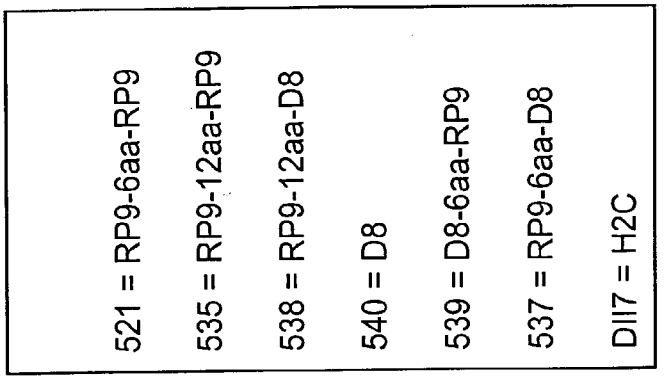


FIG. 11A

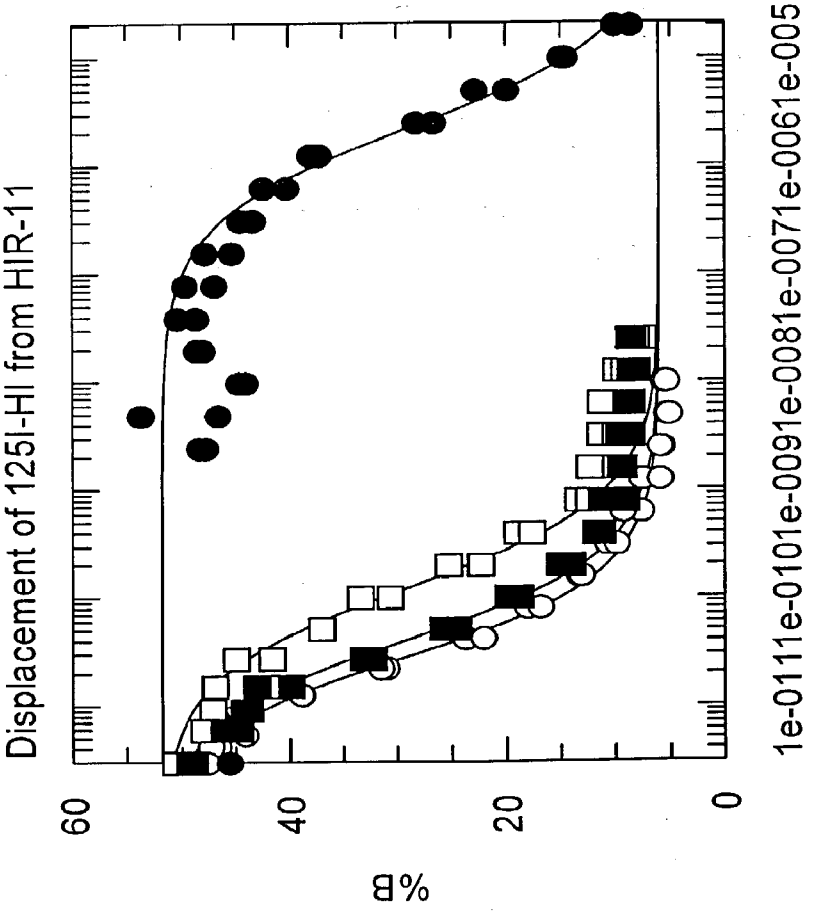



FIG. 11B

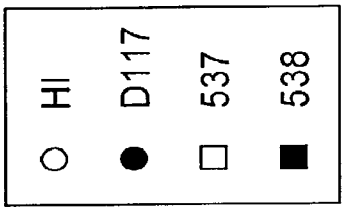
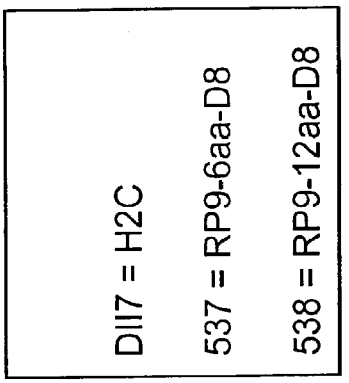



FIG. 11C



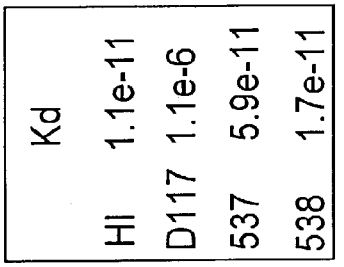




FIG. 11D

FIG. 12A

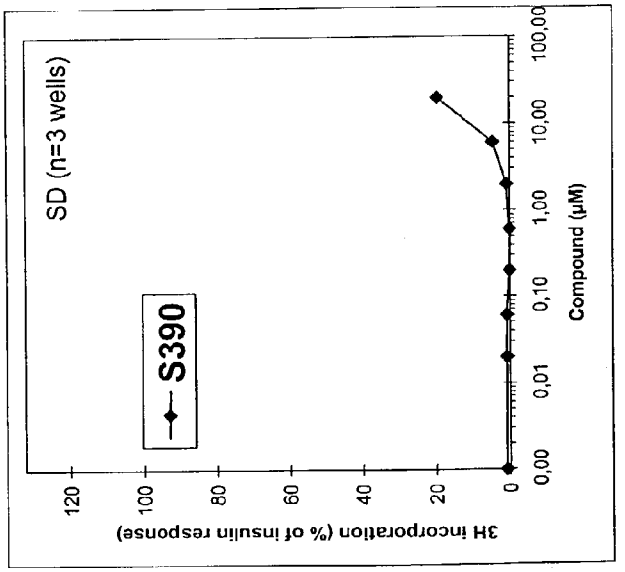
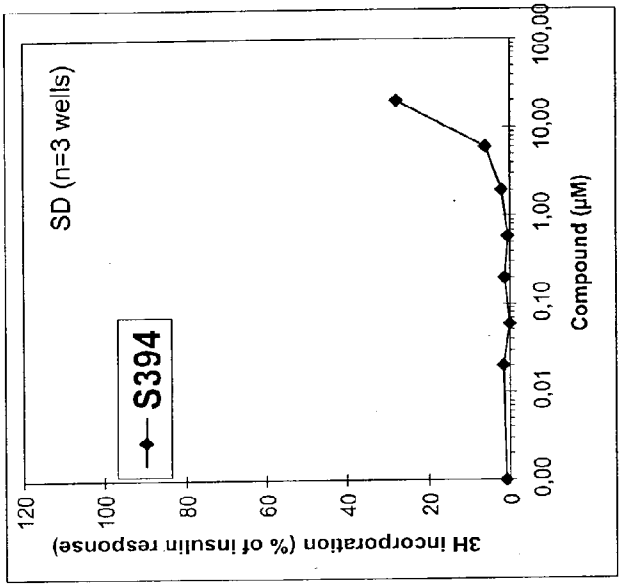



FIG. 12B




S390 = ESFYDWFERQLG
S394 = GSLDESFYDWFERQ

FIG. 12C

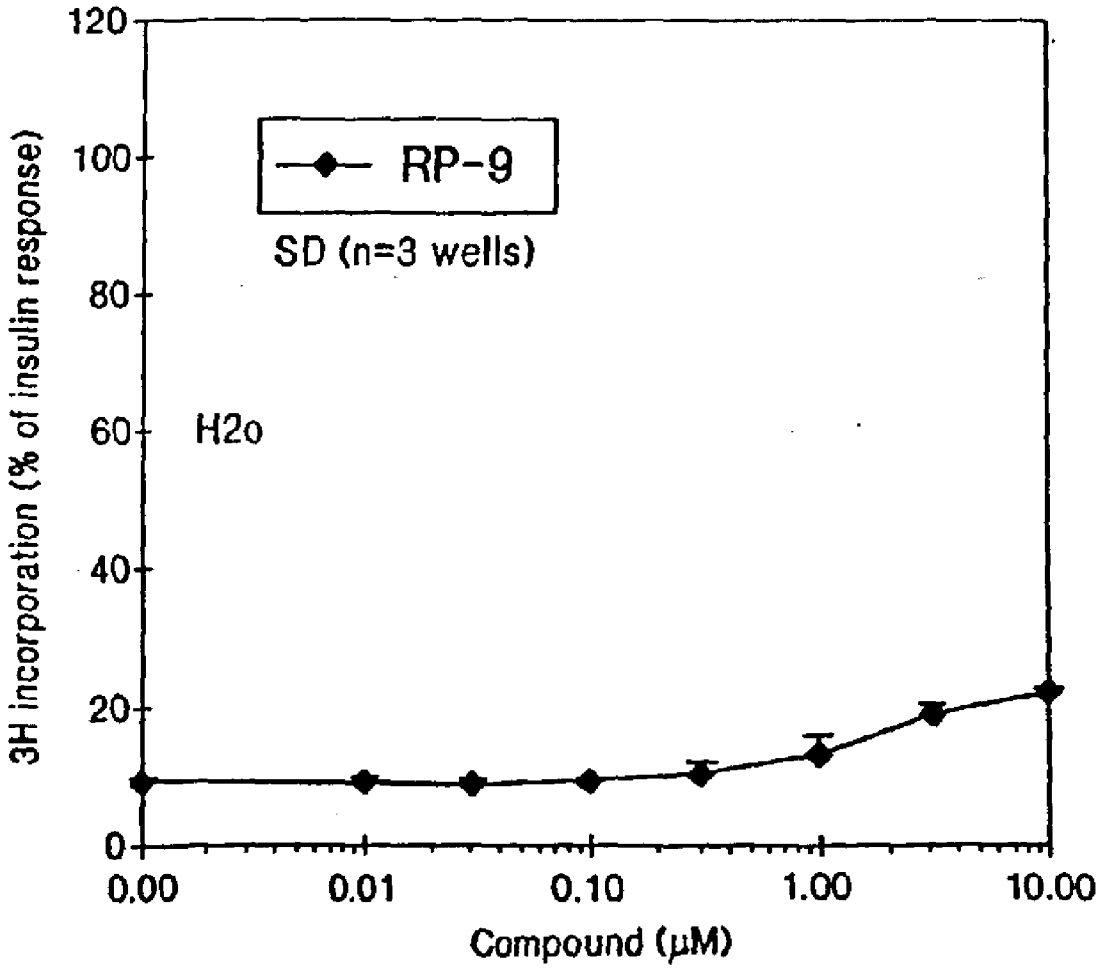

120
100
80
60
40
20
0
3H incorporation (% of insulin response)
RP-9
SD (n=3 wells)
H2o
0.00
0.01
0.10
1.00
10.00
Compound (μM)

FIG. 12D

FIG. 13A

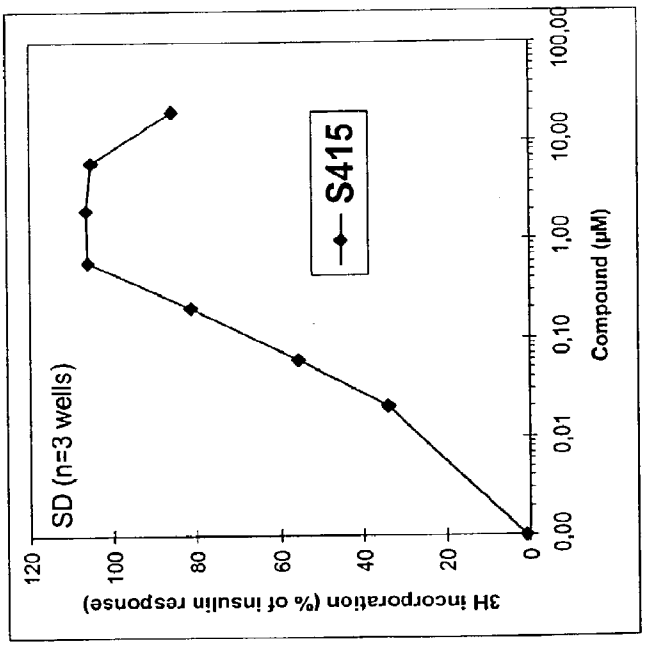
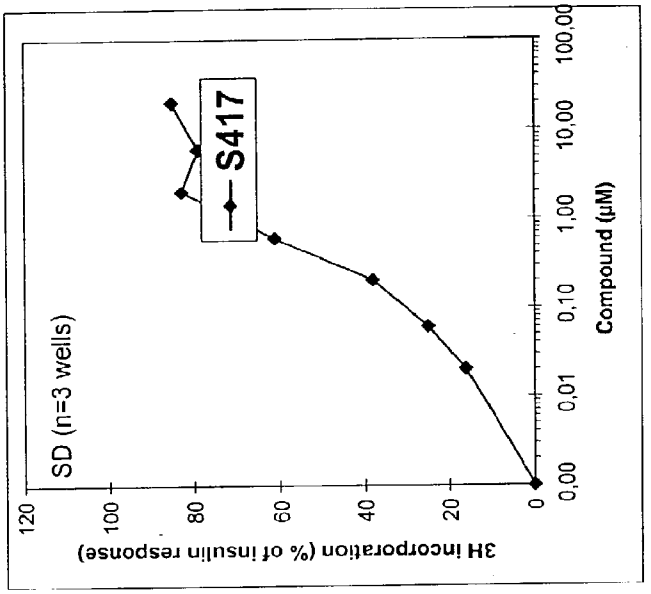



FIG. 13B




| | |
|---|---|
| S415 | $(ESFYDWFERQLGK)_2$-23 |
| S417 | 23-$(ESFYDWFERQLG)_2$ |

FIG. 13C

FIG. 14A

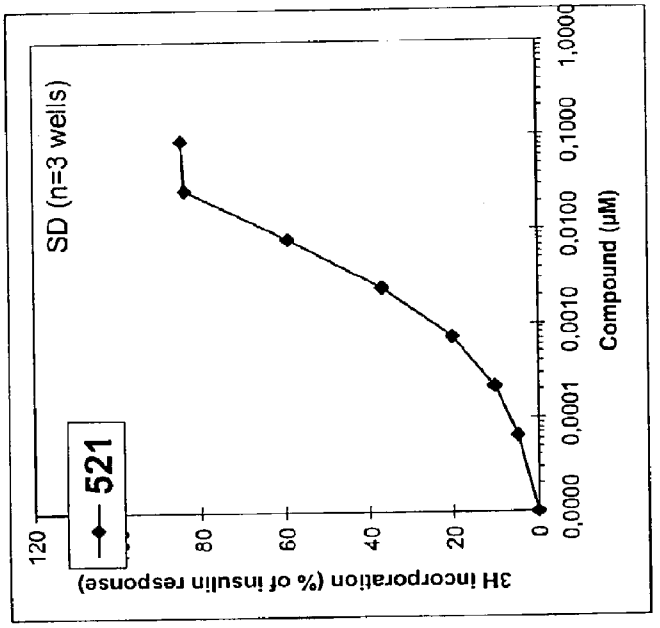
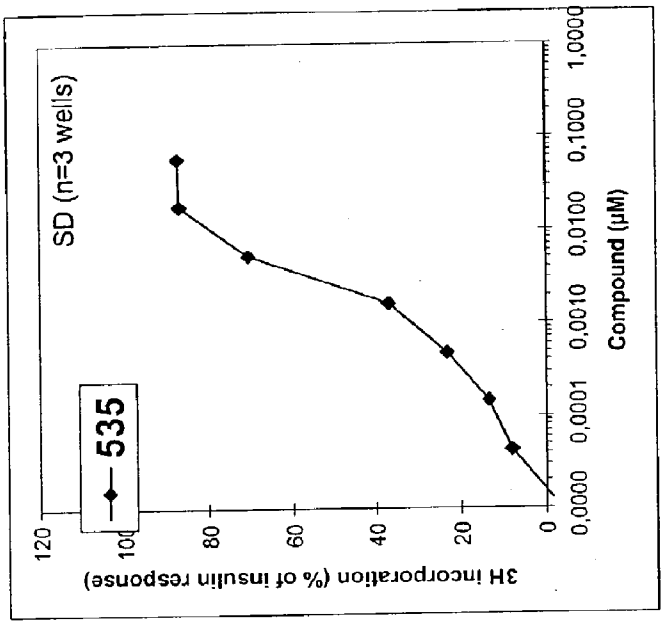



FIG. 14B




521 = RP9-6aa-RP9
535 = RP9-12aa-RP9

FIG. 14C

FIG. 15A

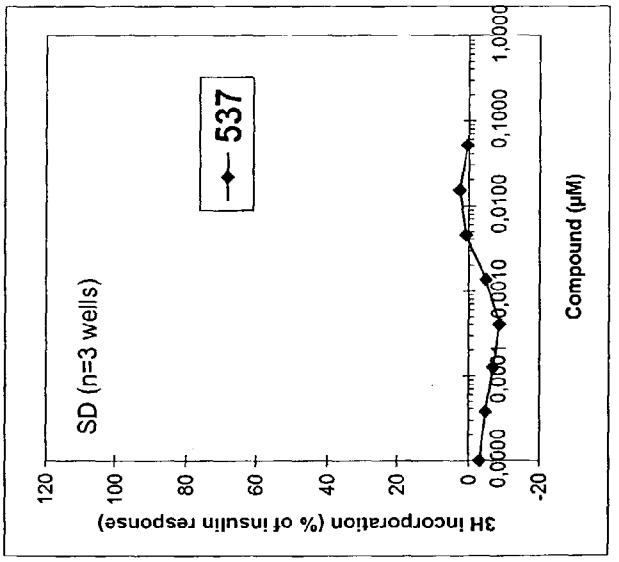
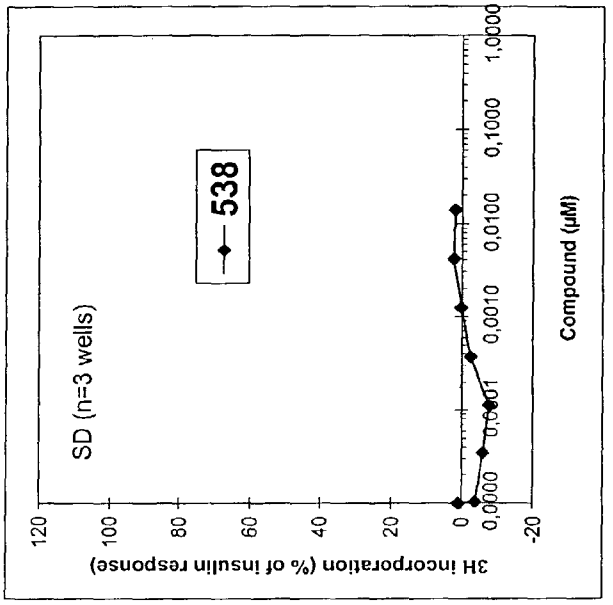



FIG. 15B




537 = RP9-6aa-D8
538 = RP9-12aa-D8

FIG. 15C

FIG. 16A

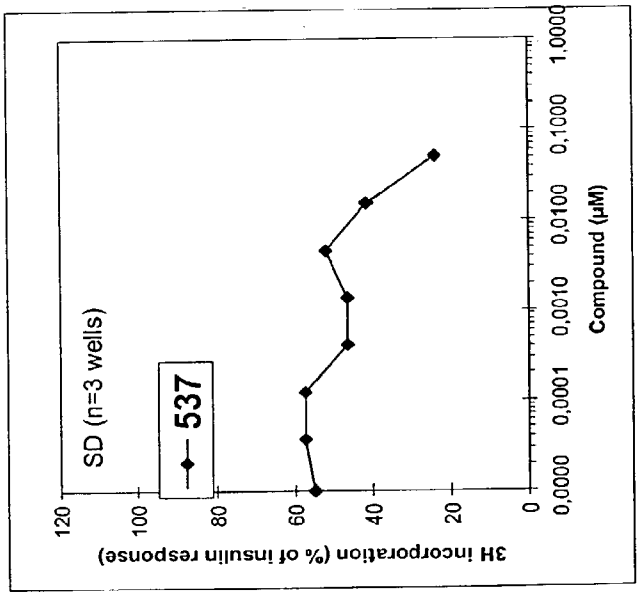
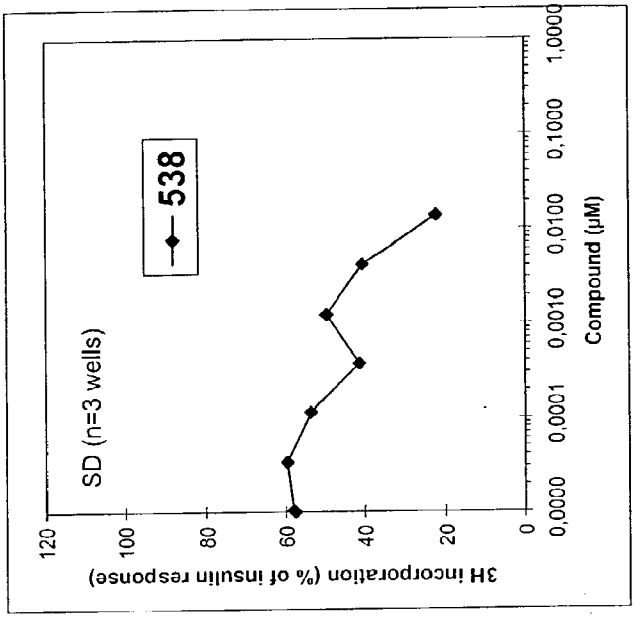


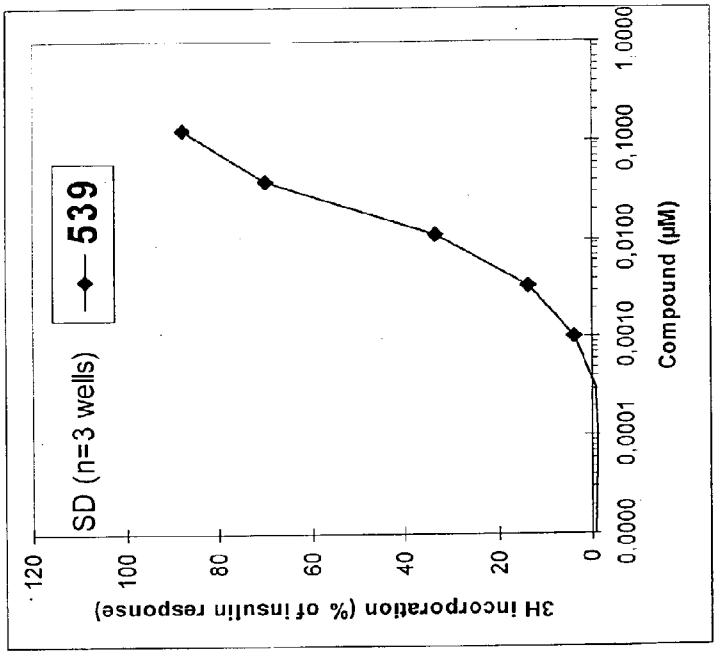

FIG. 16B




537 = RP9-6aa-D8
538 = RP9-12aa-D8

FIG. 16C

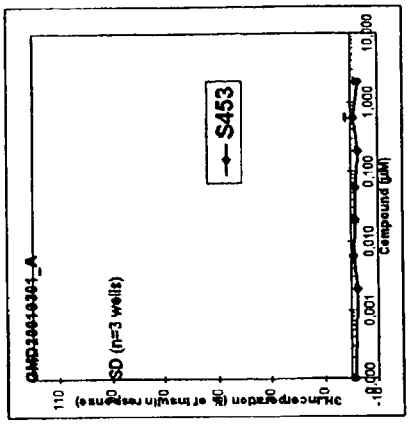
FIG. 17A




539 = D8-6aa-RP9

FIG. 17B

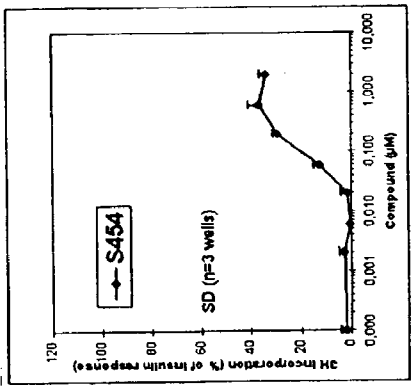
FIG. 18A




Site1 — Site2

C-N

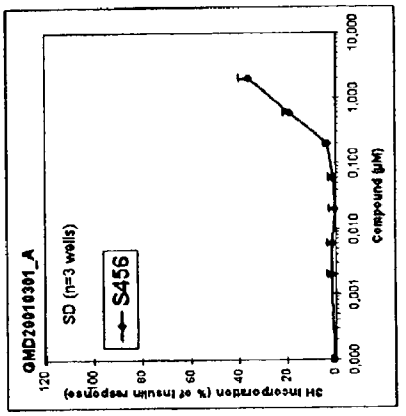
FIG. 18B




Site1 — Site2

N-N

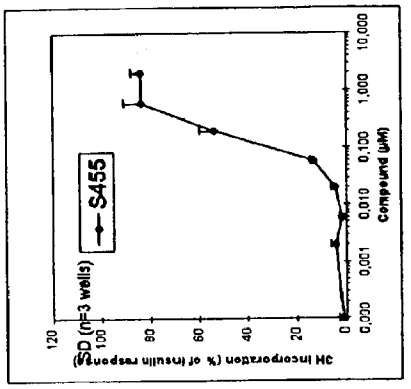
FIG. 18C




Site1 — Site2

C-C

FIG. 18D




Site1 — Site2

N-C

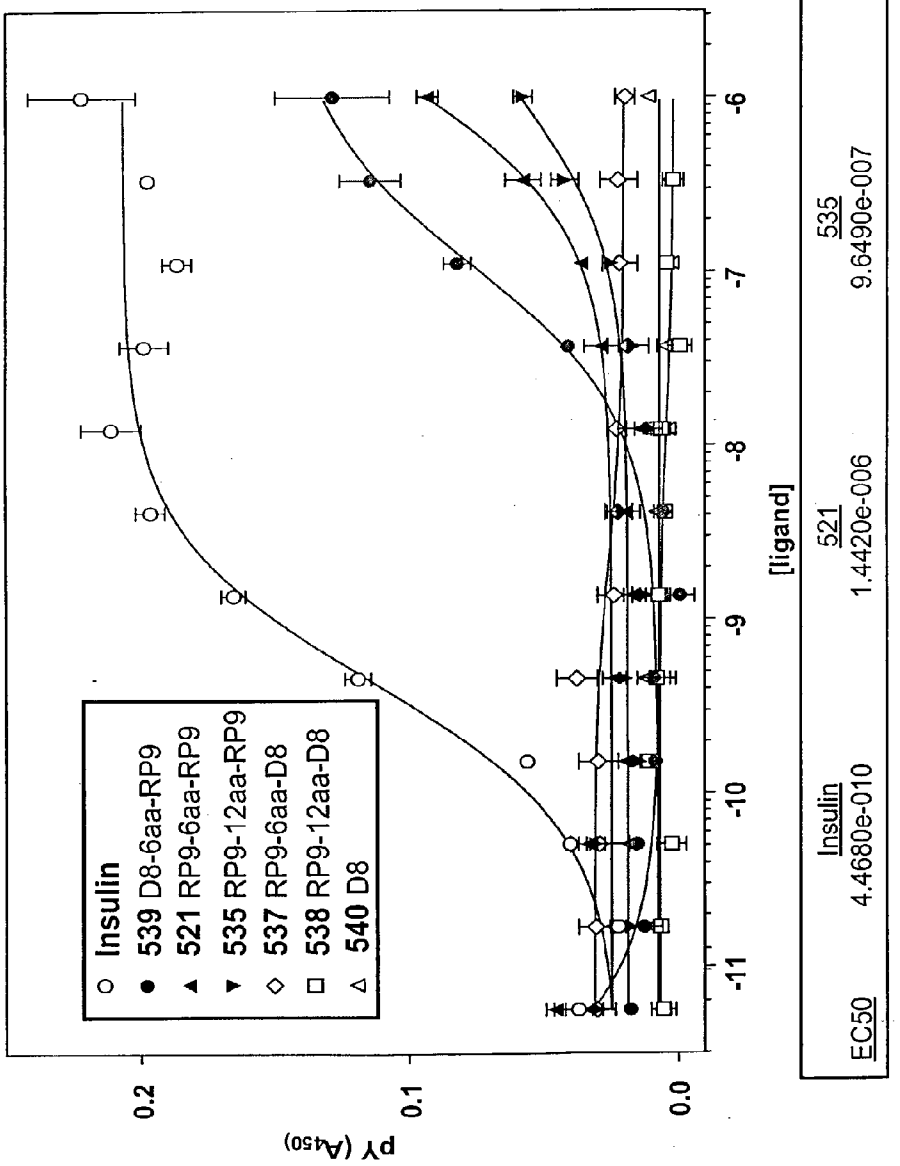
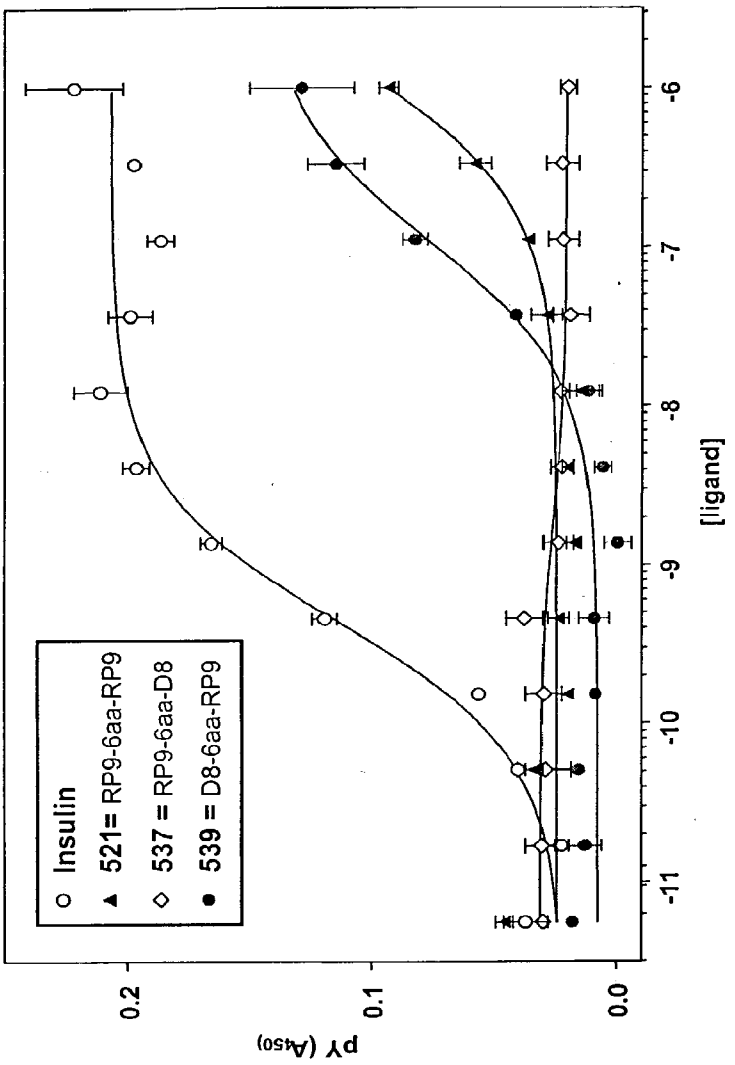



FIG. 19A

FIG. 19B

FIG. 20A




| | Insulin | 521 | 539 |
|---|---|---|---|
| EC50 | 4.4690e-010 | 1.4420e-006 | 1.1000e-007 |

FIG. 20B

FIG. 21A

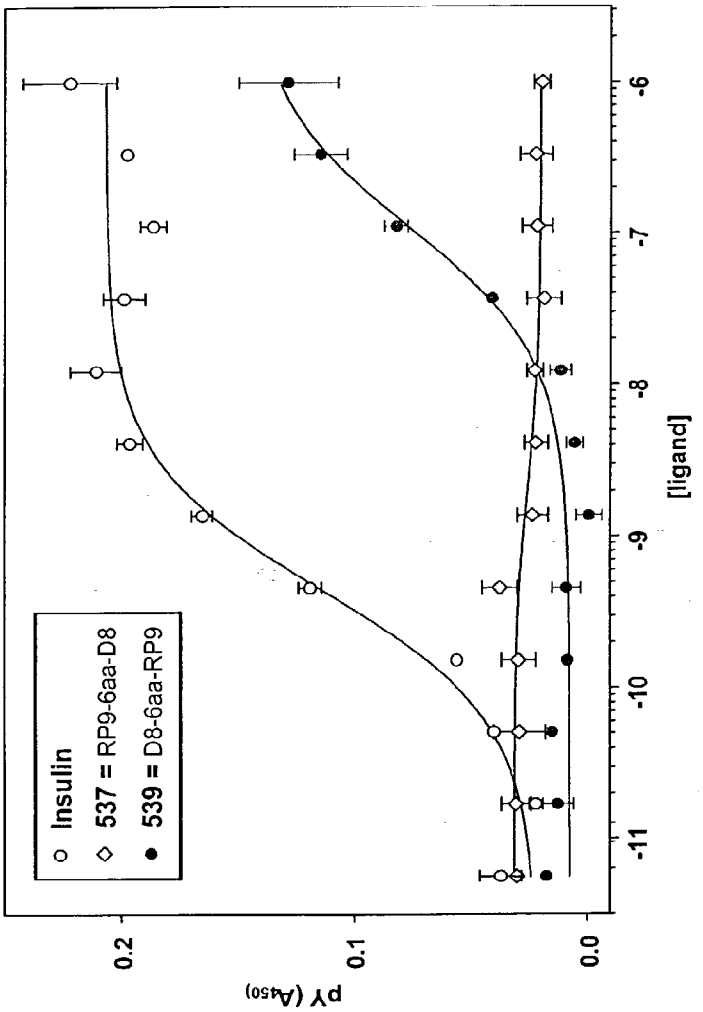
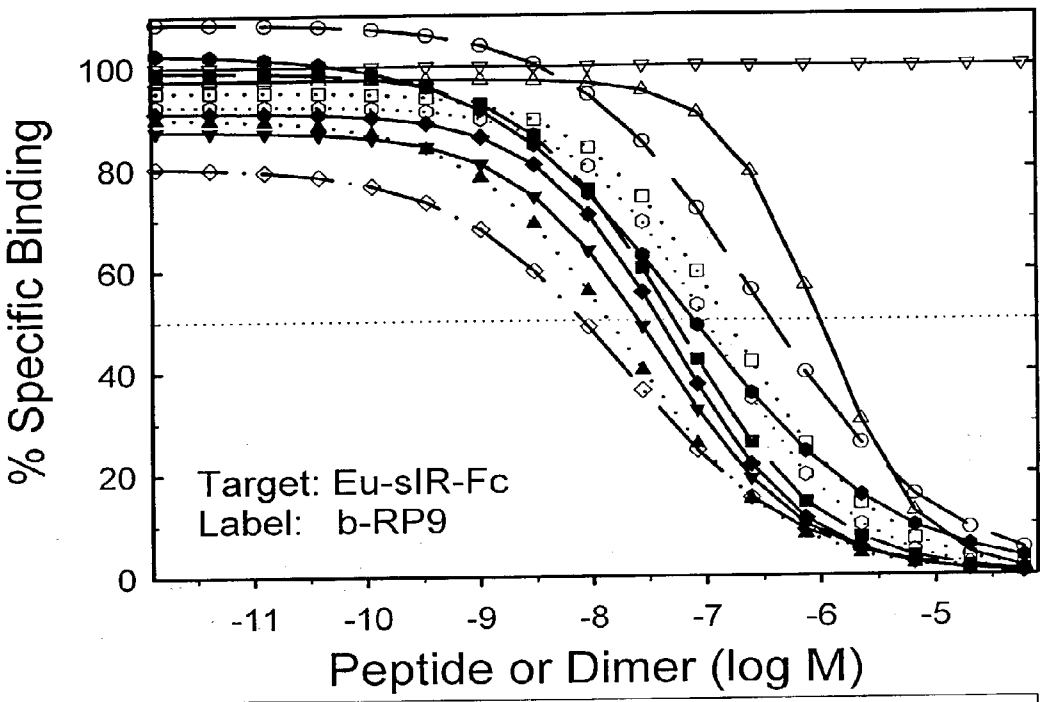



FIG. 21B

| | Insulin | 539 |
|---|---|---|
| EC50 | 4.4680e-010 | 1.1000e-007 |

FIG. 22A




| Symbol | Peptide or Dimer | Sequence |
|---|---|---|
| △ | H2C | FHENFYDWFVQRVSKK |
| □ | S291 | (Lig-GGG-H2C)$_2$-9 |
| ▼ | RP9 | GSLDESFYDWFERQLGKK |
| ■ | S375 | (RP9-Lig)-14-(RP9-Lig) |
| ◇ | S337 | (RP9-Lig)$_2$-23 |
| ○ | S391 | truncated-(-GSLDE)RP9(-KK) |
| ⬢ | S390 | truncated(-GSLD)RP9(-KK) |
| ⬡ | S414 | (truncated(-GSLD)RP9(-KK))$_2$-14 |
| ◆ | S175 | GRVDWLQRNANFYDWFVAELG |
| ▲ | S380 | (EE-short-S175-Lig)$_2$-9 |
| ▽ | Linker 23 | |

FIG. 22B

FIG. 23A

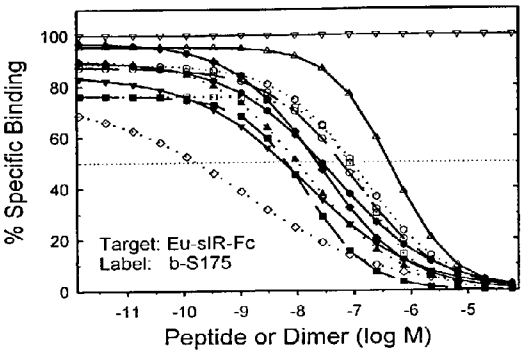
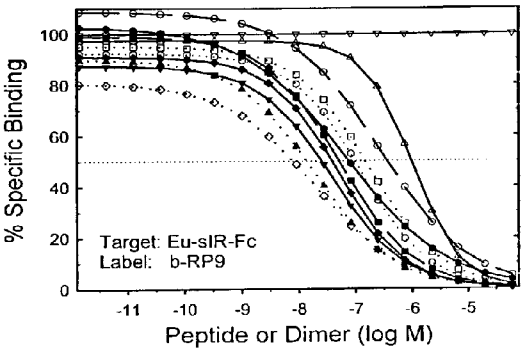


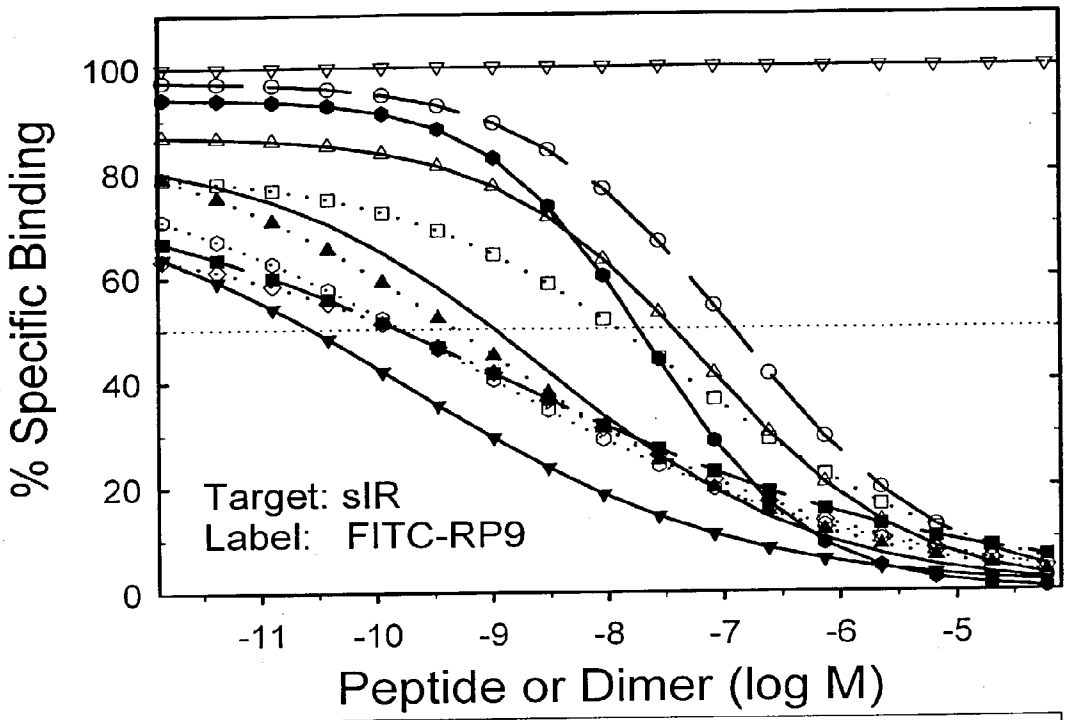

FIG. 23B




| Symbol | Peptide or Dimer | Sequence |
|---|---|---|
| △ | H2C | FHENFYDWFVQRVSKK |
| □ | S291 | (Lig-GGG-H2C)$_2$-9 |
| ▼ | RP9 | GSLDESFYDWFERQLGKK |
| ■ | S375 | (RP9-Lig)-14-(RP9-Lig) |
| ◇ | S337 | (RP9-Lig)$_2$-23 |
| ○ | S391 | truncated-(-GSLDE)RP9(-KK) |
| ⬢ | S390 | truncated(-GSLD)RP9(-KK) |
| ○ | S414 | (truncated(-GSLD)RP9(-KK))$_2$-14 |
| ◆ | S175 | GRVDWLQRNANFYDWFVAELG |
| ▲ | S380 | (EE-short-S175-Lig)$_2$-9 |
| ▽ | Linker 23 | |

FIG. 23C

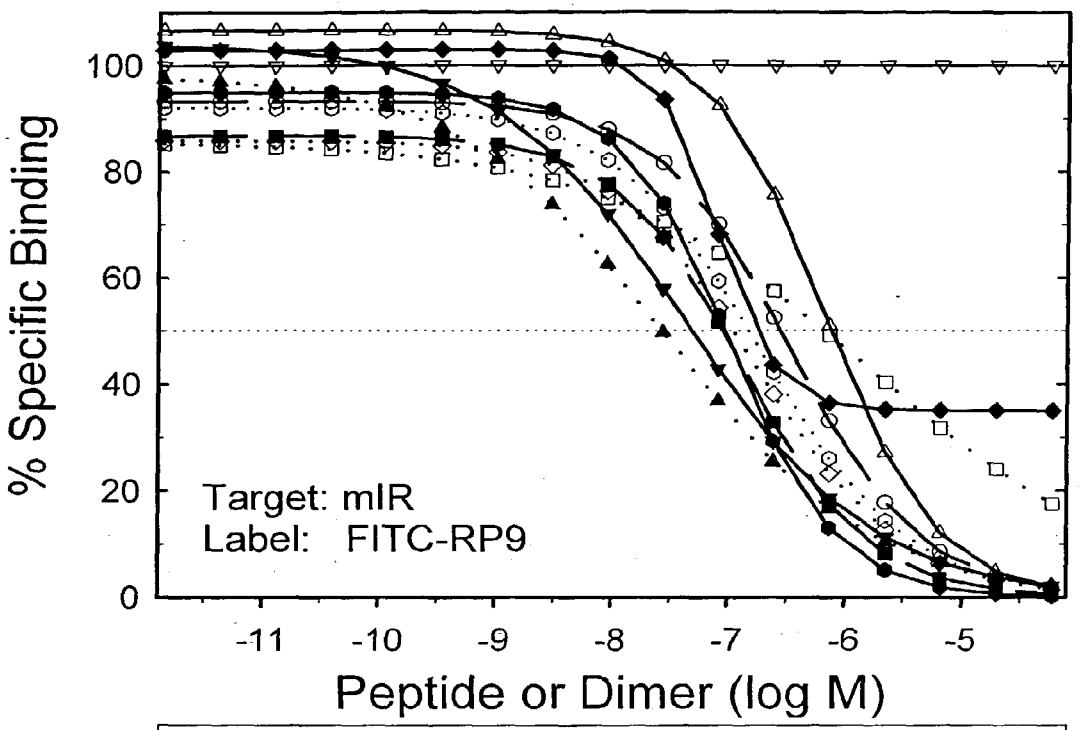
FIG. 24A




| Symbol | Peptide or Dimer | Sequence |
|---|---|---|
| △ | H2C | FHENFYDWFVQRVSKK |
| □ | S291 | (Lig-GGG-H2C)$_2$-9 |
| ▼ | RP9 | GSLDESFYDWFERQLGKK |
| ■ | S375 | (RP9-Lig)-14-(RP9-Lig) |
| ◇ | S337 | (RP9-Lig)$_2$-23 |
| ○ | S391 | truncated-(-GSLDE)RP9(-KK) |
| ⬢ | S390 | truncated(-GSLD)RP9(-KK) |
| ⬡ | S414 | (truncated(-GSLD)RP9(-KK))$_2$-14 |
| ◆ | S175 | GRVDWLQRNANFYDWFVAELG |
| ▲ | S380 | (EE-short-S175-Lig)$_2$-9 |
| ▽ | Linker 23 | |

FIG. 24B

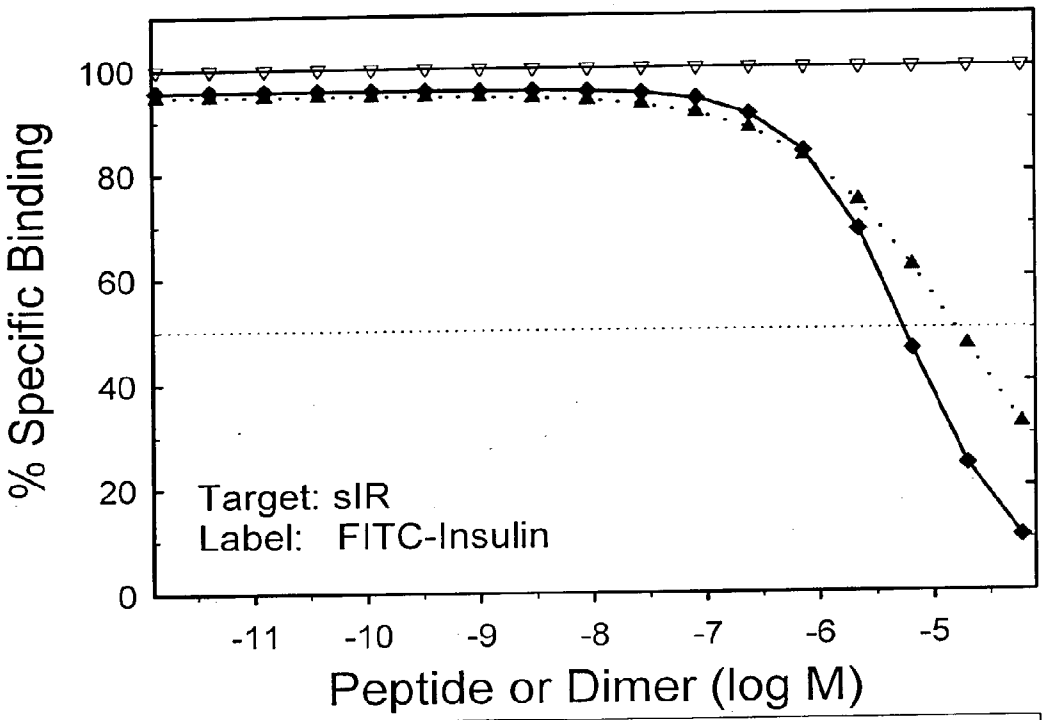
FIG. 25A




| Symbol | Peptide or Dimer | Sequence |
|---|---|---|
| △ | H2C | FHENFYDWFVQRVSKK |
| □ | S291 | (Lig-GGG-H2C)$_2$-9 |
| ▼ | RP9 | GSLDESFYDWFERQLGKK |
| ■ | S375 | (RP9-Lig)-14-(RP9-Lig) |
| ◇ | S337 | (RP9-Lig)$_2$-23 |
| ○ | S391 | truncated-(-GSLDE)RP9(-KK) |
| ⬢ | S390 | truncated(-GSLD)RP9(-KK) |
| ⬡ | S414 | (truncated(-GSLD)RP9(-KK))$_2$-14 |
| ◆ | S175 | GRVDWLQRNANFYDWFVAELG |
| ▲ | S380 | (EE-short-S175-Lig)$_2$-9 |
| ▽ | Linker 23 | |

FIG. 25B

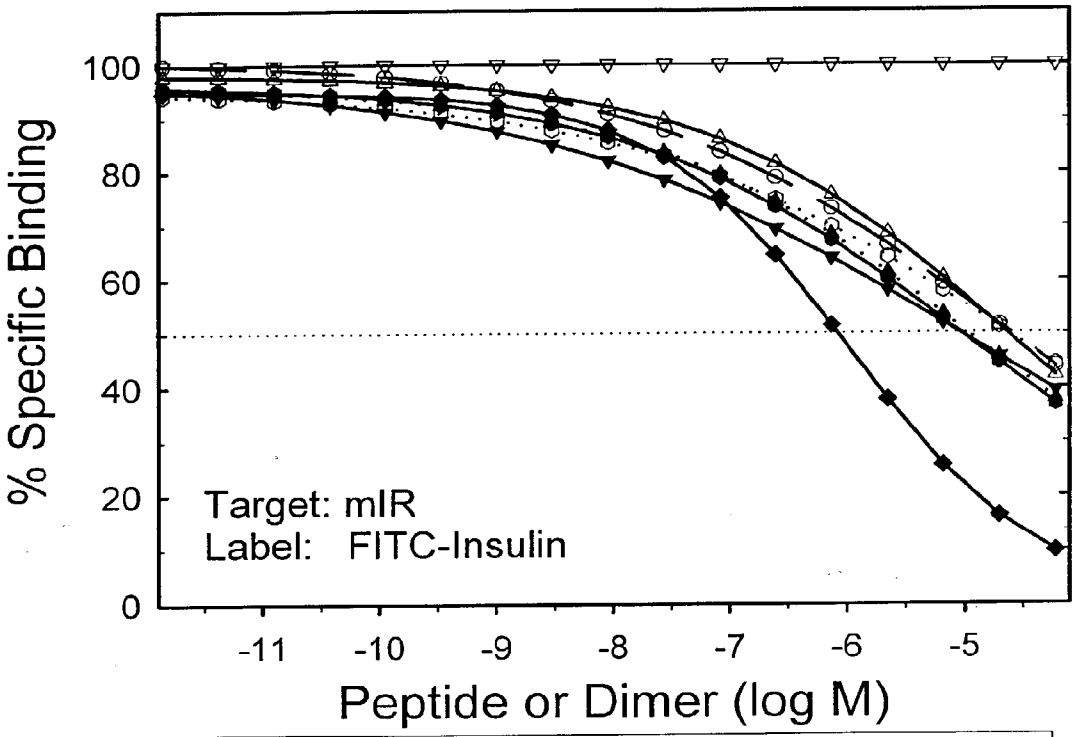
FIG. 26A




| Symbol | Peptide or Dimer | Sequence |
|---|---|---|
| △ | H2C | FHENFYDWFVQRVSKK |
| □ | S291 | (Lig-GGG-H2C)$_2$-9 |
| ▼ | RP9 | GSLDESFYDWFERQLGKK |
| ■ | S375 | (RP9-Lig)-14-(RP9-Lig) |
| ◇ | S337 | (RP9-Lig)$_2$-23 |
| ○ | S391 | truncated-(-GSLDE)RP9(-KK) |
| ● | S390 | truncated(-GSLD)RP9(-KK) |
| ⬡ | S414 | (truncated(-GSLD)RP9(-KK))$_2$-14 |
| ◆ | S175 | GRVDWLQRNANFYDWFVAELG |
| ▲ | S380 | (EE-short-S175-Lig)$_2$-9 |
| ▽ | Linker 23 | |

FIG. 26B

FIG. 27A




| Symbol | Peptide or Dimer | Sequence |
|---|---|---|
| △ | H2C | FHENFYDWFVQRVSKK |
| □ | S291 | (Lig-GGG-H2C)$_2$-9 |
| ▼ | RP9 | GSLDESFYDWFERQLGKK |
| ■ | S375 | (RP9-Lig)-14-(RP9-Lig) |
| ◇ | S337 | (RP9-Lig)$_2$-23 |
| ○ | S391 | truncated-(-GSLDE)RP9(-KK) |
| ⬟ | S390 | truncated(-GSLD)RP9(-KK) |
| ⬡ | S414 | (truncated(-GSLD)RP9(-KK))$_2$-14 |
| ◆ | S175 | GRVDWLQRNANFYDWFVAELG |
| ▲ | S380 | (EE-short-S175-Lig)$_2$-9 |
| ▽ | Linker 23 | |

FIG. 27B

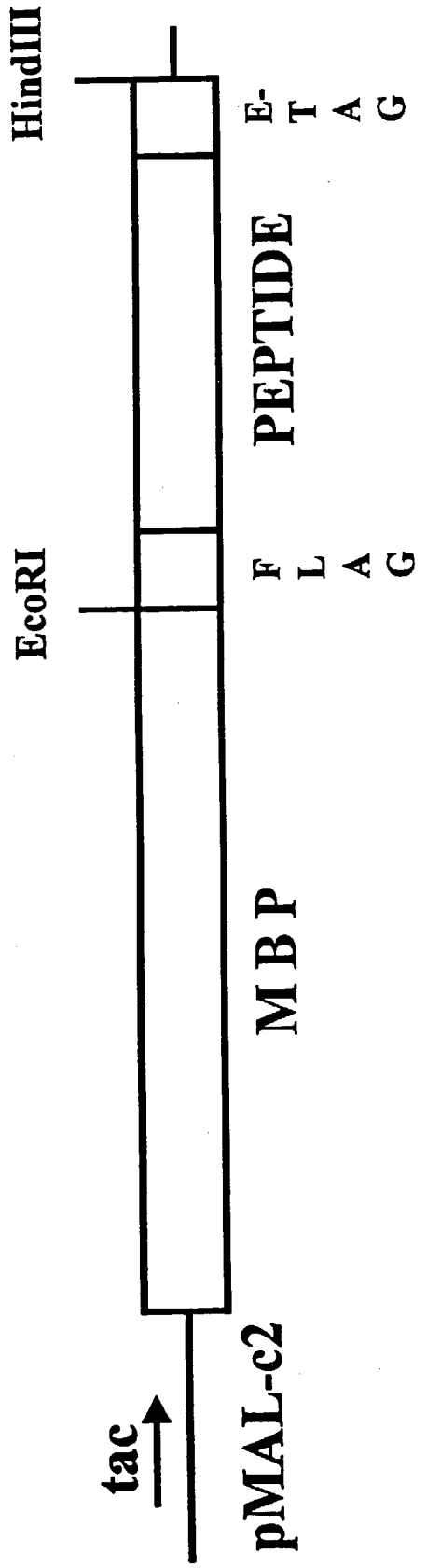



FIG. 28

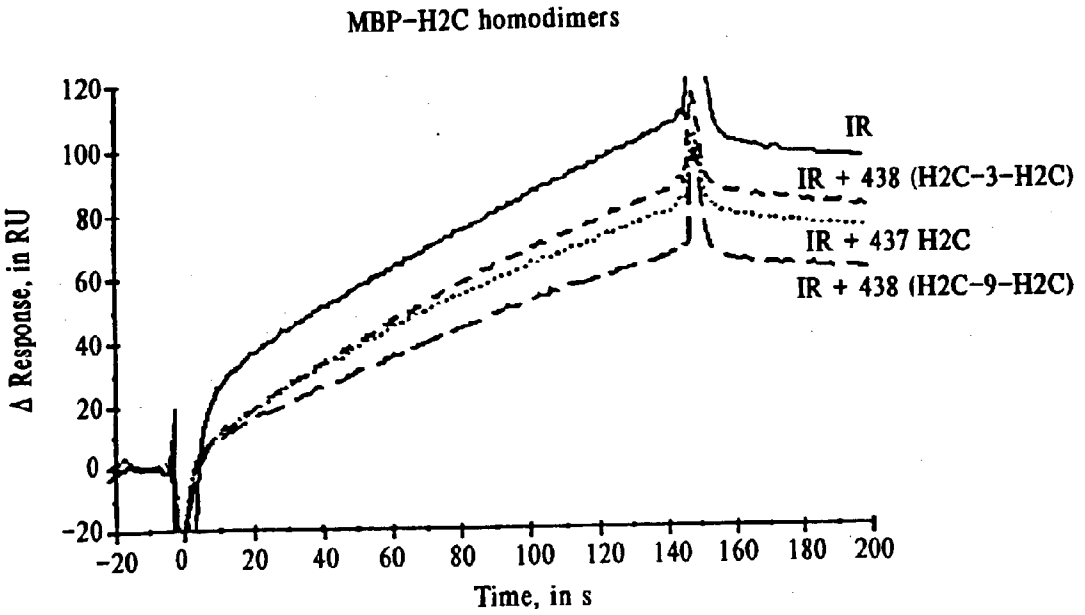

MBP-H2C homodimers
Δ Response, in RU
Time, in s
IR
IR + 438 (H2C-3-H2C)
IR + 437 H2C
IR + 438 (H2C-9-H2C)
120
100
80
60
40
20
0
-20
-20
0
20
40
60
80
100
120
140
160
180
200

FIG. 29

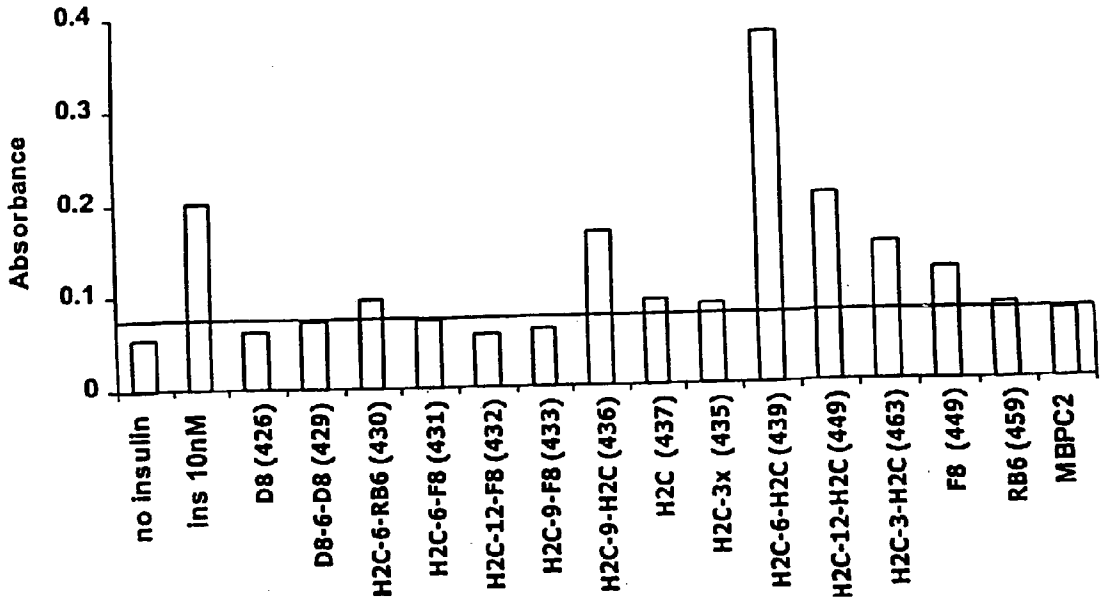

Absorbance
0.4
0.3
0.2
0.1
0
no insulin
ins 10nM
D8 (426)
D8-6-D8 (429)
H2C-6-RB6 (430)
H2C-6-F8 (431)
H2C-12-F8 (432)
H2C-9-F8 (433)
H2C-9-H2C (436)
H2C (437)
H2C-3x (435)
H2C-6-H2C (439)
H2C-12-H2C (449)
H2C-3-H2C (463)
F8 (449)
RB6 (459)
MBPC2

FIG. 30

FIG. 31A

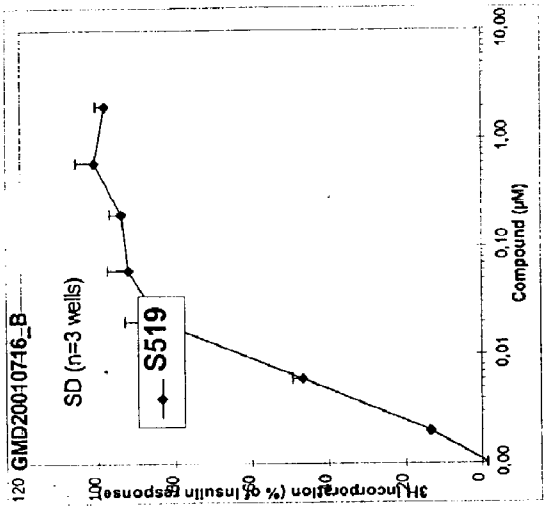



FIG. 31B

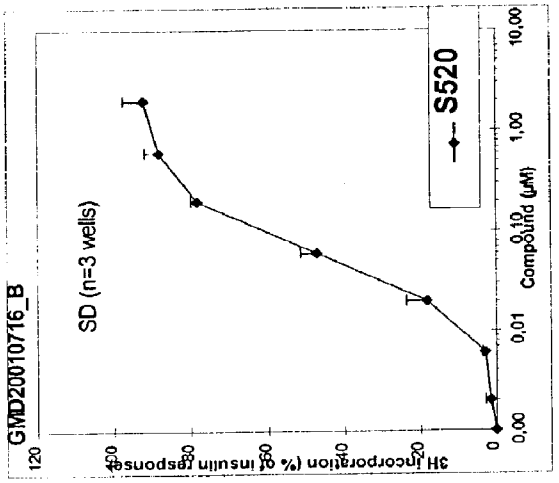
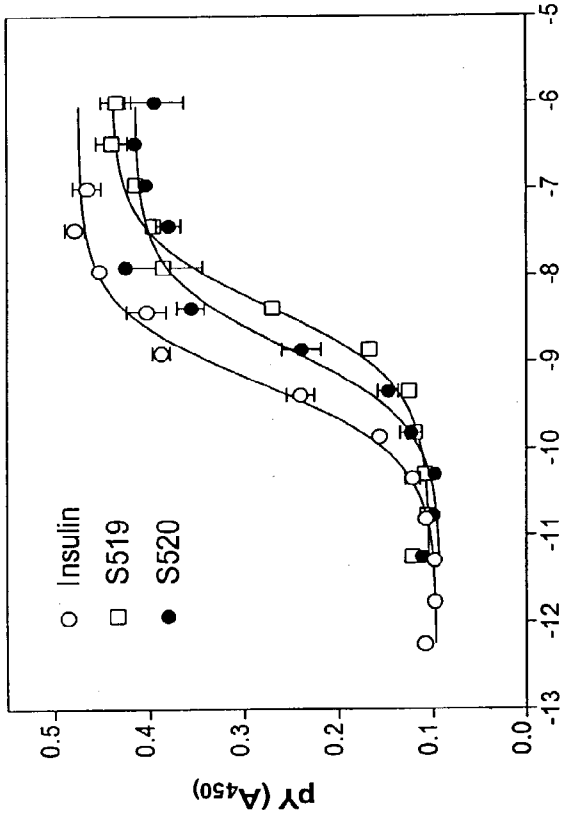



FIG. 31C

$EC_{50}$

- Insulin: 0.050 nM
- S519: 4.19 nM
- S520: 58.8 nM

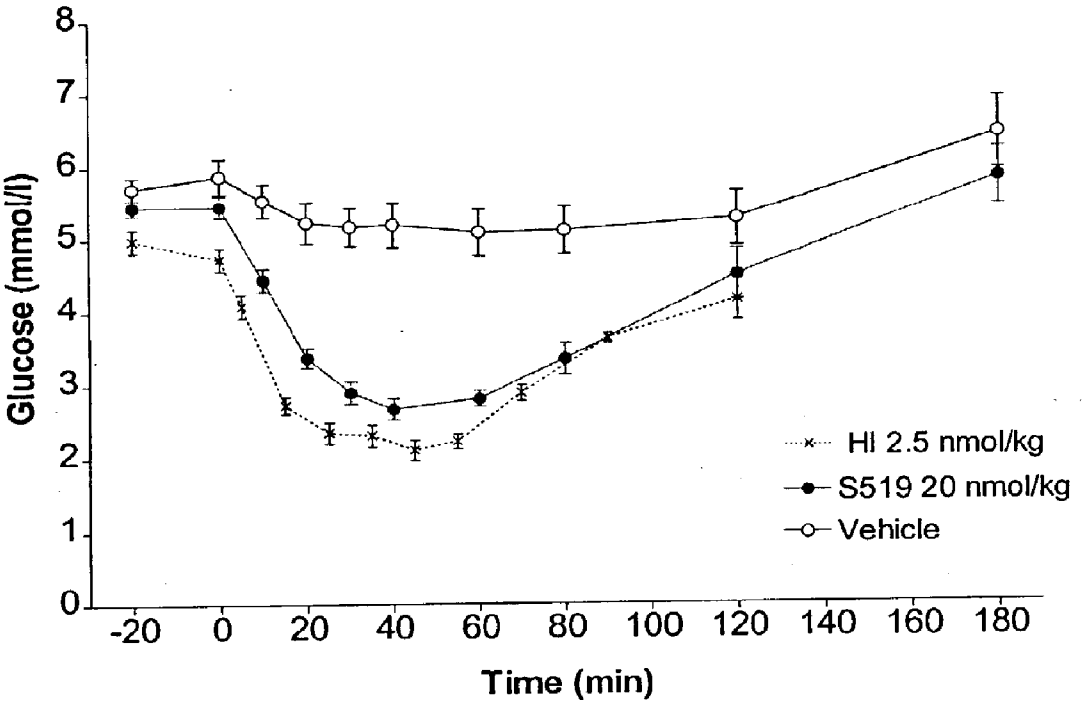
FIG. 32A




| | Insulin | S519 | S520 |
|---|---|---|---|
| Equation 1 | | | |
| Best-fit values | | | |
| BOTTOM | 0.09614 | 0.1038 | 0.09202 |
| TOP | 0.4740 | 0.4388 | 0.4145 |
| LOGEC50 | -9.237 | -8.380 | -8.852 |
| EC50 | 5.8000e-010 | 4.1660e-009 | 1.4060e-009 |

FIG. 32B

# FIG. 33


Glucose (mmol/l)
8
7
6
5
4
3
2
1
0
-20
0
20
40
60
80
100
120
140
160
180
Time (min)
HI 2.5 nmol/kg
S519 20 nmol/kg
Vehicle

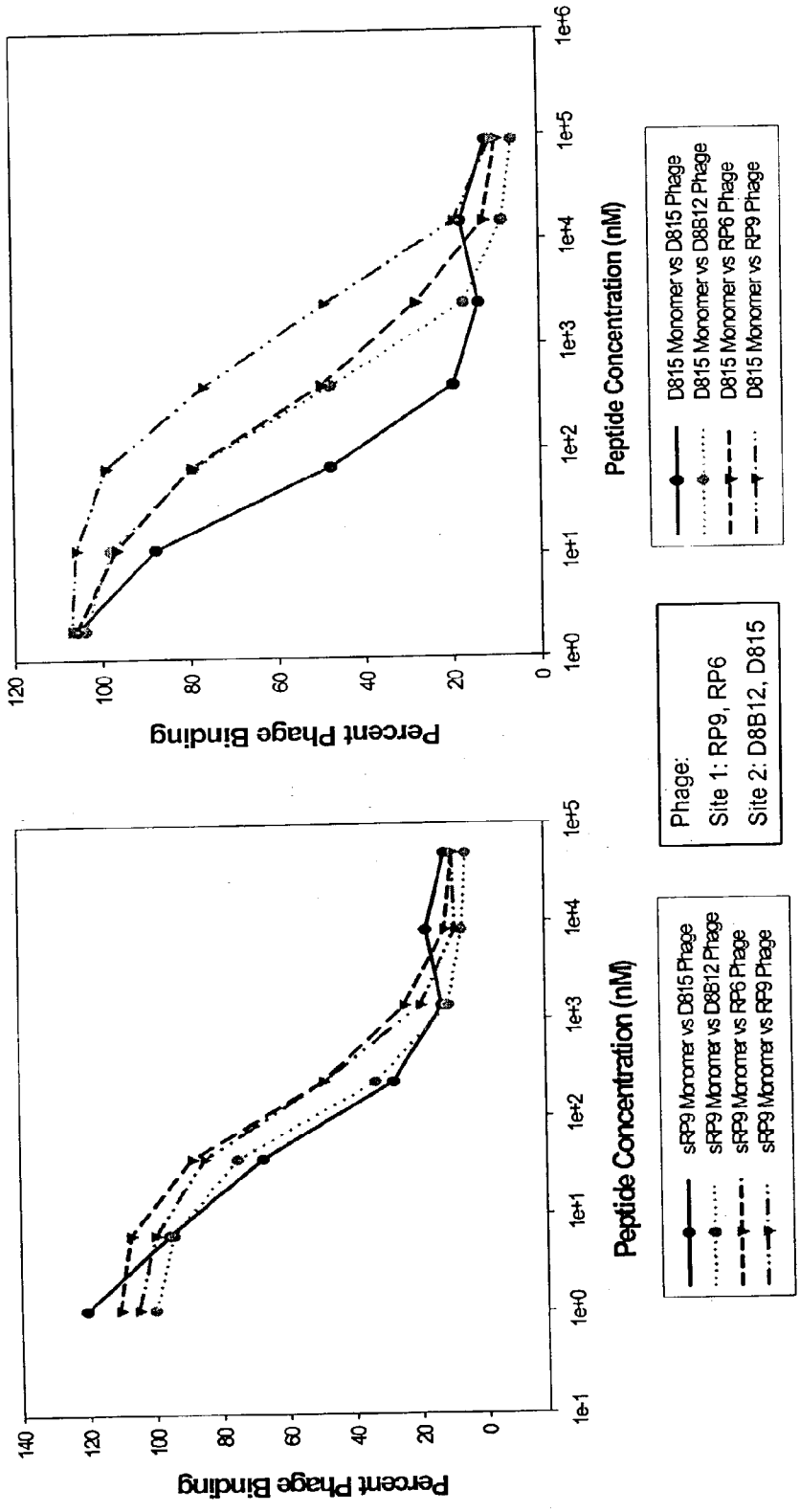



FIG. 34A

FIG. 34B

FIG. 34C

FIG. 34D

FIG. 34E

FIG. 35A

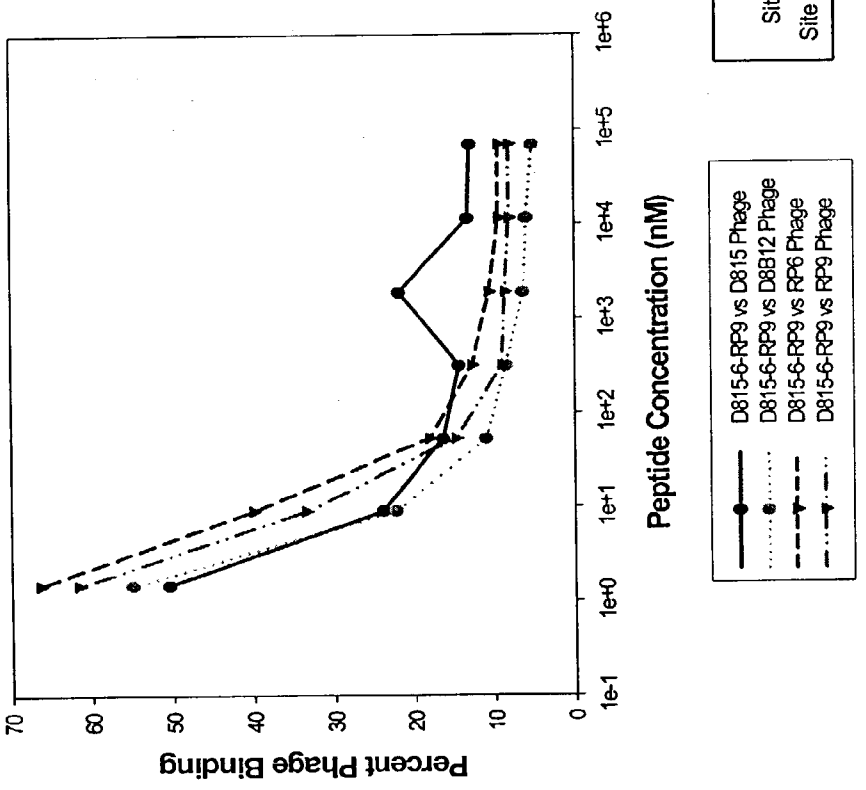



FIG. 35B

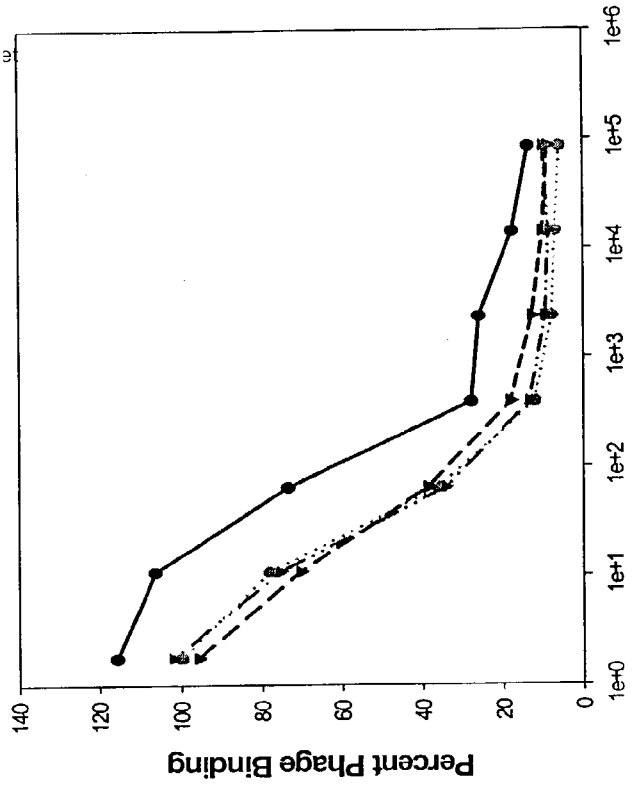



FIG. 35C

Phage:
Site 1: RP9, RP6
Site 2: D8B12, D815

FIG. 35D

FIG. 35E

FIG. 36

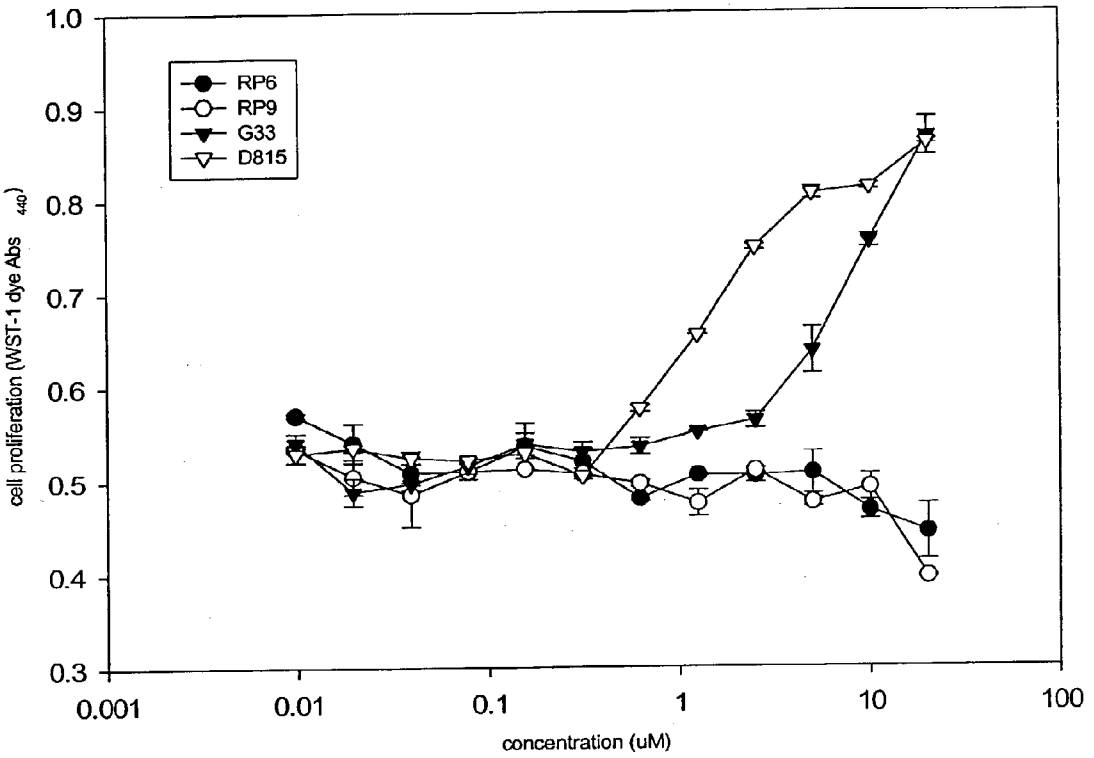

RP6
RP9
G33
D815
cell proliferation (WST-1 dye Abs 440)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.001
0.01
0.1
1
10
100
concentration (uM)

FIG. 37

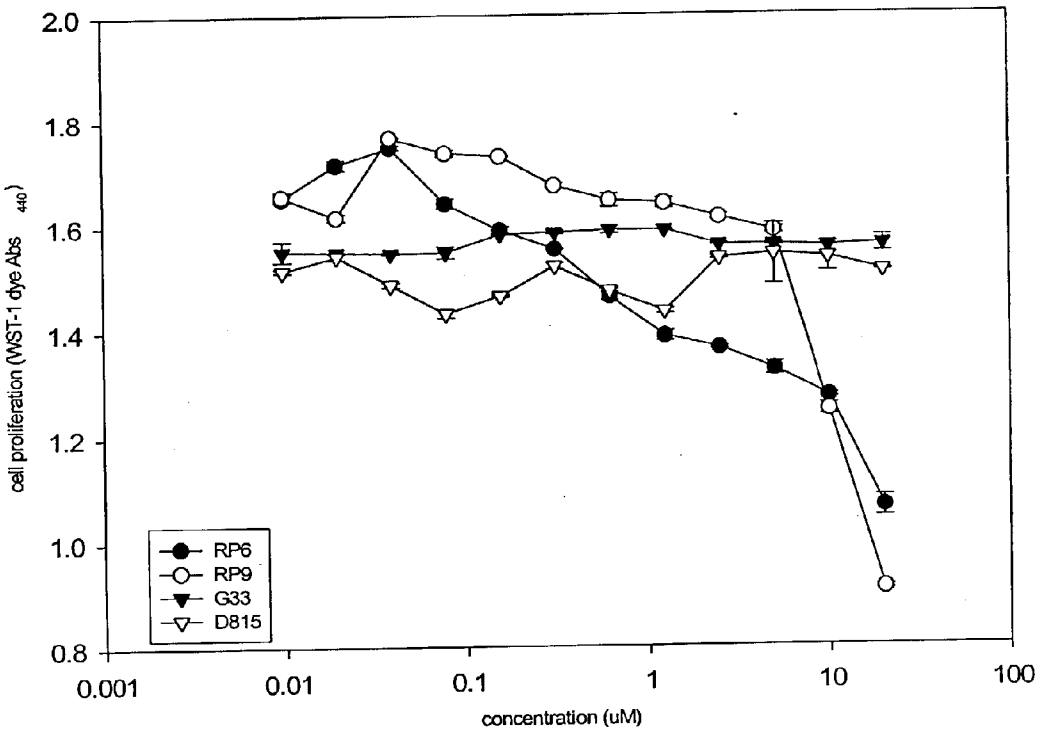
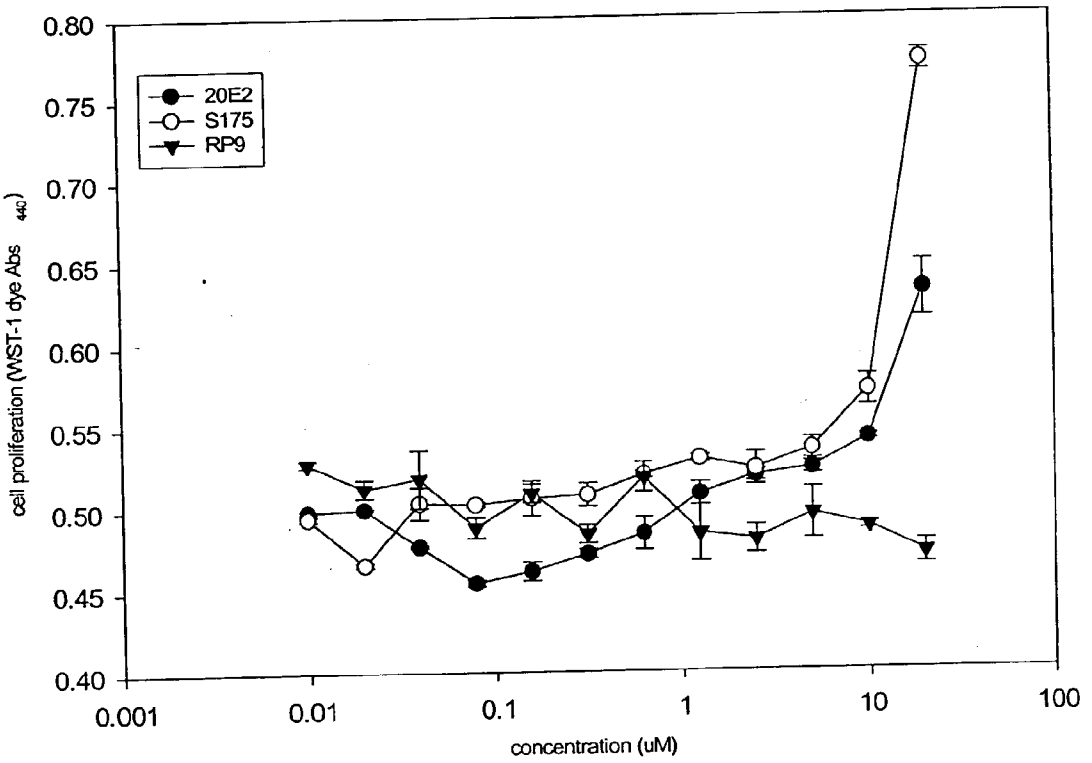

2.0
1.8
1.6
1.4
1.2
1.0
0.8
cell proliferation (WST-1 dye Abs 440)
0.001
0.01
0.1
1
10
100
concentration (uM)
RP6
RP9
G33
D815

FIG. 38


0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
20E2
S175
RP9
cell proliferation (WST-1 dye Abs 440)
0.001
0.01
0.1
1
10
100
concentration (uM)

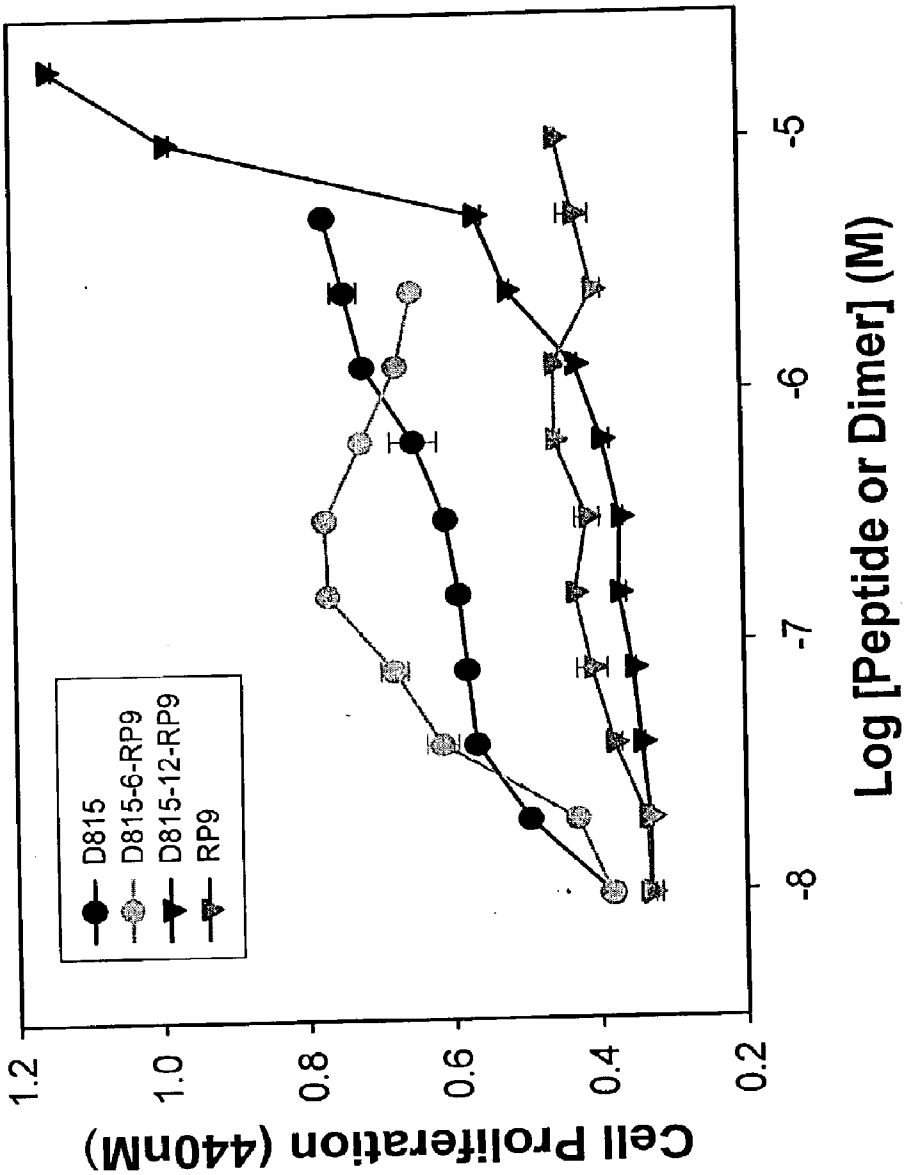

Cell Proliferation (440nM)
1.2
1.0
0.8
0.6
0.4
0.2
-5
-6
-7
-8
Log [Peptide or Dimer] (M)
D815
D815-6-RP9
D815-12-RP9
RP9

FIG. 39

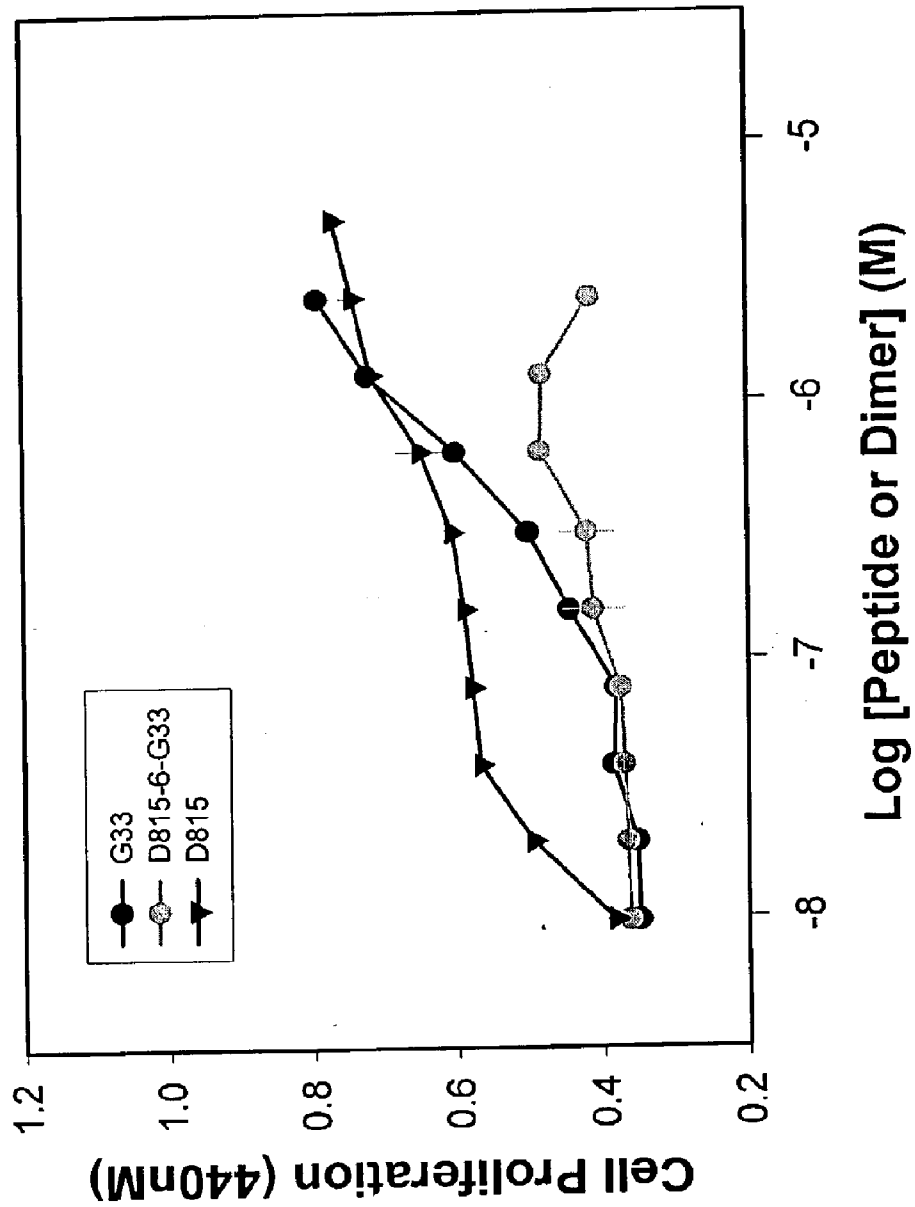

Cell Proliferation (440nM)
1.2
1.0
0.8
0.6
0.4
0.2
-5
-6
-7
-8
Log [Peptide or Dimer] (M)
G33
D815-6-G33
D815

FIG. 40

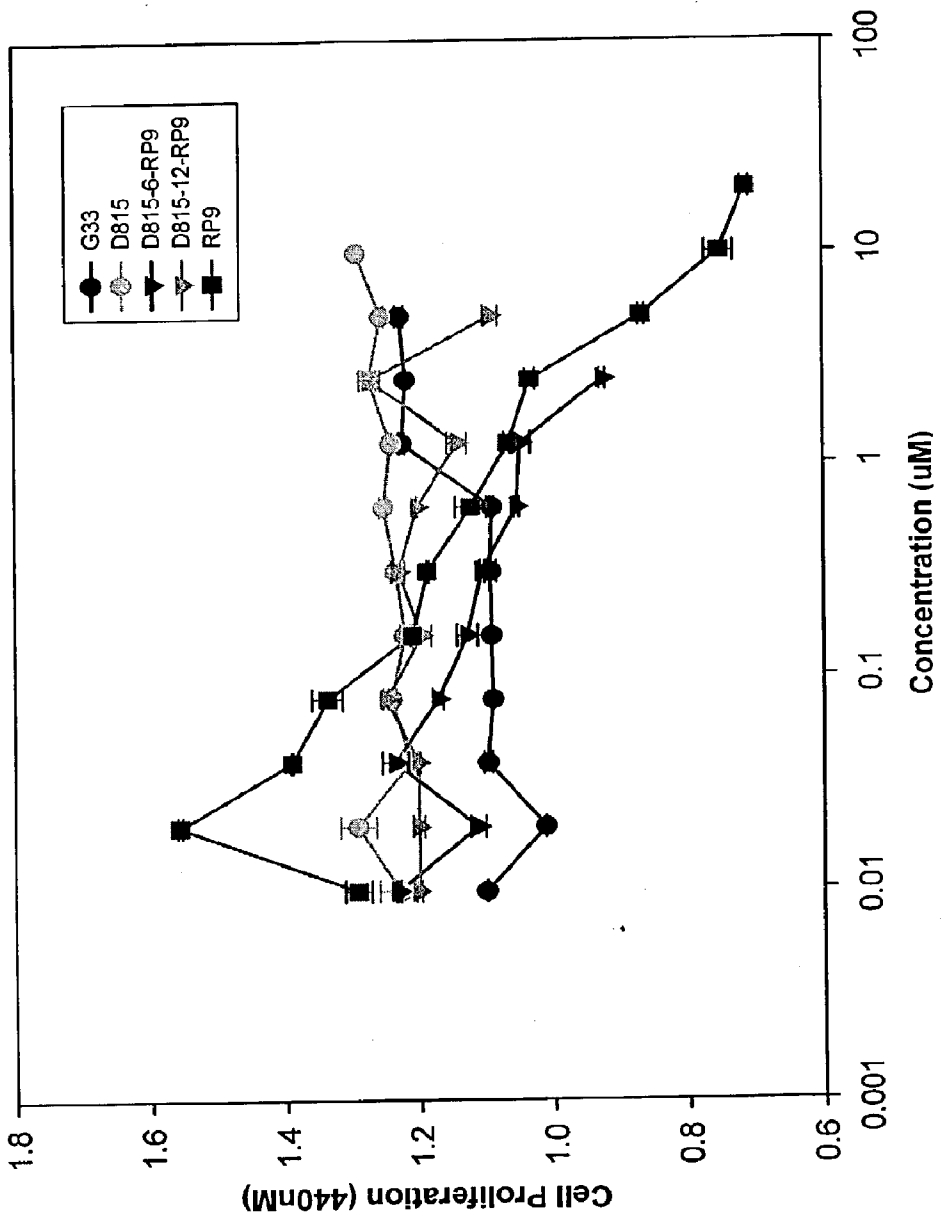
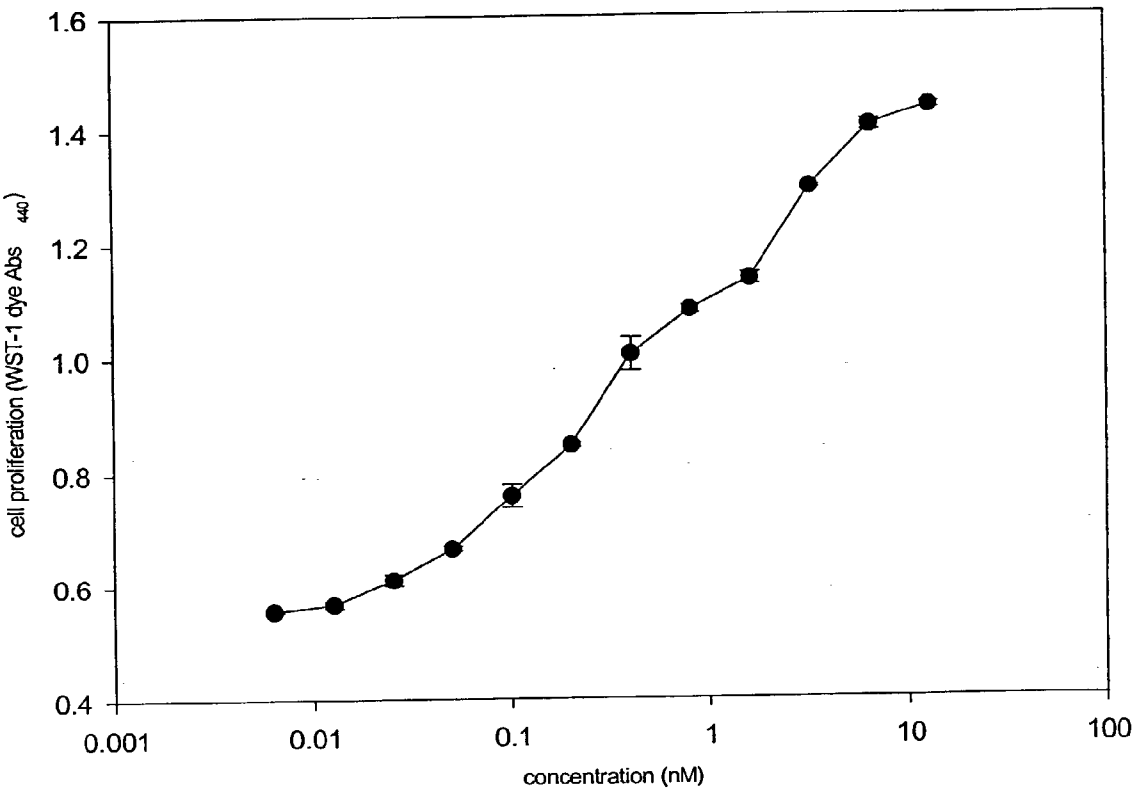

G33
D815
D815-6-RP9
D815-12-RP9
RP9
Cell Proliferation (440nM)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.001
0.01
0.1
1
10
100
Concentration (uM)

FIG. 41

FIG. 42


1.6
1.4
1.2
1.0
0.8
0.6
0.4
cell proliferation (WST-1 dye Abs 440)
0.001
0.01
0.1
1
10
100
concentration (nM)

| Clone # | Sequence | Etag | IGFR | LDH | IGFR/LDH | Binder |
|---|---|---|---|---|---|---|
| IGFR-G33-4-A1 | | 15.5 | 10.3 | 1.1 | 9.5 | HIT |
| IGFR-G33-4-A2 | | 4.8 | 2.9 | 1.2 | 2.5 | HIT |
| IGFR-G33-4-A3 | | 14.9 | 16.1 | 1.5 | 10.6 | HIT |
| IGFR-G33-4-A4 | PAMAVGYPQPCAKSTYERGRGSALESRCYQAAAGAP | 8.6 | 5.4 | 1.3 | 4.3 | HIT |
| IGFR-G33-4-A5 | | 23.5 | 12.1 | 3.4 | 3.6 | HIT |
| IGFR-G33-4-A6 | | 10.8 | 5.2 | 1.2 | 4.4 | HIT |
| IGFR-G33-4-A7 | MC | 13.3 | 5.0 | 0.8 | 6.2 | HIT |
| IGFR-G33-4-A8 | PAMACKVC*CCSVSCYDGFPRSGAHPGRRWAAAGAP | 6.0 | 1.7 | 1.0 | 1.8 | CAND |
| IGFR-G33-4-A9 | PAMAFKVSLSCGESFYEWFAGLVRDPTCGWTAAGAP | 10.8 | 6.3 | 1.1 | 5.7 | HIT |
| IGFR-G33-4-A10 | MC | 6.7 | 8.9 | 2.1 | 4.2 | HIT |
| IGFR-G33-4-A11 | AGHGACEFQVMFG*LVHLLGFPGRLGKGLAAGA | 5.8 | 5.8 | 1.2 | 4.9 | HIT |
| IGFR-G33-4-A12 | RPWRGSWLRLVGRRVECYCAERGATRGW*CAAAGAP | 3.3 | 2.6 | 1.1 | 2.4 | HIT |
| IGFR-G33-4-B1 | AGHGDFGALSCKAAVVAWVPVQTAGLRVRVAAAGAP | 8.1 | 3.2 | 1.2 | 2.6 | HIT |
| IGFR-G33-4-B2 | PAMAPRLYQGCPESFYAWTAGHVSPALYGWAAAGAP | 4.8 | 4.1 | 1.1 | 3.8 | HIT |
| IGFR-G33-4-B3 | PGHGVSVRAGVSGMLRREVAG#CVSAWEGLCGRRCA | 6.4 | 2.0 | 0.9 | 2.2 | CAND |
| IGFR-G33-4-B4 | PAMAGMDPQ#CTVASSRWFASPV#VVWRC#AAAGAP | 5.8 | 5.4 | 2.6 | 2.1 | HIT |
| IGFR-G33-4-B5 | PAMAGMFSQTCPEGFYGWFAGQASDSSLCRAAAGAP | 15.8 | 2.5 | 0.9 | 3.0 | HIT |
| IGFR-G33-4-B6 | PAMAPLGFRSCAGAY*VGCGRRVAFE#RCWAAAGAP | 7.7 | 2.3 | 1.2 | 1.9 | HIT |
| IGFR-G33-4-B7 | PAMAGILCPSCPHFLVDS#AAQDAAGQWPSAAAGAP | 7.2 | 3.3 | 1.2 | 2.8 | HIT |
| IGFR-G33-4-B8 | MC | 4.8 | 1.4 | 1.0 | 1.5 | |
| IGFR-G33-4-B9 | PAMARRIPRECGDSFYVGLRWLVENPRSDWAAAGAP | 6.2 | 1.9 | 1.0 | 1.9 | CAND |
| IGFR-G33-4-B10 | PAMADRIGVQCPDSFYGWFAVQEPGTSGGLAAAGAP | 8.5 | 3.5 | 1.0 | 3.5 | HIT |
| IGFR-G33-4-B11 | PAMAGLPS*SCRVAMYKGQAAWSCSAAAGAP | 4.9 | 3.9 | 0.9 | 4.3 | HIT |
| IGFR-G33-4-B12 | RPWRLILVTLVREASMTGSGVWYPRRGGAGPAEGA | 3.6 | 2.6 | 1.0 | 2.5 | HIT |
| IGFR-G33-4-C1 | | 27.7 | 24.5 | 1.0 | 24.5 | HIT |
| IGFR-G33-4-C2 | PAMAGSARQVCVDGVVGWREG*VVDQWL#RAAAGAP | 28.2 | 8.4 | 1.9 | 4.5 | HIT |
| IGFR-G33-4-C3 | PAMAGIMQRACEGGFTDCLWSLISGASSGRAAAGAP | 29.7 | 5.3 | 1.3 | 4.0 | HIT |
| IGFR-G33-4-C4 | RPWRVSSLRHVRVTCGELFGGQVSELFCLCAAAGAP | 7.5 | 5.6 | 1.1 | 5.0 | HIT |
| IGFR-G33-4-C5 | | 4.5 | 4.4 | 1.2 | 3.8 | HIT |
| IGFR-G33-4-C6 | PAMAGLIYMSCLAYFDDLIERRLEKPG#RFAAAGAP | 36.1 | 22.9 | 6.3 | 3.7 | HIT |
| IGFR-G33-4-C7 | PAMAGIMPQSCGETSGKCMRGQVSLRWRWSAAAGAP | 10.0 | 1.7 | 1.1 | 1.6 | CAND |
| IGFR-G33-4-C8 | PAMAFILPRSCEDYLYDFLASKVVHVFRSLAAAGAP | 9.7 | 6.9 | 1.9 | 3.6 | HIT |
| IGFR-G33-4-C9 | PAMACMSSQPCGESFYDWFAGQVRDPGWESAAAGA | 23.3 | 19.4 | 9.5 | 2.1 | HIT |
| IGFR-G33-4-C10 | RPWRGWAIRGVRHRC*GAWRGQVAQELCR#AAAGA | 30.2 | 9.3 | 4.3 | 2.2 | HIT |

FIG. 43A-1

| Clone # | Sequence | Etag | IGFR | LDH | IGFR/LDH | Binder |
|---|---|---|---|---|---|---|
| IGFR-G33-4-C11 | PAMAGIASHTCPGGFYEWFACQSRAPGWDGAAAGAP | 10.6 | 6.7 | 1.1 | 6.1 | HIT |
| IGFR-G33-4-C12 | | 19.2 | 30.2 | 5.2 | 5.9 | HIT |
| IGFR-G33-4-D1 | PAMAGRIARACPDSMFGWLAGQGSQQSGWQAAAGAP | 2.6 | 1.8 | 1.1 | 1.7 | CAND |
| IGFR-G33-4-D2 | PAMARPISPLC#RRSKDEDASRVSLPGFFCAAAGAP | 6.2 | 5.1 | 1.2 | 4.4 | HIT |
| IGFR-G33-4-D3 | MC | 31.0 | 8.5 | 1.0 | 8.3 | HIT |
| IGFR-G33-4-D4 | PAMADYKDDDDKTFYACLASLMAGTPRQYRTPWARCPAAAGAP | 4.8 | 1.7 | 1.1 | 1.5 | CAND |
| IGFR-G33-4-D5 | MC | 19.5 | 2.2 | 1.0 | 2.2 | HIT |
| IGFR-G33-4-D6 | RPWRVNTSESCL#FVCSLFSGYECWVGG*WAAAGAP | 3.4 | 1.1 | 1.0 | 1.1 | |
| IGFR-G33-4-D7 | PAMAGMGVQSCHDSFYGWFGCLFSDAEGDRAAAGAP | 20.7 | 15.2 | 7.0 | 2.2 | HIT |
| IGFR-G33-4-D8 | PAMAGDTSRACPESLNG.FCVVGVALRRWIAAAGAP | 20.1 | 7.0 | 1.0 | 7.1 | HIT |
| IGFR-G33-4-D9 | | 14.5 | 6.6 | 1.7 | 3.8 | HIT |
| IGFR-G33-4-D10 | PAMARWWRGLCGERWYHRGWVQVQFPWERGAAAGAP | 6.4 | 1.1 | 1.1 | 1.0 | |
| IGFR-G33-4-D11 | RPWRVPWVLEMPEYGNANLVFYDALQRLAAAGAP | 27.7 | 19.7 | 1.2 | 16.5 | HIT |
| IGFR-G33-4-D12 | AGHGVCYLAGVFGEALGGGRVSGFAIGQVRAAAGAP | 29.8 | 16.9 | 3.3 | 5.0 | HIT |
| IGFR-G33-4-E1 | PAMAGRIKEFCSRSFYDQVACLVKGPSWGGAAAGAP | 11.0 | 13.2 | 2.0 | 6.5 | HIT |
| IGFR-G33-4-E2 | PAMAGRIKEFCSRSFYDQVACLVKGPSWGGAAAGAP | 18.9 | 16.0 | 3.7 | 4.3 | HIT |
| IGFR-G33-4-E3 | PAMAGISSRSCAENLRFGRAWQGSDVWDCLAAAGAP | 22.4 | 21.3 | 0.9 | 22.9 | HIT |
| IGFR-G33-4-E4 | PAMASRIPQWCRDSFYEWFECQLLGPRESRAAAGAP | 14.5 | 7.3 | 1.2 | 6.1 | HIT |
| IGFR-G33-4-E5 | PAMAGAESCYRAKSFYDGLGCLVGEAWWGGAAAGAP | 7.8 | 14.3 | 1.9 | 7.4 | HIT |
| IGFR-G33-4-E6 | PAMARSGAPRCHDPFYEWFAVEAQEPLRCEAAAGAP | 6.0 | 3.1 | 1.0 | 3.1 | HIT |
| IGFR-G33-4-E7 | PAMAGMGVQSCHDSFYGWFGCLFSDAEGDRAAAGAP | 13.9 | 13.9 | 1.9 | 7.4 | HIT |
| IGFR-G33-4-E8 | PAMADISFESCLAQLLGWRAGEGSKRLWRCAAAGAP | 11.9 | 17.1 | 3.5 | 4.9 | HIT |
| IGFR-G33-4-E9 | PAMANTFLYPCRDPFYHSLADLVGVAMQCGAAAGAP | 23.2 | 24.5 | 5.2 | 4.7 | HIT |
| IGFR-G33-4-E10 | PAMARRIPRECGDSFYAGLRCLVESPRSDWAAAGAP | 9.4 | 5.8 | 1.7 | 3.3 | HIT |
| IGFR-G33-4-E11 | PAMASIVCPFCEDSFYNWFAAQVADTRGLWAAAGAP | 24.1 | 33.5 | 10.1 | 3.3 | HIT |
| IGFR-G33-4-E12 | | 1.2 | 0.9 | 1.0 | 0.9 | |
| IGFR-G33-4-F1 | PAMAWSHSHAYTESYYDWFAAQVLSAGSGRAAAGAP | 0.9 | 1.1 | 0.9 | 1.3 | |
| IGFR-G33-4-F2 | PAMAGRIKEFCSRSFYDQVACLVKGPSWGGAAAGAP | 7.2 | 8.5 | 0.9 | 9.7 | HIT |
| IGFR-G33-4-F3 | PAMARSRPPACGDSFYGWFECEVSGLGRRGAAAGAP | 2.2 | 1.4 | 1.0 | 1.4 | |
| IGFR-G33-4-F4 | PAMAGISYPACEESFYDCLASLVLSPWGSGAAAGAP | 12.1 | 5.2 | 0.8 | 6.7 | HIT |
| IGFR-G33-4-F5 | PAMAGRIKEFCSRSFYDQVACLVKGPSWGGAAAGAP | 16.7 | 24.2 | 7.3 | 3.3 | HIT |
| IGFR-G33-4-F6 | PAMAVVAGQYCRDSFYDRLSALVGDAWRCGAAAGAP | 13.6 | 7.4 | 1.9 | 3.8 | HIT |
| IGFR-G33-4-F7 | PAMACTASRFCAVSFYEWFAAQLPDLGGDSAAAGAP | 12.5 | 16.9 | 1.2 | 13.8 | HIT |
| IGFR-G33-4-F8 | PAMAGITLQSCGGGFYELLASVVGDTGCRLAAAGAP | 20.2 | 10.9 | 1.0 | 11.3 | HIT |
| IGFR-G33-4-F9 | PAMAGYICRSCQGSFYGCLAALVRDPRCSRAAAGAP | 24.7 | 33.0 | 8.8 | 3.7 | HIT |
| IGFR-G33-4-F10 | PAMAGRIKEFCSRSFYDQVACLVKGPSWGGAAAGAP | 7.1 | 10.6 | 1.2 | 9.1 | HIT |
| IGFR-G33-4-F11 | RPWRVAGAPRCHDPFYEWFAVEAQEPLRCEAAAGAP | 1.0 | 1.0 | 0.8 | 1.2 | |

FIG. 43A-2

| Clone # | Sequence | Etag | IGFR | LDH | IGFR/LDH | Binder |
|---|---|---|---|---|---|---|
| IGFR-G33-4-F12 | PAMAGMGVQSCHDSFYGWFGCLFSDAEGDRAAAGAP | 7.6 | 4.7 | 0.6 | 8.0 | HIT |
| IGFR-G33-4-G1 | PAMASICGQSCRDPFYAGLRGLLLEPLQLGAAAGAP | 17.6 | 18.5 | 1.0 | 19.5 | HIT |
| IGFR-G33-4-G2 | PAMAGVMSKCCSGSFYDWLADLVPEASWSWAAAGAP | 6.5 | 5.7 | 1.0 | 5.5 | HIT |
| IGFR-G33-4-G3 | PAMASFSGEACGGSFYDCLAGLMRDSSVSRAAAGAP | 18.4 | 7.9 | 1.1 | 7.4 | HIT |
| IGFR-G33-4-G4 | PAMASFSFYTCMETLLDGFGGQAFNRCRRTAAAGAP | 22.5 | 20.1 | 1.3 | 15.6 | HIT |
| IGFR-G33-4-G5 | PAMARVIYPTCPRDFYGGLAALVFGPHVCGAAAGAP | 22.8 | 21.7 | 1.9 | 11.5 | HIT |
| IGFR-G33-4-G6 | PAMAGIGSQACTDPFYYWFEGLVSNGGWCRAAAGAP | 5.9 | 5.3 | 1.2 | 4.3 | HIT |
| IGFR-G33-4-G7 | PAMAGRIKEFCSRSFYDQVACLVKGPSWGGAAAGAP | 18.8 | 2.1 | 1.0 | 2.1 | HIT |
| IGFR-G33-4-G8 | PAMAGAESCYRAKSFYDGLGCLVGEAWWGGAAAGAP | 23.6 | 30.3 | 3.7 | 8.2 | HIT |
| IGFR-G33-4-G9 | PAMADMMSQVCSQSMTGRFSVDFYDGLRCLAAAGAP | 17.3 | 4.6 | 0.9 | 5.1 | HIT |
| IGFR-G33-4-G10 | PAMARRIPRECGDSFYAGLRCLVESPRSDWAAAGAP | 26.8 | 24.6 | 5.4 | 4.6 | HIT |
| IGFR-G33-4-G11 | PAMARVIQEACGGSFYDGLACLVYPQGWRGAAAGAP | 3.3 | 1.5 | 0.9 | 1.7 | CAND |
| IGFR-G33-4-G12 | PAMAGGRSVACQESFYALLGCVVMGPGGGSAAAGAP | 24.1 | 32.1 | 12.1 | 2.7 | HIT |
| IGFR-G33-4-H1 | PAMAGISFRSCLQALIAGSAGNASEMGCRSAAAGAP | 5.9 | 5.8 | 1.2 | 4.8 | HIT |
| IGFR-G33-4-H2 | PAMAGIRDSYCQGAFYDWFAGLVDDGLFCQAAAGAP | 9.2 | 4.4 | 1.0 | 4.4 | HIT |
| IGFR-G33-4-H3 | PAMAGISYQSCEDSFYAWFACTVLDTRGGGAAAGAP | 17.8 | 16.0 | 1.8 | 8.9 | HIT |
| IGFR-G33-4-H4 | PAMARVIYEACGGSFYDGLACLVYPQGWRGAAAGAP | 3.1 | 3.2 | 1.1 | 2.8 | HIT |
| IGFR-G33-4-H5 | PAMADMPLLECLDPFYSWFAGQVSDPRFCGAAAGAP | 20.1 | 7.5 | 0.9 | 8.0 | HIT |
| IGFR-G33-4-H6 | PAMARVIQEACGGSFYDGLACLVYPQGWRGAAAGAP | 5.1 | 2.4 | 0.8 | 2.9 | HIT |
| IGFR-G33-4-H7 | PAMAGRIKEFCSRSFYDQVACLVKGPSWGGAAAGAP | 12.9 | 11.1 | 1.1 | 9.8 | HIT |
| IGFR-G33-4-H8 | MC | 23.4 | 23.5 | 1.6 | 14.7 | HIT |
| IGFR-G33-4-H9 | PAMAHISFHSCLEALQDPEWGQPSAAWRNCAAAGAP | 1.2 | 1.1 | 0.8 | 1.3 | |
| IGFR-G33-4-H10 | PAMAMTAQESCPDSFYECLAVLVGDRWGGWAAAGAP | 7.9 | 10.4 | 2.8 | 3.7 | HIT |
| IGFR-G33-4-H11 | PAMAHISFHSCLEALQDPEWGQPSAAWRNCAAAGAP | 16.8 | 23.7 | 1.3 | 18.1 | HIT |
| IGFR-G33-4-H12 | PAMAGTISQCCEENFYAGLAHLAGVGQWGCAAAGAP | 20.4 | 19.0 | 4.7 | 4.0 | HIT |

FIG. 43A-3

| Clone | # | E-Tag | IGFR | IR | Sp/Irr | Binders |
|---|---|---|---|---|---|---|
| B10 | DDDKTFYACLEFLLSGNPEGNSGPWDRCR | 2.6 | 29.0 | 1.0 | 29.0 | HIT |
| D1 | MC | 17.0 | 26.8 | 1.1 | 25.1 | HIT |
| A4 | DDDKIFYSCLASLLHGGPQRNTGPWERCR | 25.2 | 25.6 | 1.0 | 25.6 | HIT |
| A6 | DDDKTFYSCLASLLTGPREQNRGAWERCR | 22.3 | 23.3 | 1.1 | 21.2 | HIT |
| B1 | DDDKSFYSCLASLLTASRLPSRGAWDGCH | 18.0 | 22.8 | 0.9 | 25.3 | HIT |
| E4 | DDDKSFYSCLGSLLTGAPQPIRGAWDRCR | 20.8 | 20.6 | 1.1 | 19.1 | HIT |
| C11 | DDDKSFYSCLASLWSGTGGSSRGRWEGCR | 22.7 | 20.4 | 1.3 | 16.2 | HIT |
| C2 | DDDKTFYSCLGALLAGTGERNLRPWGRCR | 19.9 | 19.1 | 1.0 | 19.1 | HIT |
| B6 | DDDKTFYSCLGSLLTGPSDPKRGPWGGCR | 22.6 | 19.0 | 1.2 | 15.3 | HIT |
| A12 | DDDKTFYKCLASVLTGSTQTKRRPWEGCR | 13.9 | 18.8 | 1.0 | 18.4 | HIT |
| D2 | DDDKPFYSCLATLLTDPPQSQRGAWGRCR | 22.5 | 17.2 | 1.1 | 16.1 | HIT |
| C1 | DDDKSFYSCLAALLTGSSQSSGGAWMLCR | 21.6 | 16.9 | 1.1 | 15.2 | HIT |
| F10 | DDDKSFYSCLASLVAGTPWPKGGSWERCR | 11.4 | 16.6 | 0.9 | 18.2 | HIT |
| B11 | DDDKSFYSCLASLVTGIPRSNSGTQVFCR | 7.8 | 16.1 | 0.8 | 20.8 | HIT |
| A9 | DDDKSFYSCMASLLTGTPESRRGMGERCG | 16.3 | 15.9 | 1.3 | 11.8 | HIT |
| A10 | DDDKAFYSCLASLLTGSPPAQGGPWDRCR | 8.3 | 15.6 | 1.0 | 16.2 | HIT |
| B9 | DDDKFFYSCLASLLSDTPQRRRGPGVRCR | 5.9 | 14.3 | 0.9 | 15.4 | HIT |
| A3 | DDDKTFYSCLASLLAGNPQPNRAGWEYCR | 14.1 | 13.3 | 0.9 | 15.1 | HIT |
| F2 | DDDKTFYSCLGSLLLGPPQKNPGPGERCR | 9.6 | 13.3 | 0.9 | 14.8 | HIT |
| A7 | DDDKMFYSCLESLLIGRWPRNGGSLSRCR | 10.9 | 13.2 | 1.1 | 12.5 | HIT |
| G3 | DDDKSFYSCLTFLLTGTPQANDASWERCR | 9.6 | 13.2 | 1.0 | 13.8 | HIT |
| A11 | DDDKSFYSCMAALLSGAPQKSRGRWERCG | 4.7 | 12.7 | 0.8 | 15.0 | HIT |
| B7 | DDDKAFYRCLAYLLAGRPQASGGGGVRCR | 19.7 | 12.5 | 0.9 | 13.5 | HIT |
| D5 | DDDKAFYSCLAALRERSPQMSRGTWGGCR | 21.8 | 11.8 | 1.3 | 9.3 | HIT |
| B8 | DDDKTFYACLAALLGGTAELHDGSLECRG | 11.8 | 11.8 | 1.3 | 9.0 | HIT |
| D10 | DDDKTFYSCLGSLLTGTLPPARGARNICR | 15.1 | 11.6 | 1.0 | 11.6 | HIT |
| D8 | DDDKTFYSCLSSLLAGSPLPRRDLWAGCR | 11.1 | 9.2 | 0.9 | 10.6 | HIT |
| D12 | DDDKAFYSCMASLLAGTPEAQGSAWVRCR | 5.4 | 7.4 | 0.8 | 9.0 | HIT |
| A5 | DDDKNFYACMESLVSVAPPSSRDPFECRR | 16.0 | 7.3 | 1.3 | 5.5 | HIT |
| F6 | DDDKSFYSCLASLVSGTA.PNRGPWERCR | 4.2 | 6.9 | 1.0 | 7.1 | HIT |
| G5 | DDDKIFYSCLASLLDDTAQSRRGQWARCR | 4.8 | 6.4 | 1.3 | 5.1 | HIT |
| C6 | DDDKIFYSCLGALLSGTPQTSHVTSGRCR | 13.6 | 5.9 | 1.1 | 5.4 | HIT |

FIG. 43B-1

| Clone # | | E-Tag | IGFR | IR | Sp/Irr | Binders |
|---|---|---|---|---|---|---|
| D3 | TDDKTFYFCLASLLAGTQQQSRGAWERCG | 5.1 | 5.9 | 0.9 | 6.5 | HIT |
| A2 | DDDKAFYSCLASVLTGSPHPGRSRWERCR | 18.6 | 5.5 | 0.9 | 6.0 | HIT |
| B2 | DDDKTFYSCLESMLTGTPPPCRGHGERGR | 8.0 | 5.5 | 1.0 | 5.6 | HIT |
| D7 | DDDKTFESCLEALVSGGSRRERGLWYRCR | 10.6 | 5.5 | 1.1 | 5.1 | HIT |
| C12 | DDDKAFYSCLSSLLAGTRERHRDTWPRCG | 12.0 | 5.3 | 1.0 | 5.3 | HIT |
| B5 | DDDKTFHSCLAALVTGTPQQKRGPWERCR | 20.0 | 5.1 | 1.3 | 3.9 | HIT |
| E2 | DDDKTFYLCLASLQTVTRLGDRVPWERCR | 18.0 | 4.6 | 0.7 | 6.4 | HIT |
| F3 | DDDKTFYSCLVSLLNGTAKPNRGQWEGCR | 3.7 | 4.5 | 1.4 | 3.2 | HIT |
| H2 | DDDKSFYSCLASLSNCTPGLLRCQWERCR | 7.2 | 4.4 | 0.8 | 5.4 | HIT |
| B4 | DDDKTFYSCLSSLLASTPQPNRGAWLCRR | 11.9 | 4.3 | 1.2 | 3.6 | HIT |
| G12 | DDDKSFYSCLASLSNCTPGLLRCQWERCR | 2.2 | 4.2 | 1.0 | 4.4 | HIT |
| F5 | MC | 8.4 | 4.0 | 0.9 | 4.3 | HIT |
| A1 | DDDKTFYSCLGALLSGAPQTYRGPGAGCR | 8.1 | 3.9 | 1.0 | 3.9 | HIT |
| E11 | DDDKTFYSCLVSLLNGTAKPNRGQWEGCR | 4.9 | 3.8 | 1.0 | 3.9 | HIT |
| F9 | DDDKHFYSCLSSLLTAPPQSTRGPAGRHC | 3.9 | 3.7 | 1.0 | 3.6 | HIT |
| E3 | DDDKTFYSCLASLLNGNTQPNGGQWVRCR | 1.7 | 3.6 | 0.8 | 4.5 | HIT |
| G4 | DDDKVFYTCLASLSTGTPQRQSGEWQRCR | 9.4 | 3.3 | 1.3 | 2.5 | HIT |
| E5 | DDDKTFYSCLVSLLNGTAKPNRGQWEGCR | 4.6 | 3.2 | 0.9 | 3.7 | HIT |
| C10 | DDDKPFYSCLASLIQGTPLPERGMWERCR | 6.3 | 3.0 | 1.0 | 3.0 | HIT |
| C7 | MC | 10.6 | 2.9 | 1.0 | 3.0 | HIT |
| H5 | DDDKTFYSCVSWLLTGARQRDGVWERCR | 5.5 | 2.9 | 1.3 | 2.2 | HIT |
| H3 | DDDKTFYSCLVSLLNGTAKPNRGQWEGCR | 4.6 | 2.8 | 1.1 | 2.5 | HIT |
| C3 | DDDKAFYGCLAALLTGARQPSRGVGERCF | 6.0 | 2.7 | 1.5 | 1.8 | HIT |
| F1 | DDDKTFYSCLVSLLNGTAKPNRGQWEGCR | 1.5 | 2.6 | 0.8 | 3.4 | HIT |
| B3 | DDDKTFYSCLASLLAGSPQPKRAGWEYCR | 8.6 | 2.5 | 1.0 | 2.5 | HIT |
| D11 | DDDKPFYSCLESLVTGRPQADRGVWERCR | 4.9 | 2.4 | 0.9 | 2.7 | HIT |
| E10 | DDDKTFYSCLTSLSRGSAHGLSGRWERCR | 5.0 | 2.3 | 0.9 | 2.7 | HIT |
| F12 | DDDDKTFYFCLATLLTGPPVPNREPWACYR | 2.5 | 2.2 | 0.8 | 2.7 | HIT |
| D4 | DDDKIFYSCLRTLGTNPPEPVRGPFDRCG | 3.0 | 2.1 | 1.1 | 1.9 | HIT |
| E6 | DDDKTFYSCLVSLLNGTAKPNRGQWEGCR | 2.7 | 2.1 | 1.4 | 1.5 | HIT |
| F11 | NDDKSFYSCVASLVNEGPSQVGVLGERCR | 3.5 | 2.1 | 1.2 | 1.7 | HIT |
| A8 | DDDKTFYSCLASMLTGPPHPDRVPWDRCR | 8.3 | 2.0 | 1.0 | 1.9 | HIT |
| F7 | DDDKKFYSCLVELVNGTSPPARGLWERCR | 2.7 | 2.0 | 1.0 | 2.1 | HIT |
| F8 | DDDKVFYSCLESLVSGTPEVNGRAWERCR | 2.0 | 1.8 | 1.0 | 1.7 | CAND |
| E12 | YDDKRFYYCLASLASGTLQTNREQWERCR | 1.0 | 1.7 | 1.0 | 1.7 | CAND |
| H1 | DDDKTFYSCLESLLNGTPQRNRGQWDPCS | 1.9 | 1.7 | 0.9 | 1.9 | CAND |
| H8 | DDDKTFYTCLQALITGYERPVGGRWESCR | 1.2 | 1.7 | 1.2 | 1.4 | CAND |

FIG. 43B-2

| Clone | # | E-Tag | IGFR | IR | Sp/Irr | Binders |
|---|---|---|---|---|---|---|
| G9 | DDDKAFYSCLATLLYGNPPSSRGQWHRCR | 1.5 | 1.6 | 1.1 | 1.5 | CAND |
| D6 | DDDKVFYSCLESLVSGTPEVNGRAWERCR | 1.3 | 1.5 | 1.1 | 1.5 | CAND |
| H6 | GDDKTFYACLSSLLYGTADWSQGQRDRCR | 3.2 | 1.5 | 1.3 | 1.1 | |
| E7 | DDDKSFYSCMESLWTDTPQPNRGRWERCR | 3.2 | 1.4 | 1.1 | 1.3 | |
| F4 | DDDKTFYSCLASLLTVSPEPSRGPWERCR | 1.4 | 1.4 | 1.2 | 1.1 | |
| G6 | MC | 1.4 | 1.4 | 1.0 | 1.5 | |
| G11 | DDDKTFYSCMVQLLTGTPEKSCVTWERWR | 1.6 | 1.4 | 1.0 | 1.4 | |
| H4 | DDDKTFYSCLVSLLNGTAKPNRGQWEGCR | 4.7 | 1.4 | 1.0 | 1.4 | |
| B8 | DDDKTFYSCVASLVVGTAQPQCGPWQGWG | 9.1 | 1.3 | 1.0 | 1.3 | |
| C8 | DDDKTFYSCLAGLVTGPPRQNWGAGDACR | 7.7 | 1.3 | 1.4 | 1.0 | |
| E1 | DDDKTFYSCMSSLSTTAPQPKSGRWDRCR | 2.3 | 1.3 | 0.9 | 1.4 | |
| G10 | DDDKTFYSCLASLVNGSLQPNRAPGELCR | 1.2 | 1.3 | 1.2 | 1.2 | |
| H10 | DDDKTFYSCLVSLLNGTAKPNRGQWEGCR | 3.5 | 1.3 | 1.0 | 1.3 | |
| H11 | DDDKTFYSCLVSLLNGTAKPNRGQWEGCR | 2.6 | 1.3 | 1.0 | 1.3 | |
| D9 | DDDKSFYSCLESLLNGGPQQKRGPWEGCR | 1.1 | 1.2 | 0.9 | 1.3 | |
| E9 | DDDKTFYSCLASLLNGNTQPNGGQWVRCR | 1.1 | 1.2 | 1.1 | 1.1 | |
| C9 | MC6 | 1 | 1.1 | 1.1 | 1.0 | |
| G1 | DDDKSFYSCLASLSNCTPGLLRCQWERCR | 0.9 | 1.1 | 0.9 | 1.3 | |
| H7 | DDDKNFYSCLSALLNGNTVSDRGQGERCF | 2.2 | 1.1 | 0.9 | 1.1 | |
| H9 | DDTFYSCLASLVNGSPQAYGGPREHCR | 3.1 | 1.1 | 1.1 | 1.0 | |
| C5 | DDDKIFYSCLAWLMTGPAPPYRGPWSCWS | 3.3 | 1.0 | 1.0 | 0.9 | |
| G7 | DDDKIFYACLSSLSTGTWQPKRGPGERCR | 0.9 | 1.0 | 0.9 | 1.1 | |
| H12 | DDDKSFYSCLASLSNCTPGLLRCQWERCR | 3.7 | 1.0 | 1.0 | 1.1 | |
| C4 | DDDKTFYSCLGSLINGTPPPHRGLWQGCR | 1.1 | 0.9 | 0.9 | 1.0 | |
| G8 | DDDKTFYSCLVSLLNGTAKPNRGQWEGCR | 0.7 | 0.9 | 1.0 | 0.9 | |
| G2 | DDDKQFYSCLAFLASGIAQPQPGAWELCR | 1.1 | 0.8 | 1.0 | 0.8 | |
| B12 | DDDKTFYCCLAALLTGAPPPKGGTCERCG | 9.3 | 13.2 | 0.7 | 18.3 | HIT |

FIG. 43B-3

**IR**

| Clone | Sequence | E-Tag | IR | IGFR | IR/IGFR | IGFR/IR |
|---|---|---|---|---|---|---|
| B5 | FHENFYDWFARKDSGGSGGSGGSDLCVLEELFWGDSLFDYCTG | 17.0 | 16.9 | 0.5 | 35.8 | 0.0 |
| A3 | FHENF.DWFVRQVSGGSGGSGGSNLCVLEELFWGASLFGECSG | 13.0 | 11.8 | 0.3 | 35.8 | 0.0 |
| A8 | SHGNFSEWFVRQGYGGSGGSGGSDLCVLEELYWGASLFGYCSG | 13.2 | 13.1 | 0.4 | 33.2 | 0.0 |
| C7 | FQESFYDWFVR.VTGGSGGSGGSDLCGVEDLVWGSALSGYCAG | 15.1 | 14.7 | 0.5 | 30.6 | 0.0 |
| B4 | FHENFNDWFVREVSGGSGGSGGSDLCVLEELFWGASLFSYCSG | 13.2 | 11.7 | 0.4 | 27.6 | 0.0 |
| B11 | SHENFYDWFVR.GPGGSGGSGGSHLCVLEELFWGDSLFGACPG | 10.9 | 9.1 | 0.3 | 27.0 | 0.0 |
| A9 | FHENFYDWFARQVSGGSGGSGGSHLCVLEELFWGASLFA.CSD | 10.7 | 12.3 | 0.5 | 25.7 | 0.0 |
| A6 | FPDNFYDWFVR.VSGGSGGSGGSHLCVLEELFWGASPFGYCSG | 11.6 | 8.7 | 0.4 | 19.8 | 0.1 |
| A4 | **FQENFYDWFGRQI**SGGSGGSGGS**PLCDVEELFWGVSLFGYCTG** | 13.6 | 12.1 | 2.6 | 4.6 | 0.2 |
| C8 | FQENFYDWFVR.ASGGSGGSGGSHLCALEEQFWGSSQFRYCSG | 16.0 | 14.5 | 3.2 | 4.5 | 0.2 |
| A10 | FHENFYDWFARQVYGGSGGSGGSHLCVLEELF.GASLFATCSD | 10.6 | 6.0 | 1.5 | 3.9 | 0.3 |
| D11 | FHENFYDRIVRQVAGGSGGSGGSALCVREELF.GDSLFGDCSG | 12.4 | 5.5 | 1.5 | 3.6 | 0.3 |
| D4 | FHKNFYDWFDRQVSGGSGGSGSSRLCDLEELFWGASL.GHCSG | 15.4 | 9.8 | 3.9 | 2.5 | 0.4 |
| C1 | FHENFYDWFIRQDSGGSGGSGGSHLCAFEELLGGASPFGYCSG | 16.8 | 2.7 | 1.3 | 2.1 | 0.5 |
| D12 | | 11.7 | 8.7 | 4.6 | 1.9 | 0.5 |
| D8 | SNENFYDWFDR.VSGGSGGSGGSHLCLLEELSWGASLFGYCYG | 15.8 | 9.6 | 7.4 | 1.3 | 0.8 |
| C11 | FHESFYDWFDRQVSGGSGGSGGSHLCVLEE.ELGASVFGCCSG | 11.0 | 5.8 | 5.4 | 1.1 | 0.9 |
| C4 | FHETFYDWFDR.VSGGSGGSGEELFGGASLFGYPSG | 16.7 | 13.2 | 15.0 | 0.9 | 1.1 |
| D1 | SHENFYDWFGRQVSGGSGGSGGSNLCDLDEVS.GASLCGYRSG | 16.2 | 5.5 | 7.1 | 0.8 | 1.3 |
| C6 | FH.NFYDWFFCQVPEWIPMTLAVLTCAVLEEPIWGDSLFGYG.E | 16.1 | 1.7 | 2.2 | 0.8 | 1.3 |
| A5 | SHENFYDWFVRQV.GGSGGSGGSHLCDLEELLGGASLMGSCSG | 16.0 | 8.7 | 12.9 | 0.7 | 1.5 |
| B8 | SHENFYDWFVR.VSGGAAAGAPPAMASHENFYDWFVR.VSGG | 15.2 | 8.9 | 13.9 | 0.6 | 1.6 |
| D2 | FHENFYDWFIR.VGGGSGGSGGSDLCVLEDDCSRAAAGAP | 13.9 | 8.4 | 13.1 | 0.6 | 1.6 |
| A2 | DYKDASVSGTFHDAFYEWFWR.VGS | 13.4 | 6.9 | 12.6 | 0.6 | 1.8 |
| C12 | FHENFYDLVPSAGSWWIRWLWRF.PVRLGRTVLGCFSDR.LFW | 9.2 | 4.4 | 6.8 | 0.6 | 1.5 |
| B9 | FHENFYDWFDRQVSGGSGGSVRAAAGAP | 17.8 | 8.2 | 16.3 | 0.5 | 2.0 |
| B1 | VHENFYDWFDRQVSGGSGGSGGSQLCDL.EVIWGASLFGYCTG | 18.1 | 7.3 | 13.4 | 0.5 | 1.8 |

FIG. 44A-1

| Clone | Sequence | E-Tag | IR | IGFR | IR/IGFR | IGFR/IR |
|---|---|---|---|---|---|---|
| B2 | FHENFYDWFDR.VSGGSGGSGGSHLCVPEEQFWGASRFGYCSA | 16.2 | 7.0 | 13.6 | 0.5 | 1.9 |
| D9 | FHDNFYDWFVRQVSGGSGGSGGSRQCQGASL.GYCSG | 17.0 | 6.6 | 13.8 | 0.5 | 2.1 |
| D5 | OUT OF FRAME | 16.1 | 4.8 | 10.5 | 0.5 | 2.2 |
| C9 | FHEDFYDWFVR.VPGGSGGSGGSHLCVPGYCSS | 17.2 | 5.1 | 14.2 | 0.4 | 2.8 |
| B7 | FRENFYDWFVC.VSGGSGGSGGSNLCVLEEAAAGAP | 15.9 | 4.0 | 10.6 | 0.4 | 2.7 |
| D6 | OUT OF FRAME | 15.6 | 4.2 | 12.8 | 0.3 | 3.0 |
| C5 | GHDNFYDWFVRQVSGGSGGSGGSHLCV.GAFPWGYCSD | 15.2 | 3.6 | 10.7 | 0.3 | 3.0 |
| D3 | FH.NFYDWFVRQVYGGSGGSGTGAAAGAP | 16.2 | 3.5 | 12.0 | 0.3 | 3.4 |
| C10 | BAD SEQUENCE | 11.2 | 2.5 | 7.6 | 0.3 | 3.1 |
| A7 | FHENFYDWFGRQVYGGSGGSGGSPVCILGELS.GGALFGDCSG | 15.5 | 1.8 | 5.1 | 0.3 | 2.9 |
| A12 | FHENFYDWFVR.LSGGSGGSGGSHLCVPEERLWGDPLFGYCSG | 8.7 | 1.2 | 3.5 | 0.3 | 3.0 |
| D7 | FH.NFYDWFVRQVSGGSGGSGGSHPAR | 16.2 | 3.0 | 11.9 | 0.2 | 4.0 |
| A11 | FHENFYDWFVRQVTGGSGGSGGSHLCVLEELS.GAALPGYCSG | 11.8 | 1.0 | 4.0 | 0.2 | 4.1 |
| D10 | VQGSFYDWFVRQVSGGSGGSGGSHLC.GSG | 12.7 | 1.0 | 6.3 | 0.2 | 6.6 |
| C3 | FHENFYEWFVRQVSGGSGGSGGSHRCDVEELH.CASG | 16.8 | 0.6 | 2.5 | 0.2 | 4.2 |
| A1 | DYKDGGYWGSFYEGLM.LVQSGTSG | 13.6 | 1.7 | 12.5 | 0.1 | 7.1 |
| B6 | OUT OF FRAME | 12.7 | 1.0 | 8.1 | 0.1 | 8.1 |
| **Non-Binders:** | | | | | | |
| B12 | FHENFYDWFDRQVSGGSGGSGGSHRCVLEERFWGASLFG.CSG | 7.8 | 0.5 | 1.6 | 0.3 | 3.3 |
| B10 | SHENFYDWFVHQVSGGSGGSGGSHLCVLEERF.GPSLFGYCSG | 10.8 | 0.6 | 1.4 | 0.4 | 2.3 |
| B3 | FHANFYDWFFRQVSGGSGGSGGSDLCVLQDMF.GGSGAAAGAP | 16.9 | 0.7 | 1.2 | 0.6 | 1.7 |
| C2 | FQDNFYDWFVRQISGGSGGSGGSHLCVLESWF.GASLFGYCSG | 14.8 | 0.5 | 0.8 | 0.6 | 1.7 |

FIG. 44A-2

**IGFR**

| Clone | Sequence | E-Tag | IR | IGFR | IR/IGFR | IGFR/IR |
|---|---|---|---|---|---|---|
| H11 | FHETFYD.LGRLVFGGSGGSGGSHLCVPEELFWGTSLLSYCSG | 9.3 | 0.4 | 4.0 | 0.1 | 11.4 |
| F11 | FHENFYDWFVRQVSGGSGGSGGSHL.GSG | 12.5 | 0.8 | 5.2 | 0.2 | 6.5 |
| E2 | FHENFYDWFVRQVSGGSGGSGGSHRCGLEEPV.GASLVGYCAG | 13.4 | 1.3 | 7.5 | 0.2 | 5.7 |
| G7 | FHANFYDWFVRQV.GGSGGSGGSG | 16.1 | 2.1 | 9.8 | 0.2 | 4.7 |
| G12 | FHEDFYDWFVRQVGGGSGGSGGSHLCVREELF.GASLLGDCSG | 9.4 | 1.2 | 5.5 | 0.2 | 4.6 |
| H7 | .HENFYDWFVRQLSGGSGGSDGSHLFGYGSG | 7.2 | 0.6 | 2.7 | 0.2 | 4.5 |
| G11 | OUT OF FRAME | 11.4 | 1.4 | 5.8 | 0.2 | 4.3 |
| F7 | FHENFYDWFDRQVGGGSGGSGGFSPVRTGRTVLGGFSVRLLLW | 15.0 | 2.7 | 10.9 | 0.2 | 4.1 |
| G1 | SHDNFYDWFVR.VSGGSGGSGGSPLCVLGNCSG | 11.3 | 2.8 | 10.6 | 0.3 | 3.8 |
| E8 | FYDNFYHWFDR.VSGGSGGSGGSHLCVLEERVCGASLFDYRSG | 13.5 | 0.9 | 3.4 | 0.3 | 3.7 |
| E9 | FSEHFYDWFARQVSGGSGGSGGSHLCVLDERF.GASMVGYCSG | 14.5 | 0.7 | 2.3 | 0.3 | 3.6 |
| G2 | FPENFYDWFDRQVSGGSGGGASLFG.GSG | 15.3 | 3.8 | 13.1 | 0.3 | 3.5 |
| E3 | FHENFYDWFDRQVSGGSSGSGGSHQCVQEERFWGASLCGYCSG | 15.9 | 1.9 | 6.7 | 0.3 | 3.5 |
| E12 | FHDSFYDWFVRQVSGGSGGSGGSHLCGLEELF.GASRFGDCSG | 10.0 | 2.3 | 6.8 | 0.3 | 2.9 |
| E5 | OUT OF FRAME | 14.7 | 3.7 | 9.6 | 0.4 | 2.6 |
| F8 | FHGDFYDWFVR.VSGGSGGSGGSHLCVLEELYCSG | 13.7 | 3.6 | 9.5 | 0.4 | 2.6 |
| E6 | FHDNFYDWFVR.VSGGSGGSGGSHLCVVEERFWGGSPIGYCSG | 13.3 | 3.0 | 7.3 | 0.4 | 2.5 |
| G8 | OUT OF FRAME | 13.9 | 4.5 | 10.5 | 0.4 | 2.4 |
| E1 | FQDNFYDWFVRQVSGGSGGSGGSHRCVLEGCSG | 13.4 | 5.8 | 13.3 | 0.4 | 2.3 |
| H12 | FHENFYDWFDRQVSGGSACLFGYCSG | 9.8 | 3.9 | 8.5 | 0.5 | 2.2 |
| F2 | YHENFYDWFVR.VSGGSG | 14.4 | 6.2 | 12.8 | 0.5 | 2.1 |
| H6 | VHESFYDWFVR.VAGGSGGSGGSHLCDVDCSG | 11.5 | 4.8 | 9.6 | 0.5 | 2.0 |
| H4 | FHDNFYDWFDRQVSGGSGGSGSPFG.RSD | 11.2 | 5.3 | 10.0 | 0.5 | 1.9 |
| H5 | FH.HFYDWFDRQVSGGSGGSGGSLLCVGEEPFWGASLFAYCSG | 11.8 | 4.4 | 8.5 | 0.5 | 1.9 |
| E7 | FHENFYDWFVRQVSGGSGGSGG | 15.4 | 7.8 | 14.0 | 0.6 | 1.8 |
| F5 | FHESFYDWFVR.VPGGSGGSGGSQLCVQEELFEGDSLLGDCSG | 16.8 | 7.3 | 12.9 | 0.6 | 1.8 |
| F10 | | 13.9 | 5.9 | 10.8 | 0.5 | 1.8 |
| E10 | FHENFYEWFDRQVSGGSGGVLDERF.GACPSGYCSG | 10.6 | 5.1 | 8.9 | 0.6 | 1.8 |

FIG. 44B-1

| Clone | Sequence | E-Tag | IR | IGFR | IR/IGFR | IGFR/IR |
|---|---|---|---|---|---|---|
| F4 | FHDNFYDWFVR.VAGGSGGSGGSHLCVPEELFWGASLFGYCSG | 15.7 | 3.1 | 5.6 | 0.6 | 1.8 |
| H2 | FGEDFYDWFVR.VSGGSGGSGGSHLCVLDELFWDASPFGFCPG | 11.4 | 2.5 | 4.6 | 0.6 | 1.8 |
| E11 | .HDNFYGWFDRQVSGGSGGSGGSHLCVLDELLWGASLFGYCS | 11.7 | 1.3 | 2.2 | 0.6 | 1.7 |
| F12 | FQENFYDWFVR.VSGDELSGGASQCGSCSG | 10.6 | 7.0 | 9.6 | 0.7 | 1.4 |
| F9 | SHESFYDWFVRQVSGGSGGSGGSDLCVWEELCGGAPLVG.GSS | 16.0 | 9.9 | 13.3 | 0.7 | 1.3 |
| E4 | FPENFYDWFDRQVSGGSGGSGGSSG | 16.4 | 13.4 | 15.8 | 0.8 | 1.2 |
| H10 | FRENFYDWFERQVSGGSGGSGGSHLCVLEELSWGASTFGSCSG | 10.8 | 7.8 | 9.1 | 0.9 | 1.2 |
| F3 | IHVDFYDWFAR.VSGGSGGSGGSSLCVLDELFWDASLFGDCAG | 14.2 | 3.9 | 4.6 | 0.8 | 1.2 |
| G6 | FHASFYDWFDRQVSGGSGGSGGSHLCDLEGLFWGAAPFGYCSG | 16.2 | 11.0 | 12.1 | 0.9 | 1.1 |
| H3 | SDANFYDWFLR.VSGGSGGSGGSHLCALEEQFWDASLFGDCSG | 13.1 | 9.8 | 11.1 | 0.9 | 1.1 |
| G5 | FHDKFYDWFVS.VAGGSGGSGGSHLCVLEDRFWGSSLSGYCSG | 14.7 | 7.1 | 7.9 | 0.9 | 1.1 |
| H9 | FHDNFYDWFVRQVTDGSGGSGGSQLCVVEDLFWDASRFGYC.G | 13.1 | 8.2 | 8.0 | 1.0 | 1.0 |
| G3 | VSEDFYEWFVR.ASGGSGGSGGSNLCVLEELFWGSSLIGDCSG | 13.7 | 11.7 | 2.5 | 4.6 | 0.2 |
| G4 | FPENFYDWFVRQVSGGSGGSGGSHLCVLEEL.WGASMFGYCSG | 10.0 | 4.3 | 0.7 | 6.0 | 0.2 |
| F6 | FQENFYDWFVRQVSGGSGGSGGSHLCVLEALFWGASLFG.CSG | 5.6 | 9.0 | 0.4 | 21.2 | 0.0 |
| **Non-Binders:** | | | | | | |
| H1 | DYKDGRGGRRF.GRSSVVLWKRL.R | 1.2 | 0.7 | 0.5 | 1.5 | 0.7 |
| G10 | DTKTFIGITGVLPRLSAV.GFWGGSW | 1.7 | 0.3 | 0.3 | 0.8 | 1.2 |
| G9 | CHENFYVWFVSQVAGGSGGSGGSRLCIM.ELFRGASLFGYSSG | 2.0 | 0.4 | 0.5 | 0.9 | 1.1 |
| F1 | FHANFYDWFVR.VSGGSGGSGGSHLCVLEELVSGPSLLGYCSG | 14.5 | 0.6 | 1.5 | 0.4 | 2.3 |
| H8 | FHEKFYDWFDL.LSGGSGGSGGSHLCVREEPFWGASLFGYCPG | 9.7 | 0.6 | 1.5 | 0.4 | 2.3 |

FIG. 44B-2

## IGFR Binders with change in Cys

| Clone | Sequence | E-Tag | IR | IGFR | IR/IGFR | IGFR/IR |
|---|---|---|---|---|---|---|
| F8(X14) | HLCVLEELFWGASLFGYCSG | | | | | |
| D8(X6) | WLDQEWAWVQCEVYGRGCPS | | | | | |
| G1(X4) | SHDNFYDWFVR.VSGGSGGSGGSPLCVLGNCSG | 11.3 | 2.8 | 10.6 | 0.3 | 3.8 |
| E8(X6) | FYDNFYHWFDR.VSGGSGGSGGSHLCVLEERVCGASLFDYRSG | 13.5 | 0.9 | 3.4 | 0.3 | 3.7 |
| E3(X10) | FHENFYDWFDRQVSGGSSGSGGSHQCVQEERFWGASLCGYCSG | 15.9 | 1.9 | 6.7 | 0.3 | 3.5 |
| F8(X6) | FHGDFYDWFVR.VSGGSGGSGGSHLCVLEELYCSG | 13.7 | 3.6 | 9.5 | 0.4 | 2.6 |
| E1(X4) | FQDNFYDWFVRQVSGGSGGSGGSHRCVLEGCSG | 13.4 | 5.8 | 13.3 | 0.4 | 2.3 |
| H12(X4) | FHENFYDWFDRQVSGGSACLFGYCSG | 9.8 | 3.9 | 8.5 | 0.5 | 2.2 |
| H6(X3) | VHESFYDWFVR.VAGGSGGSGGSHLCDVDCSG | 11.5 | 4.8 | 9.6 | 0.5 | 2.0 |
| E10(X4) | FHENFYEWFDRQVSGGSGGVLDERF.GACPSGYCSG | 10.6 | 5.1 | 8.9 | 0.6 | 1.8 |
| F12(X2) | FQENFYDWFVR.VSGDELSGGASQCGSCSG | 10.6 | 7.0 | 9.6 | 0.7 | 1.4 |
| F9(X5) | SHESFYDWFVRQVSGGSGGSGGSDLCVWEELCGGAPLVG.GSS | 16.0 | 9.9 | 13.3 | 0.7 | 1.3 |

## IGFR Binders with loss of F8

| | | | | | | |
|---|---|---|---|---|---|---|
| G7 | FHANFYDWFVRQV.GGSGGSGGSG | 16.1 | 2.1 | 9.8 | 0.2 | 4.7 |
| F2 | YHENFYDWFVR.VSGGSG | 14.4 | 6.2 | 12.8 | 0.5 | 2.1 |
| E7 | FHENFYDWFVRQVSGGSGGSGG | 15.4 | 7.8 | 14.0 | 0.6 | 1.8 |
| E4 | FPENFYDWFDRQVSGGSGGSGGSSG | 16.4 | 13.4 | 15.8 | 0.8 | 1.2 |

## IGFR Binders with loss of Cys in F8

| | | | | | | |
|---|---|---|---|---|---|---|
| F11 | FHENFYDWFVRQVSGGSGGSGGSHL.GSG | 12.5 | 0.8 | 5.2 | 0.2 | 6.5 |
| H7 | .HENFYDWFVRQLSGGSGGSDGSHLFGYGSG | 7.2 | 0.6 | 2.7 | 0.2 | 4.5 |
| F7 | FHENFYDWFDRQVGGGSGGSGGFSPVRTGRTVLGGFSVRLLLW | 15.0 | 2.7 | 10.9 | 0.2 | 4.1 |
| G2 | FPENFYDWFDRQVSGGSGGGASLFG.GSG | 15.3 | 3.8 | 13.1 | 0.3 | 3.5 |
| H4 | FHDNFYDWFDRQVSGGSGGSGSPFG.RSD | 11.2 | 5.3 | 10.0 | 0.5 | 1.9 |

FIG. 44B-3

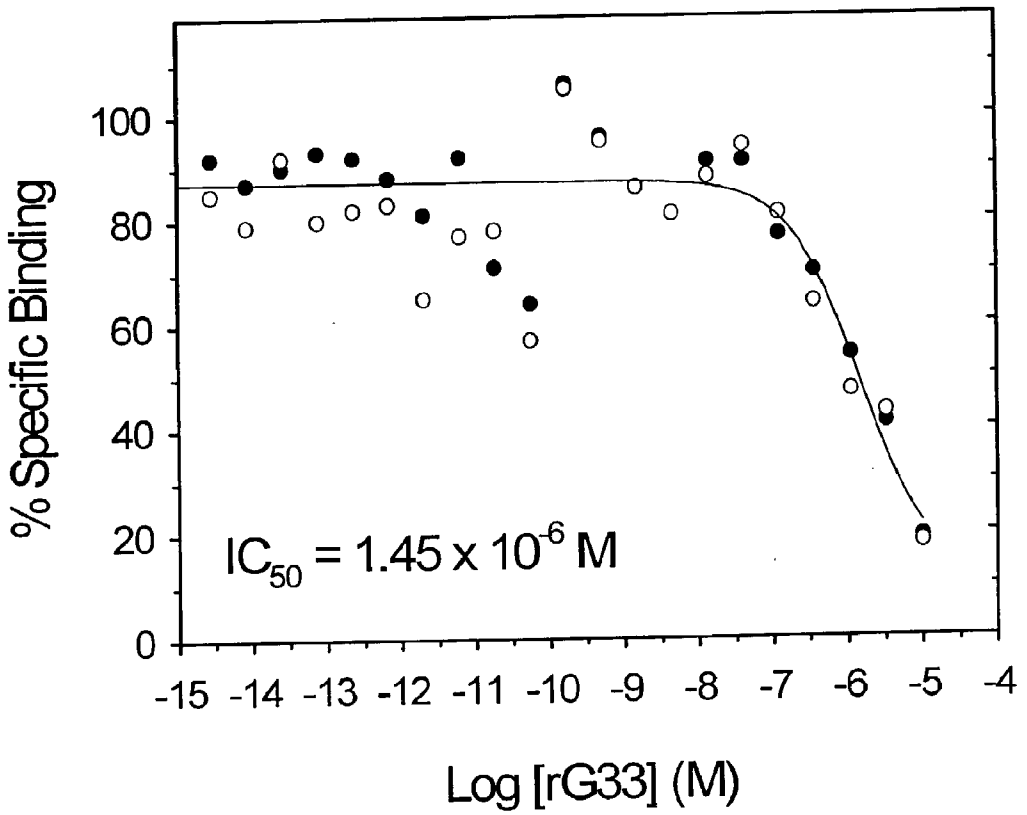

% Specific Binding
100
80
60
40
20
0
IC50 = 1.45 x 10-6 M
-15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
Log [rG33] (M)

FIG. 45

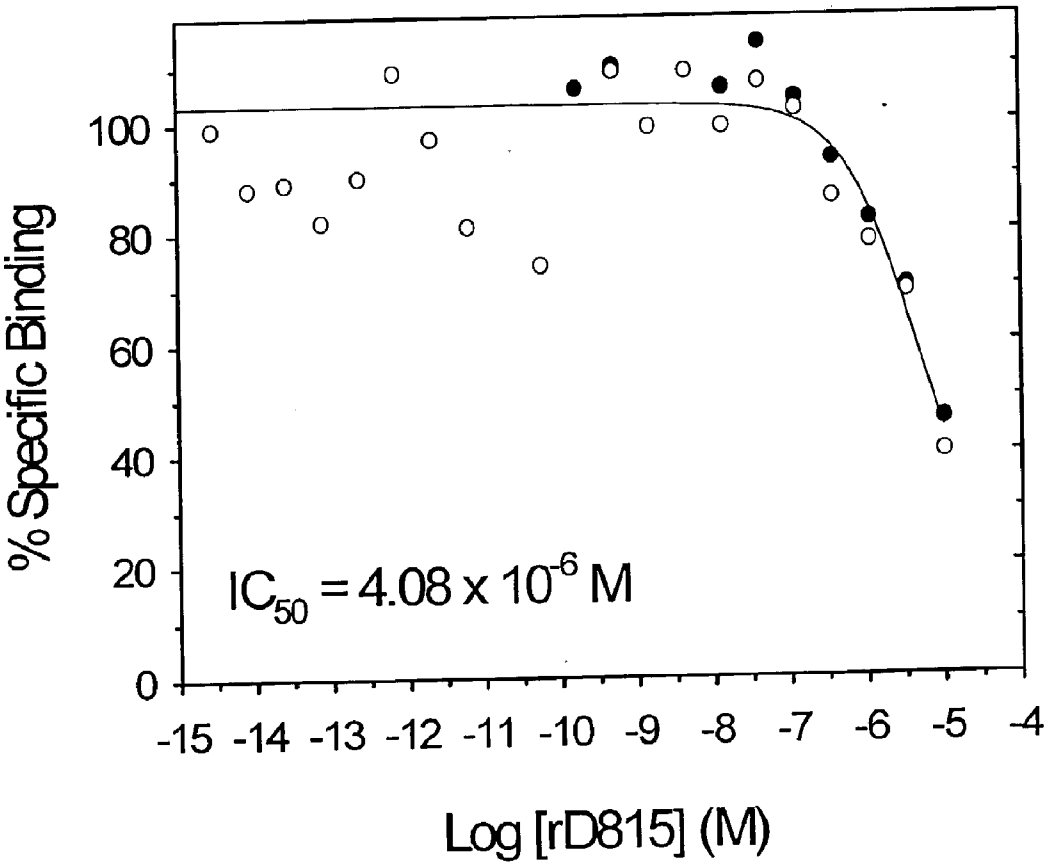

% Specific Binding
100
80
60
40
20
0
$IC_{50} = 4.08 \times 10^{-6}$ M
-15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
Log [rD815] (M)

FIG. 46

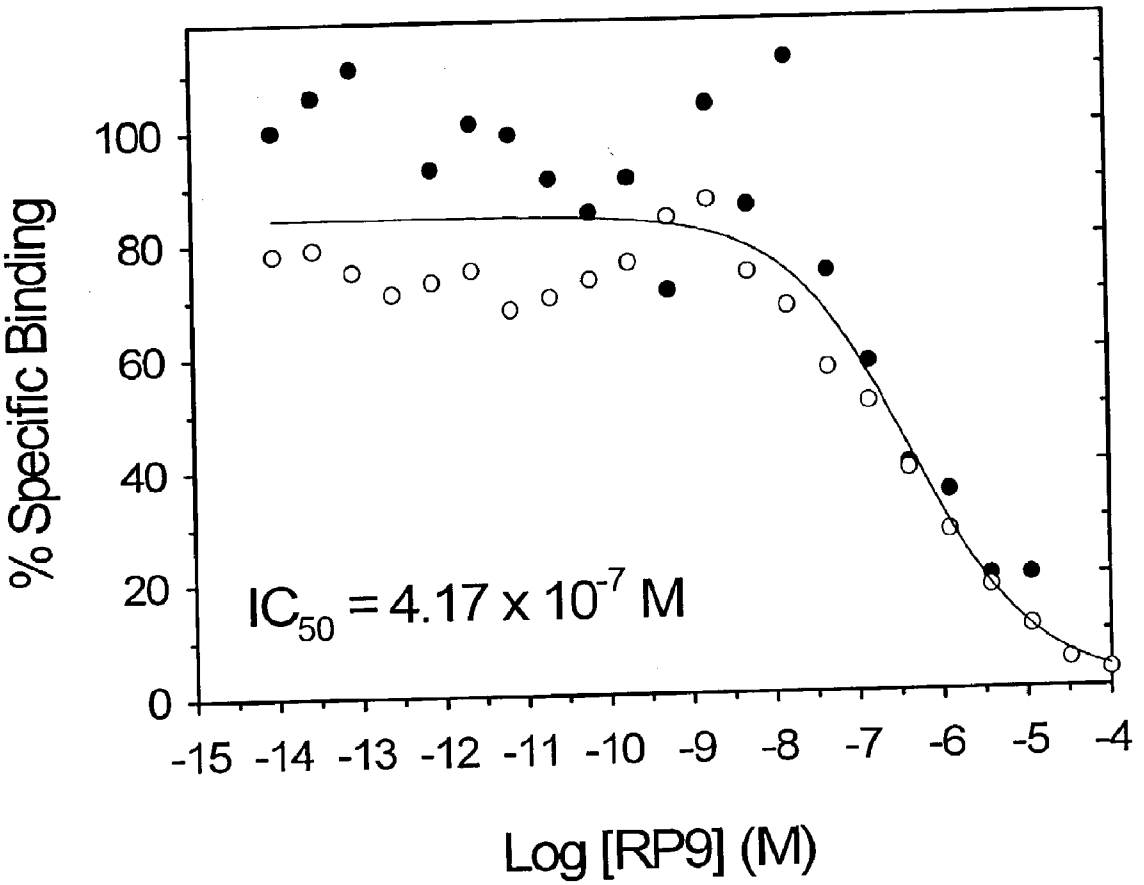

% Specific Binding
100
80
60
40
20
0
$IC_{50} = 4.17 \times 10^{-7}$ M
-15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
Log [RP9] (M)

FIG. 47

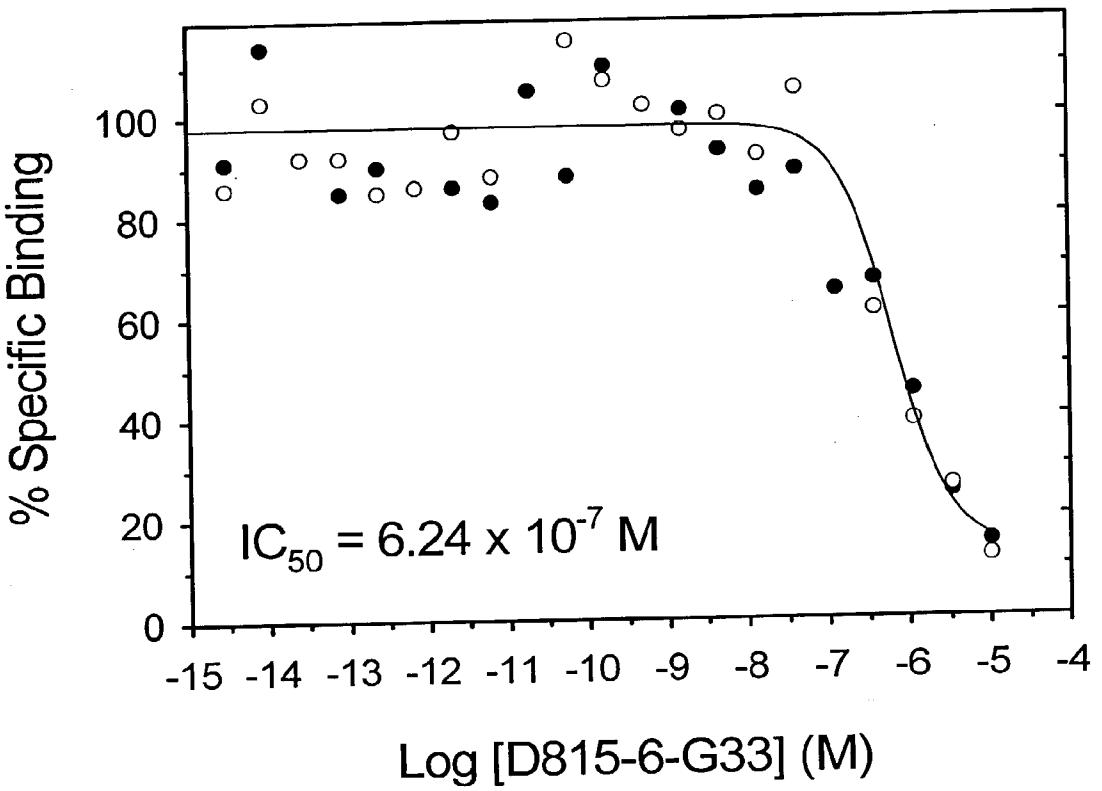

% Specific Binding
100
80
60
40
20
0
$IC_{50} = 6.24 \times 10^{-7}$ M
-15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
Log [D815-6-G33] (M)

FIG. 48

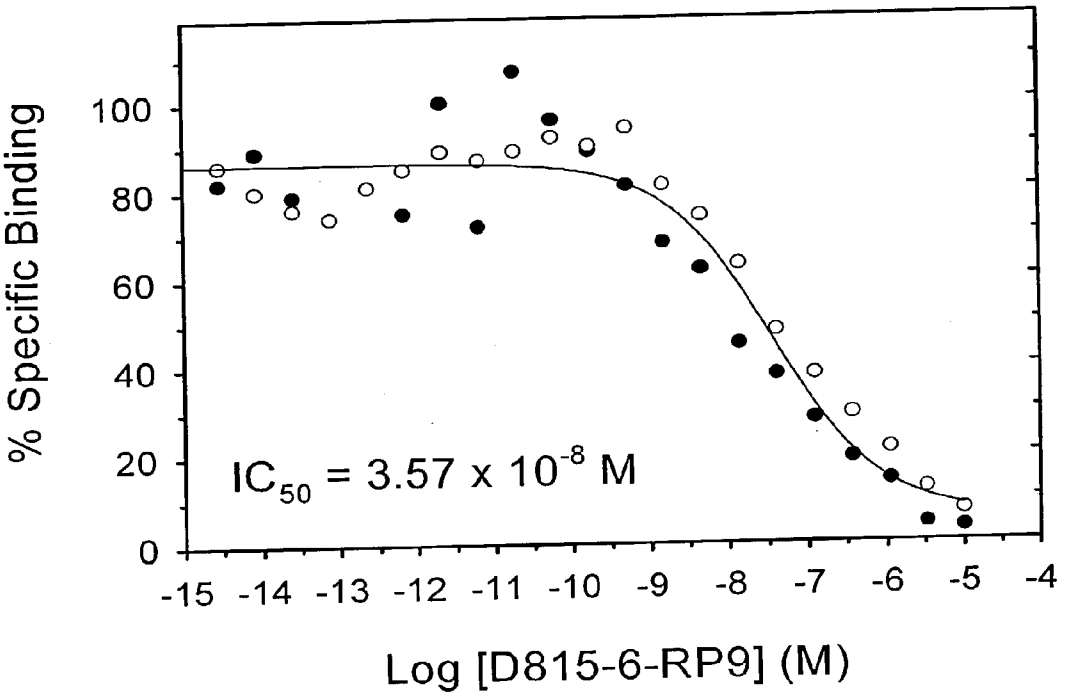

% Specific Binding
100
80
60
40
20
0
$IC_{50}$ = 3.57 x $10^{-8}$ M
-15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
Log [D815-6-RP9] (M)

FIG. 49

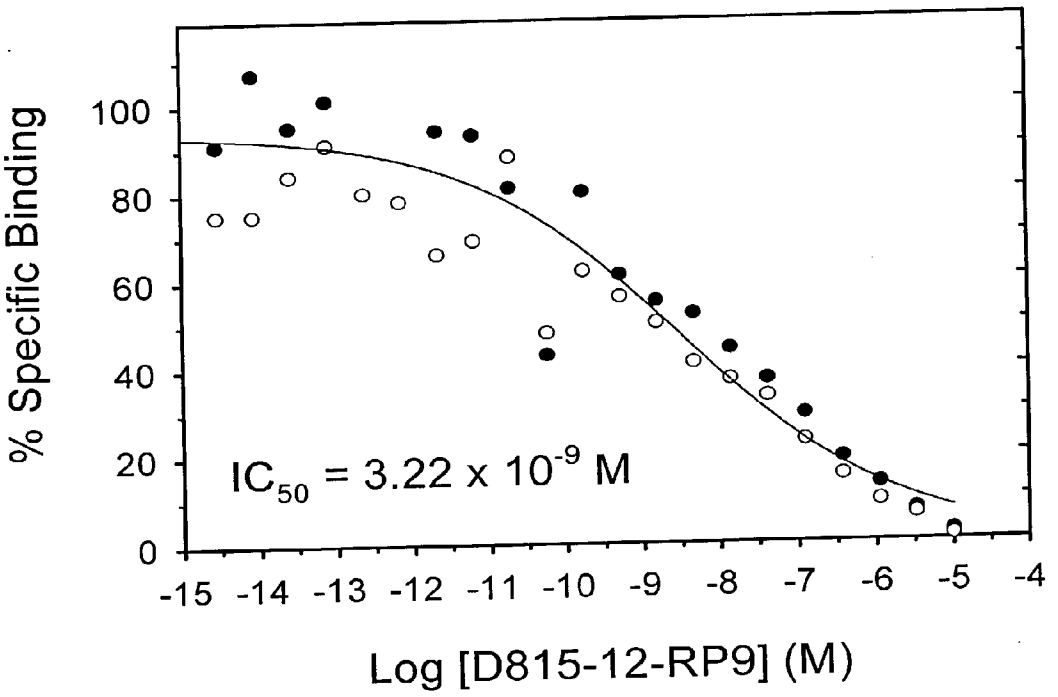

% Specific Binding
100
80
60
40
20
0
IC50 = 3.22 x 10-9 M
-15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
Log [D815-12-RP9] (M)

FIG. 50

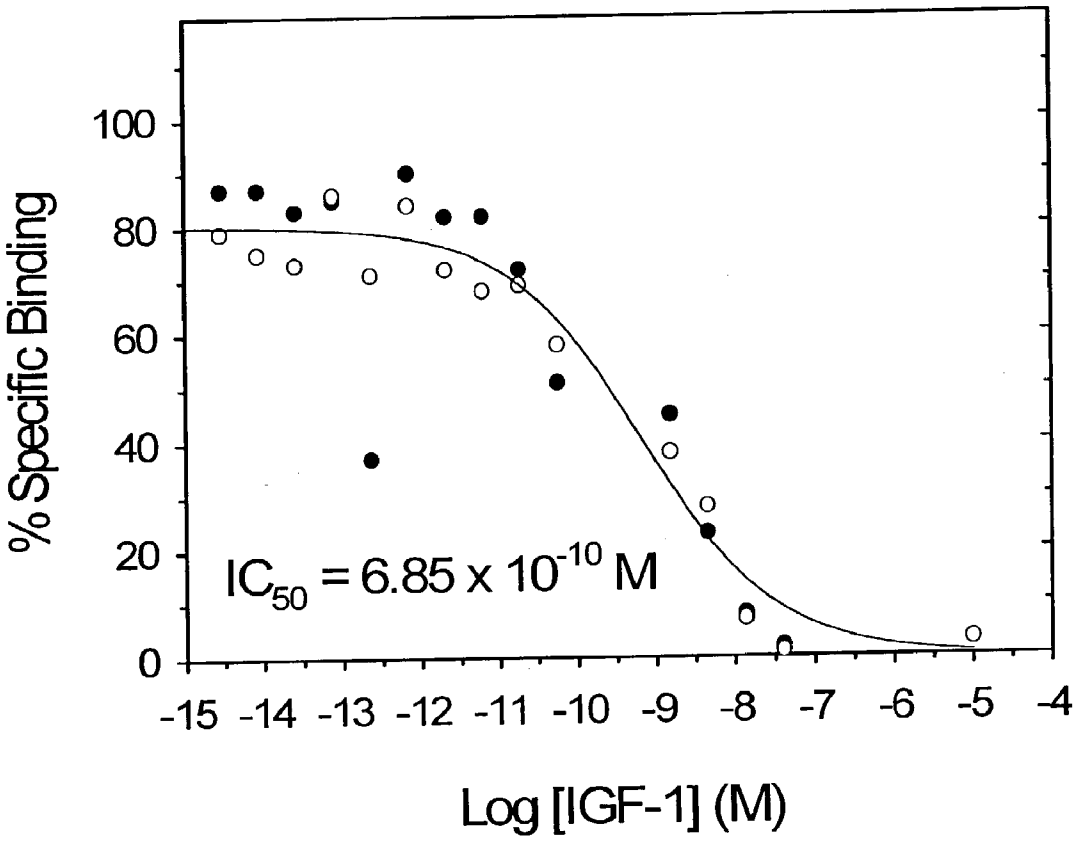

% Specific Binding
100
80
60
40
20
0
$IC_{50} = 6.85 \times 10^{-10}$ M
-15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
Log [IGF-1] (M)

FIG. 51

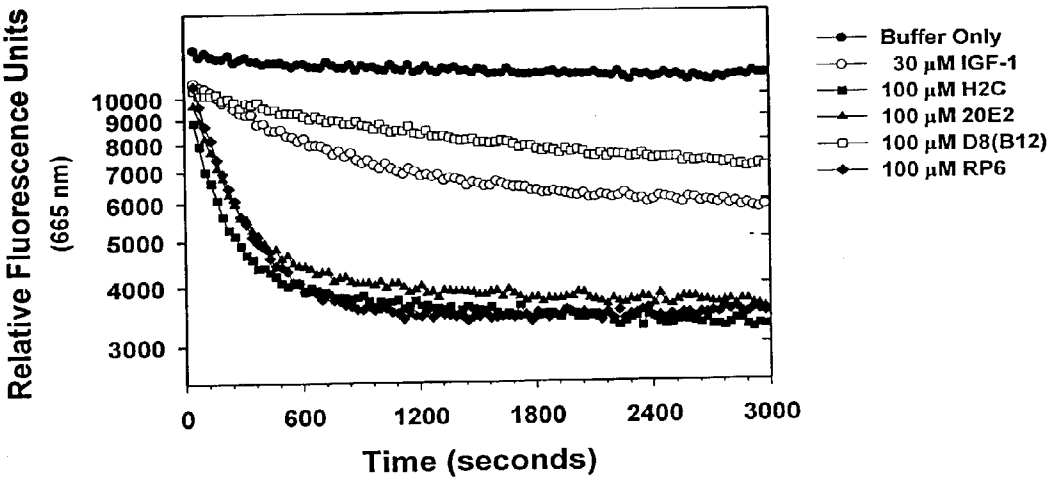

Relative Fluorescence Units
(665 nm)
10000
9000
8000
7000
6000
5000
4000
3000
0
600
1200
1800
2400
3000
Time (seconds)
Buffer Only
30 µM IGF-1
100 µM H2C
100 µM 20E2
100 µM D8(B12)
100 µM RP6

FIG. 52

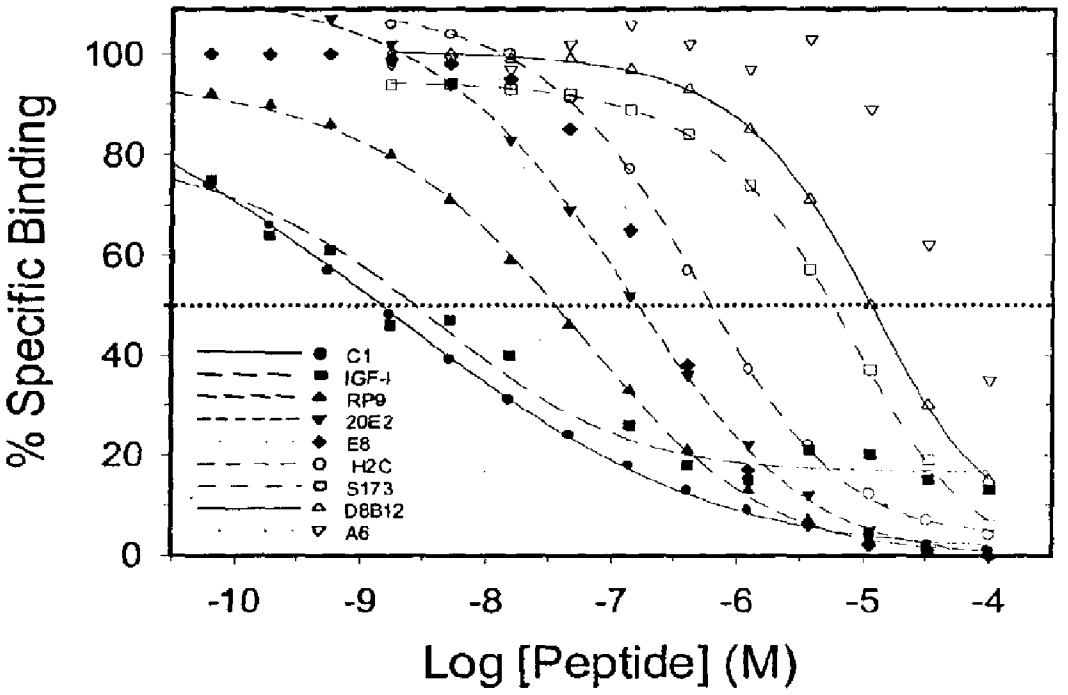

% Specific Binding
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
-4
Log [Peptide] (M)
C1
IGF-I
RP9
20E2
E8
H2C
S173
D8B12
A6

FIG. 53

Figure 54:

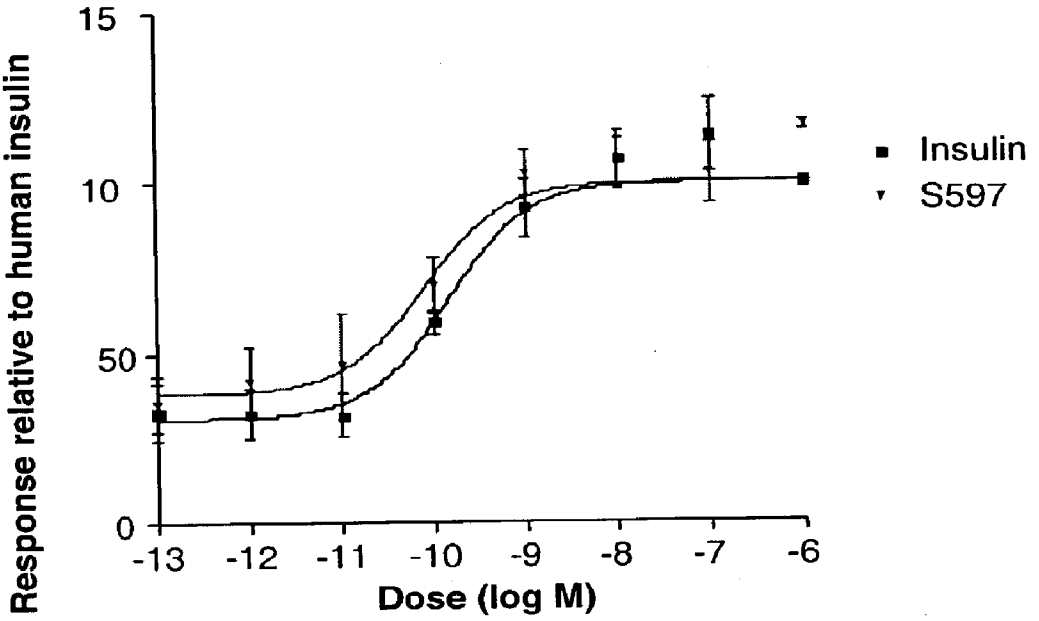

15
10
50
0
Response relative to human insulin
-13
-12
-11
-10
-9
-8
-7
-6
Dose (log M)
Insulin
S597

Figure 55:

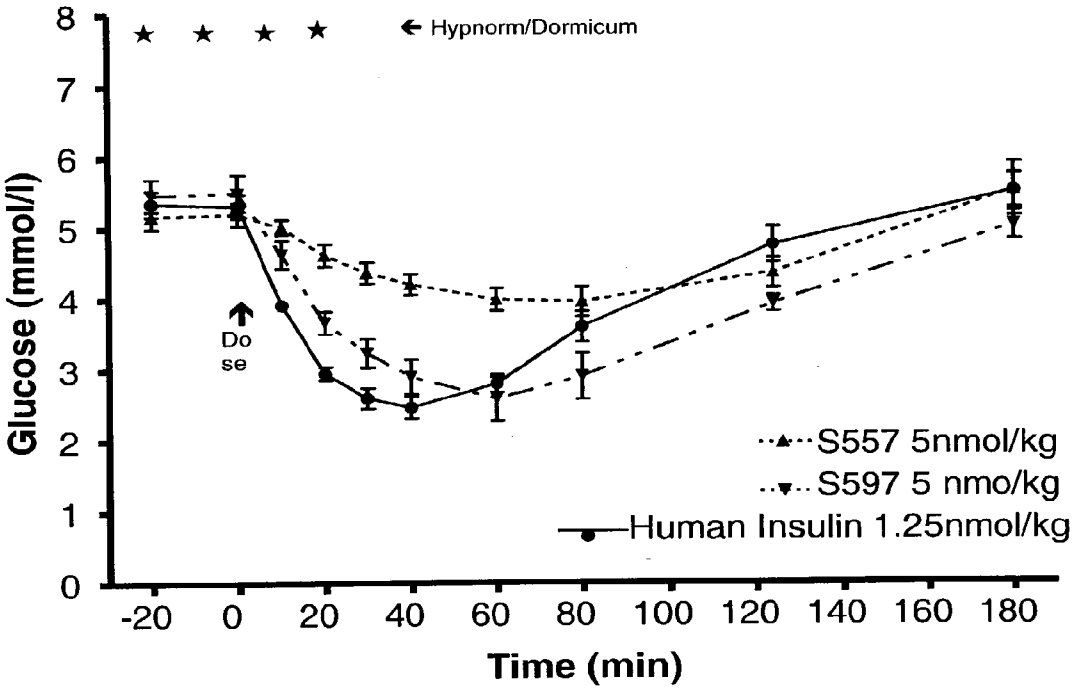

Hypnorm/Dormicum
Dose
S557 5nmol/kg
S597 5 nmo/kg
Human Insulin 1.25nmol/kg
Glucose (mmol/l)
Time (min)
0
1
2
3
4
5
6
7
8
-20
0
20
40
60
80
100
120
140
160
180

Figure 56:

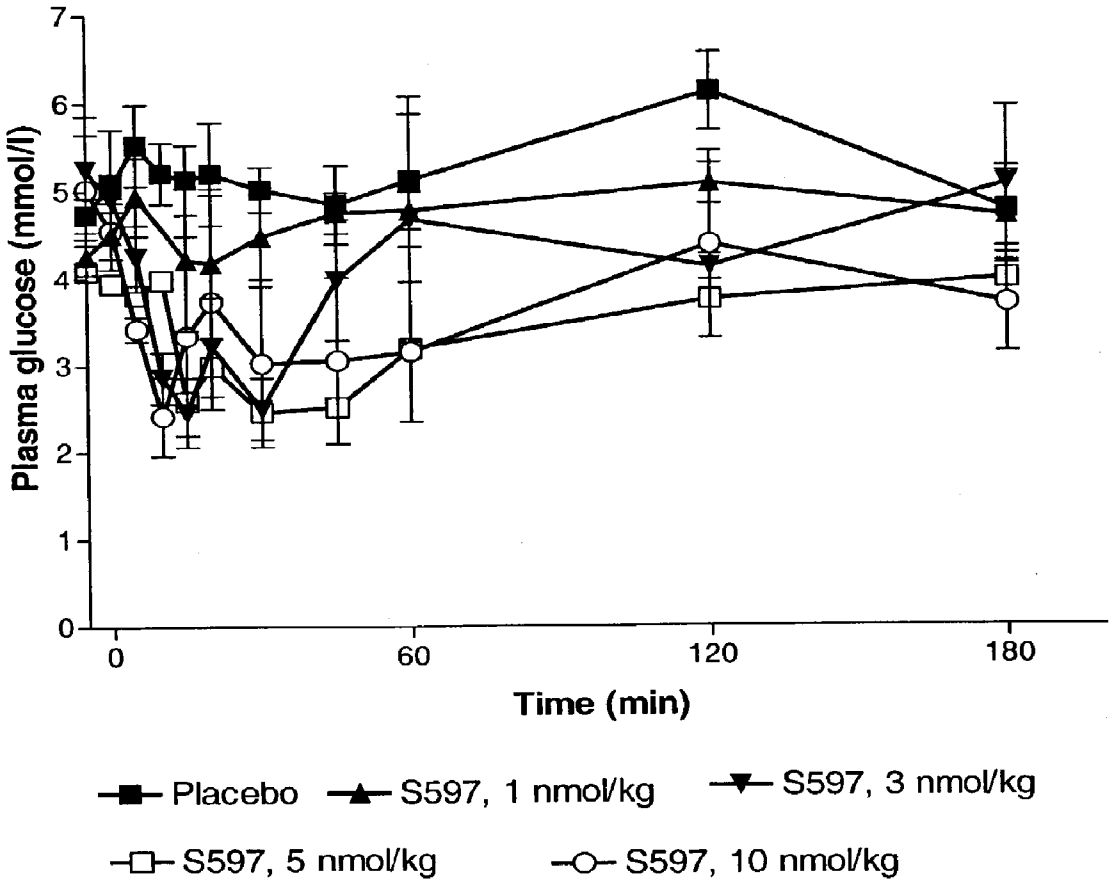
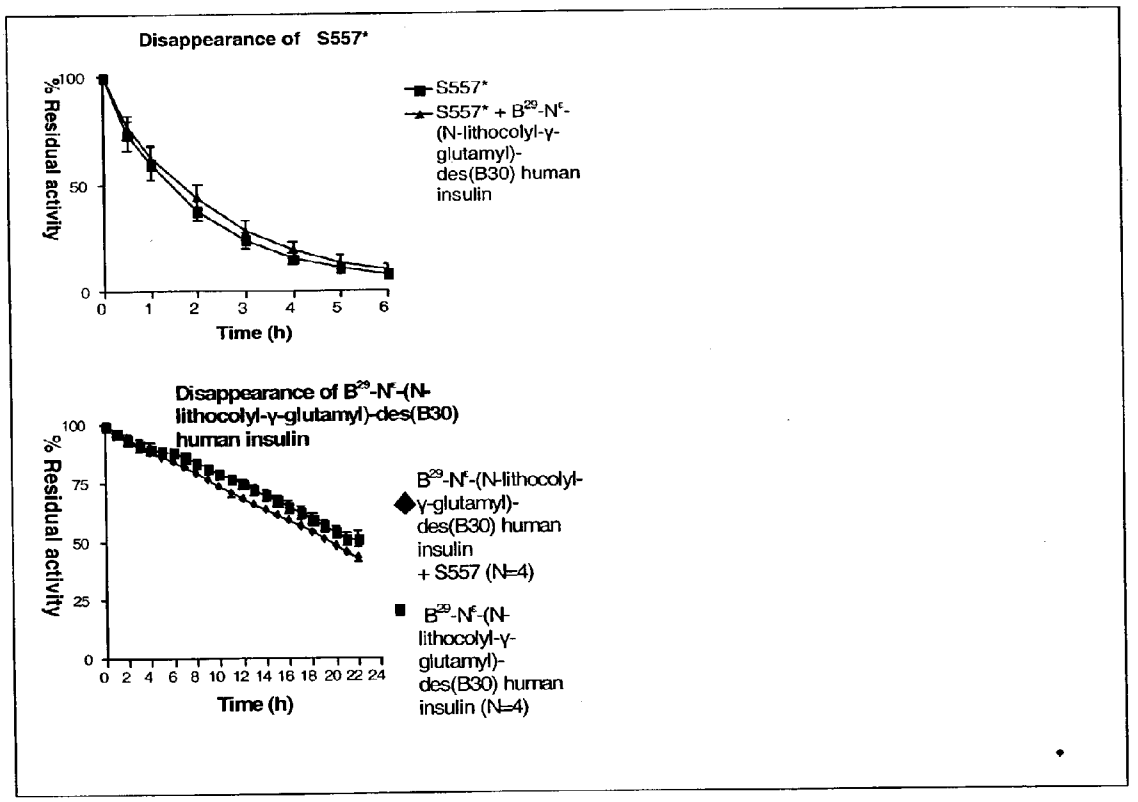

Plasma glucose (mmol/l)
0
1
2
3
4
5
6
7
0
60
120
180
Time (min)
Placebo
S597, 1 nmol/kg
S597, 3 nmol/kg
S597, 5 nmol/kg
S597, 10 nmol/kg

Figure 57


Disappearance of S557*
% Residual activity
100
50
0
0 1 2 3 4 5 6
Time (h)
S557*
S557* + B29-Nε-(N-lithocolyl-γ-glutamyl)-des(B30) human insulin
Disappearance of B29-Nε-(N-lithocolyl-γ-glutamyl)-des(B30) human insulin
% Residual activity
100
75
50
25
0
0 2 4 6 8 10 12 14 16 18 20 22 24
Time (h)
B29-Nε-(N-lithocolyl-γ-glutamyl)-des(B30) human insulin + S557 (N=4)
B29-Nε-(N-lithocolyl-γ-glutamyl)-des(B30) human insulin (N=4)

# INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

[0001] This application is a continuation of U.S. application Ser. No. 10/253,471, filed Sep. 24, 2002, which is a continuation-in-part of U.S. application Ser. No. 09/962,756, filed Sep. 24, 2001, which is a continuation-in-part of U.S. application Ser. No. 09/538,038 filed Mar. 29, 2000, which is a continuation-in-part of U.S. application Ser. No. 09/146,127, filed Sep. 2, 1998, both of which are incorporated by reference in their entirety.

## FIELD OF THE INVENTION

[0002] This invention relates to the field of hormone receptor activation or inhibition. More specifically, this invention relates to the identification of molecular structures, especially peptides, which are capable of acting at either the insulin or insulin-like growth factor receptors as agonists or antagonists. Also related to this invention is the field of molecular modeling whereby useful molecular models are derived from known structures.

## BACKGROUND OF THE INVENTION

[0003] Insulin is a potent metabolic and growth promoting hormone that acts on cells to stimulate glucose, protein, and lipid metabolism, as well as RNA and DNA synthesis. A well-known effect of insulin is the regulation of glucose levels in the body. This effect occurs predominantly in liver, fat, and muscle tissue. In the liver, insulin stimulates glucose incorporation into glycogen and inhibits the production of glucose. In muscle and fat tissue, insulin stimulates glucose uptake, storage, and metabolism. Defects in glucose utilization are very common in the population, giving rise to diabetes.

[0004] Insulin initiates signal transduction in target cells by binding to a specific cell-surface receptor, the insulin receptor (IR). The binding leads to conformational changes in the extracellular domain of IR, which are transmitted across the cell membrane and result in activation of the receptor's tyrosine kinase activity. This, in turn, leads to autophosphorylation of tyrosine kinase of IR, and the binding of soluble effector molecules that contain SH2 domains such as phophoinositol-3-kinase, Ras GTPase-activating protein, and phospholipase Cγ to IR (Lee and Pilch, 1994, *Am. J. Physiol.* 266:C319-C334).

[0005] Insulin-like growth factor 1 (IGF-1) is a small, single-chain protein (MW=7,500 Da) that is involved in many aspects of tissue growth and repair. Recently, IGF-1 has been implicated in various forms cancer including prostate, breast, colorectal, and ovarian cancers. It is similar in size, sequence, and structure to insulin, but has 100-1,000-fold lower affinity for IR (Mynarcik et al., 1997, *J. Biol. Chem.* 272:18650-18655).

[0006] Clinically, recombinant human IGF-1 has been investigated for the treatment of several diseases, including type I diabetes (Carroll et al., 1997, *Diabetes* 46:1453-1458; Crowne et al., 1998, *Metabolism* 47:31-38), amyotropic lateral sclerosis (Lai et al., 1997, *Neurology* 49:1621-1630), and diabetic motor neuropathy (Apfel and Kessler, 1996, *CIBA Found. Symp.* 196:98-108). Other potential therapeutic applications of IGF-1, such as osteoporosis (Canalis, 1997, *Bone* 21:215-216), immune modulation (Clark, 1997, *Endocr. Rev.* 18:157-179) and nephrotic syndrome (Feld and Hirshberg, 1996, *Pediatr. Nephrol.* 10:355-358), are also under investigation.

[0007] A number of studies have analyzed the role of endogenous IGF-1 in various disease states. Interestingly, several reports have shown that IGF-1 promotes the growth of normal and cancerous prostate cells both in vitro and in vivo (Angelloz-Nicoud and Binoux, 1995, *Endocrinol.* 136:5485-5492; Figueroa et al., 1995, *J. Clin. Endocrinol. Metab.* 80:3476-3482; Torring et al., 1997, *J. Urol.* 158:222-227). Additionally, elevated serum IGF-1 levels correlate with increased risks of prostate cancer, and may be an earlier predictor of cancer than is prostate-specific antigen (PSA) (Chan et al., 1998, *Science* 279:563-566). Recent studies have indicated a connection between IGF-1 levels and other cancers such as breast, colorectal, and ovarian. Serum IGF-1 levels are regulated by the presence of IGF binding proteins (IGFBP) which bind to IGF-1 and prevent its interaction with the IGF-1 receptor (IGF-1R; reviewed in Conover, 1996, *Endocr. J.* 43S:S43-S48 and Rajaram et al., 1997, *Endocr. Rev.* 18:801-831). Interestingly, PSA has been shown to be a protease that cleaves IGFBP-3, resulting in an increase of free IGF-1 in serum (Cohen et al., 1992, *J. Clin. Endocrinol. Metab.* 75:1046-1053; Cohen et al., 1994, *J. Endocrinol.* 142:407-415; Lilja, 1995, *J. Clin. Lab. Invest. Suppl.* 220:47-56). Clearly, regulation of IGF-1R activity can play an important role in several disease states, indicating that there are potential clinical applications for both IGF-1 agonists and antagonists.

[0008] IGF-1R and IR are related members of the tyrosine-kinase receptor superfamily of growth factor receptors. Both types of receptors are composed of two α and two β subunits which form a disulfide-linked heterotetramer (β-α-α-β). These receptors have an extracellular ligand binding domain, a single transmembrane domain, and a cytoplasmic domain displaying the tyrosine kinase activity. The extracellular domain is composed of the entire α subunits and a portion of the N-terminus of the β subunits, while the intracellular portion of the β subunits contains the tyrosine kinase domain. Another family member is insulin-related receptor (IRR), for which no natural ligand is known.

[0009] While similar in structure, IGF-1R and IR serve different physiological functions. IR is primarily involved in metabolic functions whereas IGF-1R mediates growth and differentiation. However, both insulin and IGF-1 can induce both mitogenic and metabolic effects. Whether each ligand elicits both activities via its own receptor, or whether insulin exerts its mitogenic effects through its weak affinity binding to IGF-1R, and IGF-1 its metabolic effects through IR, remains controversial (De Meyts, 1994, *Horm. Res.* 42:152-169).

[0010] IR is a glycoprotein having molecular weight of 350-400 kDa (depending of the level of glycosylation). It is synthesized as a single polypeptide chain and proteolytically cleaved to yield a disulfide-linked monomer α-β insulin receptor. Two α-β monomers are linked by disulfide bonds between the α-subunits to form a dimeric form of the receptor (β-α-α-β-type configuration). The α subunit is comprised of 723 amino acids, and it can be divided into two large homologous domains, L1 (amino acids 1-155) and L2 (amino acids 313-468), separated by a cysteine rich region (amino acids 156-312) (Ward et al., 1995, *Prot. Struct.*

*Funct. Genet.* 22:141-153). Many determinants of insulin binding seem to reside in the α-subunit. A unique feature of IR is that it is dimeric in the absence of ligand.

[0011] The sequence of IR is highly homologous to the sequence of IGF-1R. The sequence identity level varies from about 40% to 70%, depending on the position within the α-subunit. The three-dimensional structures of both receptors may therefore be similar. The crystal structure of the first three domains of IGF-1R has been determined (Garrett et al., 1998, *Nature* 394:395-399). The L domains consist of a single-stranded right-handed β-helix (a helical arrangement of β-strands), while the cysteine-rich region is composed of eight disulfide-bonded modules.

[0012] The β-subunit of the insulin receptor has 620 amino acid residues and three domains: extracellular, transmembrane, and cytosolic. The extracellular domain is linked by disulfide bridges to the α-subunit. The cytosolic domain includes the tyrosine kinase domain, the three-dimensional structure of which has been solved (Hubbard et al., 1994, *Nature* 372:746-754).

[0013] To aid in drug discovery efforts, a soluble form of a membrane-bound receptor was constructed by replacing the transmembrane domain and the intracellular domain of IR with constant domains from immunoglobulin Fc or λ subunits (Bass et al., 1996, *J. Biol. Chem.* 271:19367-19375). The recombinant gene was expressed in human embryonic kidney 293 cells. The expressed protein was a fully processed heterotetramer and the ability to bind insulin was similar to that of the full-length holoreceptor.

[0014] IGF-1 and insulin competitively cross-react with IGF-1R and IR. (L. Schäffer, 1994, *Eur. J. Biochem.* 221:1127-1132). Despite 45% overall amino acid identity, insulin and IGF-1 bind only weakly to each other's receptor. The affinity of each peptide for the non-cognate receptor is about 3 orders of magnitude lower than that for the cognate receptor (Mynarcik, et al., 1997, *J. Biol. Chem.* 272:18650-18655). The differences in binding affinities may be partly explained by the differences in amino acids and unique domains which contribute to unique tertiary structures of ligands (Blakesley et al., 1996, *Cytokine Growth Factor Rev.* 7(2):153-9).

[0015] Both insulin and IGF-1 are expressed as precursor proteins comprising, among other regions, contiguous A, B, and C peptide regions, with the C peptide being an intervening peptide connecting the A and B peptides. A mature insulin molecule is composed of the A and B chains connected by disulfide bonds, where the connecting C peptide has been removed during post-translational processing. IGF-1 retains its smaller C-peptide as well as a small D extension at the C-terminal end of the A chain, making the mature IGF-1 slightly larger than insulin (Blakesley, 1996). The C region of human IGF-1 appears to be required for high affinity binding to IGF-1R (Pietrzkowski et al., 1992, *Cancer Res.* 52(23):6447-51). Specifically, tyrosine 31 located within this region appears to be essential for high affinity binding. Furthermore, deletion of the D domain of IGF-1 increased the affinity of the mutant IGF-1 for binding to the IR, while decreasing its affinity for the IGF-1R (Pietrzkowski et al., 1992). A further distinction between the two hormones is that, unlike insulin, IGF-1 has very weak self-association and does not hexamerize (De Meyts, 1994).

[0016] The α-subunits, which contain the ligand binding region of IR and IGF-1R, demonstrate between 47-67% overall amino acid identity. Three general domains have been reported for both receptors from sequence analysis of the α subunits, L1-Cys-rich-L2. The cysteine residues in the C-rich region are highly conserved between the two receptors; however, the cysteine-rich domains have only 48% overall amino acid identity.

[0017] Despite the similarities observed between these two receptors, the role of the domains in specific ligand binding are distinct. Through chimeric receptor studies, (domain swapping of the IR and IGF-1R α-subunits), researchers have reported that the sites of interaction of the ligands with their specific receptors differ (T. Kjeldsen et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:4404-4408; A. S. Andersen et al., 1992, *J. Biol. Chem.* 267:13681-13686). For example, the cysteine-rich domain of the IGF-1R was determined to be essential for high-affinity IGF binding, but not insulin binding. When amino acids 191-290 of IGF-1R region was introduced into the corresponding region of the IR (amino acids 198-300), the modified IR bound both IGF-1 and insulin with high affinity. Conversely, when the corresponding region of the IR was introduced into the IGF-1R, the modified IGF-1R bound to IR but not IGF-1.

[0018] A further distinction between the binding regions of the IR and IGF-1R is their differing dependence on the N-terminal and C-terminal regions. Both the N-terminal and C-terminal regions (located within the putative L1 and L2 domains) of the IR are important for high-affinity insulin binding but appear to have little effect on IGF-1 binding for either IR or IGF-1R. Replacing residues in the N-terminus of IGF-1R (amino acids 1-62) with the corresponding residues of IR (amino acids 1-68) confers insulin-binding ability on IGF-1R. Within this region, residues Phe-39, Arg-41 and Pro-42 are reported as major contributors to the interaction with insulin (Williams et al., 1995). When these residues are introduced into the equivalent site of IGF-1R, the affinity for insulin is markedly increased, whereas, substitution of these residues by alanine in IR results in markedly decreased insulin affinity. Similarly, the region between amino acids 704-717 of the C-terminus of IR has been shown to play a major role in insulin specificity. Substitution of these residues with alanine also disrupts insulin binding (Mynarcik et al., 1996, *J. Biol. Chem.* 271(5):2439-42; C. Kristensen et al., 1999, *J. Biol. Chem.* 274(52):37351-37356).

[0019] Further studies of alanine scanning of the receptors suggest that insulin and IGF-1 may use some common contacts to bind to IGF-1R but that those contacts differ from those that insulin utilizes to bind to IR (Mynarcik et al., 1997). Hence, the data in the literature has led one commentator to state that even though "the binding interfaces for insulin and IGF-1 on their respective receptors may be homologous within this interface the side chains which make actual contact and determine specificity may be quite different between the two ligand-receptor systems" (De Meyts, 1994).

[0020] Based on data for binding of insulin and insulin analogs to various insulin receptor constructs, a binding model has been proposed. This model shows insulin receptor with two insulin binding sites that are positioned on two different surfaces of the receptor molecule, such that each alpha-subunit is involved in insulin binding. In this way, activation of the insulin receptor is believed to involve cross-connection of the alpha-subunits by insulin. A similar

mechanism may operate for IGF-1R, but one of the receptor binding interactions appears to be different (Schäffer, 1994, *Eur. J. Biochem.* 221:1127-1132).

[0021] The identification of molecular structures having a high degree of specificity for one or the other receptor is important to developing efficacious and safe therapeutics. For example, a molecule developed as an insulin agonist should have little or no IGF-1 activity in order to avoid the mitogenic activity of IGF-1 and a potential for facilitating neoplastic growth. It is therefore important to determine whether insulin and IGF-1 share common three-dimensional structures but which have sufficient differences to confer selectivity for their respective receptors. Similarly, it would be desirable to identify other molecular structures that mimic the active binding regions of insulin and/or IGF-1 and which impart selective agonist or antagonist activity.

[0022] Although certain proteins are important drugs, their use as therapeutics presents several difficult problems, including the high cost of production and formulation, administration usually via injection and limited stability in the bloodstream. Therefore, replacing proteins, including insulin or IGF-1, with small molecular weight drugs has received much attention. However, to date, none of these efforts has resulted in finding an effective drug replacement.

[0023] Peptides mimicking functions of protein hormones have been previously reported. Yanofsky et al. (1996, *Proc. Natl. Acad. Sci. USA* 93:7381-7386) reported the isolation of a monomer antagonistic to IL-1 with nanomolar affinity for the IL-1 receptor. This effort required construction and use of many phage displayed peptide libraries and sophisticated phage-panning procedures.

[0024] Wrighton et al. (1996, *Science* 273:458-464) and Livnah et al. (1996, *Science* 273:464-471) reported dimer peptides that bind to the erythropoietin (EPO) receptor with full agonistic activity in vivo. These peptides are cyclical and have intra-peptide disulfide bonds; like the IL-1 receptor antagonist, they show no significant sequence identity to the natural ligand. Importantly, X-ray crystallography revealed that it was the spontaneous formation of non-covalent peptide homodimer peptides that enabled the dimerization two EPO receptors.

[0025] WO 96/04557 reported the identification of peptides and antibodies that bound to active sites of biological targets, which were subsequently used in competition assays to identify small molecules that acted as agonist or antagonists at the biological targets. Renchler et al. (1994, *Proc. Natl. Acad. Sci. USA* 91:3623-3627) reported synthetic peptide ligands of the antigen binding receptor that induced programmed cell death in human B-cell lymphoma.

[0026] Most recently, Cwirla et al. (1997, *Science* 276:1696-1698) reported the identification of two families of peptides that bound to the human thrombopoietin (TPO) receptor and were competed by the binding of the natural TPO ligand. The peptide with the highest affinity, when dimerized by chemical means proved to be as potent an in vivo agonist as TPO, the natural ligand.

## SUMMARY OF THE INVENTION

[0027] This invention relates to the identification of amino acid sequences that specifically recognize sites involved in IR or IGF-1R activation. Specific amino acid sequences are identified and their agonist or antagonist activity at IR and/or IGF-1R has been determined. Such sequences may be developed as potential therapeutics or as lead compounds to develop other more efficacious ones. In addition, these sequences may be used in high-throughput screens to identify and provide information on small molecules that bind at these sites and mimic or antagonize the functions of insulin or IGF-1. Furthermore, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which can be used to identify sequence variants that increase or modulate the binding and/or activity of the original peptide at IR or IGF-1R.

[0028] In one aspect of this invention, large numbers of peptides have been screened for their IR and IGF-1R binding and activity characteristics. Analysis of their amino acid sequences has identified certain consensus sequences which may be used themselves or as core sequences in larger amino acid sequences conferring upon them agonist or antagonist activity. Several generic amino acid sequences are disclosed which bind IR and/or IGF-1R with varying degrees of agonist or antagonist activity depending on the specific sequence of the various peptides identified within each motif group. Also provided are amino or carboxyl terminal extensions capable of modifying the affinity and/or pharmacological activity of the consensus sequences when part of a larger amino acid sequence.

[0029] The amino acid sequences of this invention which bind IR and/or IGF-1R include:

[0030] a. $X_1\ X_2\ X_3\ X_4\ X_5$ wherein $X_1$, $X_2$, $X_4$ and $X_5$ are aromatic amino acids, and $X_3$ is any polar amino acid (Formula 1; Group 1; A6 motif);

[0031] b. $X_6\ X_7\ X_8\ X_9\ X_{10}\ X_{11}\ X_{12}\ X_{13}$ wherein $X_6$ and $X_7$ are aromatic amino acids, $X_8$, $X_9$, $X_{11}$ and $X_{12}$ are any amino acid, and $X_{10}$ and $X_{13}$ are hydrophobic amino acids (Formula 2; Group 3; B6 motif);

[0032] c. $X_{14}\ X_{15}\ X_{16}\ X_{17}\ X_{18}\ X_{19}\ X_{20}\ X_{21}$ wherein $X_{14}$, and $X_{17}$ are hydrophobic amino acids, $X_{15}$, $X_{16}$, $X_{18}$ and $X_{19}$ are any amino acid, and $X_{20}$ and $X_{21}$ are aromatic amino acids (Formula 3; reverse B6; revB6).

[0033] d. $X_{22}\ X_{23}\ X_{24}\ X_{25}\ X_{26}\ X_{27}\ X_{28}\ X_{29}\ X_{30}\ X_{31}\ X_{32}\ X_{33}\ X_{34}\ X_{35}\ X_{36}\ X_{37}\ X_{38}\ X_{39}\ X_{40}\ X_{41}$ wherein $X_{22}$, $X_{25}$, $X_{28}$, $X_{29}$, $X_{30}$, $X_{33}$, $X_{34}$, $X_{35}$, $X_{36}$, $X_{37}$, $X_{38}$, $X_{40}$, and $X_{41}$ are any amino acid, $X_{35}$ and $X_{37}$ may be any amino acid for binding to IR, whereas $X_{35}$ is preferably a hydrophobic amino acid and $X_{37}$ is preferably glycine for binding to IGF-1R and possess agonist or antagonist activity. $X_{23}$ and $X_{26}$ are hydrophobic amino acids. This sequence further comprises at least two cysteine residues, preferably at $X_{25}$ and $X_{40}$ $X_{31}$ and $X_{32}$ are small amino acids (Formula 4; Group 7; E8 motif).

[0034] e.) $X_{42}\ X_{43}\ X_{44}\ X_{45}\ X_{46}\ X_{47}\ X_{48}\ X_{49}\ X_{50}\ X_{51}\ X_{52}\ X_{53}\ X_{54}\ X_{55}\ X_{56}\ X_{57}\ X_{58}\ X_{59}\ X_{60}\ X_{61}$ wherein $X_{42}$, $X_{43}$, $X_{44}$, $X_{45}$, $X_{53}$, $X_{55}$, $X_{56}$, $X_{58}$, $X_{60}$ and $X_{61}$ may be any amino acid, $X_{43}$, $X_{46}$, $X_{49}$, $X_{50}$, $X_{54}$ are hydrophobic amino acids, $X_{47}$ and $X_{59}$ are preferably cysteines, $X_{48}$ is a polar amino acid, and $X_{51}$, $X_{52}$ and $X_{57}$ are small amino acids (Formula 5; mini F8 motif).

[0035] f. $X_{62}\ X_{63}\ X_{64}\ X_{65}\ X_{66}\ X_{67}\ X_{68}\ X_{69}\ X_{70}\ X_{71}\ X_{72}\ X_{73}\ X_{74}\ X_{75}\ X_{76}\ X_{77}\ X_{78}\ X_{79}\ X_{80}\ X_{81}$ wherein $X_{62}$, $X_{65}$, $X_{68}$, $X_{69}$, $X_{71}$, $X_{73}$, $X_{76}$, $X_{77}$, $X_{78}$, $X_{80}$, and $X_{81}$ may be any

amino acid; $X_{63}$, $X_{70}$, $X_{74}$ are hydrophobic amino acids; $X_{64}$ is a polar amino acid, $X_{67}$ and $X_{75}$ are aromatic amino acids and $X_{72}$ and $X_{79}$ are preferably cysteines capable of forming a loop (Formula 6; Group 2; D8 motif).

[0036] g. H $X_{82}$ $X_{83}$ $X_{84}$ $X_{85}$ $X_{86}$ $X_{87}$ $X_{88}$ $X_{89}$ $X_{90}$ $X_{91}$ $X_{92}$ wherein $X_{82}$ is proline or alanine, $X_{83}$ is a small amino acid, $X_{84}$ is selected from leucine, serine or threonine, $X_{85}$ is a polar amino acid, $X_{86}$, $X_{88}$, $X_{89}$ and $X_{90}$ are any amino acid, and $X_{87}$, $X_{91}$ and $X_{92}$ are an aliphatic amino acid (Formula 7).

[0037] h. $X_{104}$ $X_{105}$ $X_{106}$ $X_{107}$ $X_{108}$ $X_{109}$ $X_{110}$ $X_{111}$ $X_{112}X_{113}$ $X_{114}$ wherein at least one of the amino acids of $X_{106}$ through $X_{111}$, and preferably two, are tryptophan separated by three amino acids, and wherein at least one of $X_{104}$, $X_{105}$ and $X_{106}$ and at least one of $X_{112}$, $X_{113}$ and $X_{114}$ are cysteine (Formula 8); and

[0038] i. an amino acid sequence comprising the sequence JBA5: DYKDLCQSWGVRIGWLAGLCPKK (SEQ ID NO:1541) or JBA5 minus FLAG® tag and terminal lysines: LCQSWGVRIGWLAGLCP (SEQ ID NO:1542) (Formula 9).

[0039] j. W $X_{123}$ G Y $X_{124}$W $X_{125}$ $X_{126}$ (SEQ ID NO:1543)wherein $X_{123}$ is selected from proline, glycine, serine, arginine, alanine or leucine, but more preferably proline; $X_{124}$ is any amino acid, but preferably a charged or aromatic amino acid; $X_{125}$ is a hydrophobic amino acid preferably leucine or phenylalanine, and most preferably leucine. $X_{126}$ is any amino acid, but preferably a small amino acid (Formula 10; Group 6 motif).

[0040] In one embodiment, peptides comprising a preferred amino acid sequence $FYX_3$ WF (SEQ ID NO: 1544) (Formula 1; Group 1; A6 motif) have been identified which competitively bind to sites on IR. Surprisingly, peptides comprising an amino acid sequence $FYX_3WF$ (SEQ ID NO:1544) can possess agonist or antagonist activity at IR.

[0041] This invention also identifies at least two distinct binding sites on IR based on the differing ability of certain of the peptides to compete with one another and insulin for binding to IR. Accordingly, this invention provides amino acid sequences that bind specifically to one or both sites of IR. Furthermore, specific amino acid sequences are provided which have either agonist or antagonist characteristics based on their ability to bind to the specific sites of IR.

[0042] In another embodiment of this invention, amino acid sequences which bind to one or more sites of IR or IGF-1R (e.g., Site 1 or Site 2) are covalently linked together to form multivalent ligands. These multivalent ligands are capable of forming complexes with a plurality of IR or IGF-1R. Either the same or different amino acid sequences are covalently bound together to form homo- or heterocomplexes.

[0043] In various aspects of the invention, monomer subunits are covalently linked at their N-termini or C-termini to form N—N, C—C, N—C, or C—N linked dimer peptides. In one example, dimer peptides are used to form receptor complexes bound through the same corresponding sites, e.g., Site 1-Site 1 or Site 2-Site 2 dimers. Alternatively, heterodimer peptides are used to bind to different sites on one receptor or to cause receptor complexing through different sites, e.g., Site 1-Site 2 or Site 2-Site 1 dimers. In one novel aspect of the invention, Site 2-Site 1 dimers find use as insulin agonists, while certain Site 1-Site 2 dimers find use as insulin antagonists.

[0044] In various embodiments, insulin agonists comprise Site 1-Site 1 dimer peptide sequences S325, S332, S333, S335, S337, S353, S374-S376, S378, S379, S381, S414, S415, and S418; whereas other insulin agonists comprise Site 2-Site 1 dimer peptide sequences S455, S457, S458, S467, S468, S471, S499, S510, S518, S519, and S520, as described herein below. In one preferred embodiment, an insulin agonist comprises the sequence of the S519 dimer peptide, which shows insulin-like activity in both in vitro and in vivo assays.

[0045] The present invention also provides assays for identifying compounds that mimic the binding characteristics of insulin or IGF-1. Such compounds may act as antagonists or agonists of insulin or IGF-1 function in cell based assays.

[0046] This invention further provides kits for identifying compounds that bind to IR and/or IGF-1R. Also provided are therapeutic compounds that bind the insulin receptor or the IGF-1 receptor.

[0047] Other embodiments of this invention are the nucleic acid sequences encoding the amino acid sequences of the invention. Also within the scope of this invention are vectors containing the nucleic acids and host cells which express the nucleic acids encoding the amino acid sequences which bind at IR and/or IGF-1R and possess agonist or antagonist activity.

[0048] This invention also provides amino acid sequences that bind to active sites of IR and/or IGF-1R and to identify structural criteria for conferring agonist or antagonist activity at IR or IGF-1R.

[0049] This invention further provides specific amino acid sequences that possess agonist, partial agonist, or antagonist activity at either IR or IGF-1R. Such amino acid sequences are potentially useful as therapeutics themselves or may be used to identify other molecules, especially small organic molecules, which possess agonist or antagonist activity at IR or IGF-1R.

[0050] In addition, the present invention provides structural information derived from the amino acid sequences of this invention, which may be used to construct other molecules possessing the desired activity at the relevant IR binding site.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0051] FIGS. **1**A-**1**O; **2**A-**2**E; **3**A-**3**E; **4**A-**4**I; **43**A-**43**B, **44**A-**44**B: Amino acid sequences identified by panning peptide libraries against IGF-1R and/or IR. The amino acids are represented by their one-letter abbreviation. The ratios over background are determined by dividing the signal at 405 nm (E-Tag, IGF-1R, or IR) by the signal at 405 nm for non-fat milk. The IGF-1R/IR Ratio Comparison is determined by dividing the ratio of IGF-1R by the ratio of IR. The IR/IGF-1R Ratio Comparison is determined by dividing the ratio of IR by the ratio of IGF-1R. HIT indicates binder; CAND indicates binder candidate; LDH indicates binding to lactate dehydrogenase (negative control); Sp/Irr indicates the ratio of specific binding over non-specific binding.

[0052] The design of each library is shown in the first line in bold. In the design, symbol 'X' indicates a random position, an underlined amino acid indicates a doped position at the nucleotide level, and other positions are held constant. Additional abbreviations in the B6H library are: 'O' indicates an NGY codon where Y is C or T; 'J' indicates an RHR codon where R is A or G, and H is A, C, or T; and 'U' indicates an VVY codon where V is A, C, or G, and Y is C or T. The 'h' in the 20E2 libraries indicates an NTN codon.

[0053] Symbols in the listed sequences are: Q—TAG Stop; #—TAA Stop; *—TGA Stop; and ?—Unknown Amino Acid. It is believed that a W replaces the TGA Stop Codon when expressed. Except for the 20C and A6L libraries, all libraries are designed with the short FLAG® Epitope DYKD (SEQ ID NO:1545; Hopp et al., 1988, *Bio/Technology* 6:1205-1210) at the N-terminus of the listed sequence and AAAGAP (SEQ ID NO:1546) at the C-terminus. The 20C and A6L libraries have the full length FLAG® epitope DYKDDDDK (SEQ ID NO:1547).

[0054] FIG. 1A: Formula 1 motif peptide sequences obtained from a random 40mer library panned against IR (SEQ ID NOS1-3).

[0055] FIG. 1B: Formula 1 motif peptide sequence obtained from a random 40mer library panned against IGF-1R (SEQ ID NOS4-6).

[0056] FIG. 1C: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IR (SEQ ID NOS7-29).

[0057] FIG. 1D: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IGF-1R (SEQ ID NOS30-33).

[0058] FIG. 1E: Formula 1 motif peptide sequences obtained from a 21 mer library constructed to contain $X_{1-10}$NFYDWFV$X_{18-21}$ (SEQ ID NO:34; also referred to as "A6S") panned against IR (SEQ ID NOS35-98).

[0059] FIG. 1F: Formula 1 motif peptide sequences obtained from a 21 mer library constructed to contain $X_{1-10}$NFYDWFV$X_{18-21}$ (SEQ ID NO:34; also referred to as "A6S") panned against IGF-1R (SEQ ID NOS99-166).

[0060] FIG. 1G: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the A6 peptide as indicated (referred to as "A6L" (SEQ ID NO: 167)) panned against IR (SEQ ID NOS168-216).

[0061] FIG. 1H: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the A6 peptide as indicated (referred to as "A6L" (SEQ ID NO: 167)) panned against IGF-1R (SEQ ID NOS217-244).

[0062] FIG. 1I: Formula 1 motif peptide sequences obtained from a library constructed to contain variations in the consensus core of the E4D peptide (SEQ ID NO: 245) (as indicated) panned against IR (SEQ ID NOS246-305).

[0063] FIG. 1J: Formula 1 motif peptide sequences obtained from a library constructed to contain variations in the consensus core of the E4D peptide (SEQ ID NO: 245) (as indicated) panned against IGF-1R (SEQ ID NOS306-342).

[0064] FIG. 1K: Formula 1 motif peptide sequences obtained from a library constructed using the sequence $X_{1-16}$FHENFYDWFVRQVS$X_{21-26}$ (SEQ ID NO:343; $H_2$C-A) panned against IR (SEQ ID NOS344-430).

[0065] FIG. 1L: Formula 1 motif peptide sequences obtained from a library constructed using the sequence $X_{1-16}$FHENFYDWFVRQVS$X_{21-26}$ (SEQ ID NO:343; H2C-A) panned against IGF-1R (SEQ ID NOS431-467).

[0066] FIG. 1M: Formula 1 motif peptide sequences obtained from a library constructed using the sequence $X_{1-16}$FHXXFYXWF$X_{16-21}$ (SEQ ID NO:468; H2C-B) and panned against IR (SEQ ID NOS469-575).

[0067] FIG. 1N: Formula 1 motif peptide sequences obtained from a library constructed using the sequence $X_{1-16}$FHXXFYXWF$X_{16-21}$ (SEQ ID NO:468; H2C-B) and panned against IGF-1R (SEQ ID NOS576-657).

[0068] FIG. 1O: Formula 1 motif peptide sequences obtained from other libraries panned against IR (SEQ ID NOS658-712).

[0069] FIG. 2A: Formula 4 motif peptide sequences identified from a random 20mer library panned against IR (SEQ ID NO:713).

[0070] FIG. 2B: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide (SEQ ID NO:713) as indicated (15% dope; referred to as "F815") panned against IR (SEQ ID NOS714-796).

[0071] FIG. 2C: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide (SEQ ID NO:713) as indicated (15% dope; referred to as "F815") panned against IGF-1R (SEQ ID NOS797-811).

[0072] FIG. 2D: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide (SEQ ID NO: 713)as indicated (20% dope; referred to as "F820") panned against IR (SEQ ID NOS812-861).

[0073] FIG. 2E: Formula 4 motif peptide sequences identified from other libraries panned against IR (SEQ ID NOS862-925).

[0074] FIG. 3A: Formula 6 motif peptide sequences identified from a random 20mer library and panned against IR (SEQ ID NOS926-928).

[0075] FIG. 3B: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide (SEQ ID NO: 929) as indicated (15% dope; referred to as "D815") panned against IR (SEQ ID NOS930-967).

[0076] FIG. 3C: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide (SEQ ID NO: 929) as indicated (20% dope; referred to as "D820") panned against IR (SEQ ID NOS968-1010).

[0077] FIG. 3D: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide (SEQ ID NO: 929) as indicated (20% dope; referred to as "D820") panned against IGF-1R (SEQ ID NOS1011-1059).

[0078] FIG. 3E: Formula 6 motif peptide sequences identified from other libraries panned against IR (SEQ ID NOS1060-1061).

[0079] FIG. 4A: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IGF-1R (SEQ ID NOS1062-1077).

[0080] FIG. 4B: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IR (SEQ ID NOS1078-1082).

[0081] FIG. 4C: Miscellaneous peptide sequences identified from a random 20mer library panned against IR (SEQ ID NOS1083-1086).

[0082] FIG. 4D: Miscellaneous peptide sequences identified from a random 40mer library panned against IR (SEQ ID NOS1087-1088).

[0083] FIG. 4E: Miscellaneous peptide sequences identified from a random 20mer library panned against IGF-1R (SEQ ID NOS1089-1092).

[0084] FIG. 4F: Miscellaneous peptide sequences identified from an $X_{1-4}$ C $X_{6-20}$ library and panned against IGF-1R (SEQ ID NOS1093-1113).

[0085] FIG. 4G: Miscellaneous peptide sequences identified from a library constructed to contain variations of the F8 peptide (SEQ ID NO: 1114) as indicated (F815) panned against IGF-1R (SEQ ID NOS1115-1118).

[0086] FIG. 4H: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide (SEQ ID NO: 1119) as indicated (referred to as "NNKH") panned against IR (SEQ ID NOS1120-1142).

[0087] FIG. 4I: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide (SEQ ID NO: 1119) as indicated (referred to as "NNKH") panned against IGF-1R (SEQ ID NOS1143-1154).

[0088] FIG. 5A: Summary of specific representative amino acid sequences from Formulas 1, 4, 6, and 10 (SEQ ID NOS1155-1180).

[0089] FIG. 5B: Summary of specific representative amino acid sequences from Formulas 1, 4, 6, and 10 (SEQ ID NOS1181-1220).

[0090] FIG. 6: Illustration of 2 binding site domains on IR based on competition data.

[0091] FIG. 7: Schematic illustration of potential binding schemes to the multiple binding sites on IR.

[0092] FIG. 8: Biopanning results and sequence alignments of Group 1 of IR-binding peptides (SEQ ID NOS1221-1243). The number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or IGF-1R receptor. Absorbance signals are indicated by: ++++, >30× over background; +++, 15-30×; ++, 5-15×; +, 2-5×; and 0, <2×.

[0093] FIGS. 9A-9B: Biopanning results and sequence alignments of Groups 2, 6, and 7 of IR-binding peptides (SEQ ID NOS1244-1261). The number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or IGF-1R receptor. Absorbance signals are indicated by: ++++, >30× over background; +++, 15-30×; ++, 5-15×; +, 2-5×; and 0, <2×.

[0094] FIGS. 10A-10C: Insulin competition data determined for various monomer and dimer peptides. FIG. 10A shows the competition curve. FIG. 10B shows the symbol key for the peptides. FIG. 10C shows the description of the peptides.

[0095] FIGS. 11A-11D: Insulin competition data determined for various monomer and dimer peptides. FIG. 11A shows the competition curve. FIG. 11B shows the symbol key for the peptides. FIG. 11C shows the description of the peptides. FIG. 11D shows IR binding affinity for the peptides.

[0096] FIGS. 12A-12D: Results of free fat cell assays for truncated synthetic RP9 monomer peptides, S390 and S394. FIG. 12A shows the results for peptide S390.

[0097] FIG. 12B shows the results for peptide S394. FIG. 12C shows the amino acid sequence of peptides S390 and S394 (SEQ ID NOS:1794 and 1788, respectively in order of appearance). FIG. 12D shows the results for full-length RP9 peptide.

[0098] FIGS. 13A-13C: Results of free fat cell assays for truncated synthetic RP9 dimer peptides, S415 and S417. FIG. 13A shows the results for peptide S415.

[0099] FIG. 13B shows the results for peptide S417. FIG. 13C shows the amino acid sequence of peptides S415 and S417 (SEQ ID NOS1795-1796).

[0100] FIGS. 14A-14C: Results of free fat cell assays for RP9 homodimer peptides, 521 and 535. FIG. 14A shows the results for peptide 521. FIG. 14B shows the results for peptide 535. FIG. 14C shows the amino acid sequence of peptides 521 and 535.

[0101] FIGS. 15A-15C: Results of free fat cell assays for RP9-D8 heterodimer peptides, 537 and 538. FIG. 15A shows the results for peptide 537. FIG. 15B shows the results for peptide 538. FIG. 15C shows the amino acid sequence of peptides 537 and 538.

[0102] FIGS. 16A-16C: Results of free fat cell assays for RP9-D8 heterodimer peptides 537 and 538. FIG. 16A shows the results for peptide 537. FIG. 16B shows the results for peptide 538. FIG. 16C shows the amino acid sequence of peptides 537 and 538.

[0103] FIGS. 17A-17B: Results of free fat cell assays for D8-RP9 heterodimer peptide, 539. FIG. 17A shows the results for peptide 539. FIG. 17B shows the amino acid sequence of peptide 539.

[0104] FIGS. 18A-18D: Results of free fat cell assays for Site 1/Site 2 dimer peptides with constituent monomer peptides with Site 1-Site 2 C—N (FIG. 18A), Site 1-Site 2, N—N (FIG. 18B), Site 1-Site 2, C—C (FIG. 18C), and Site 2-Site 1, C—N (FIG. 18D) orientations and linkages, respectively.

[0105] FIGS. 19A-19B: Results of human insulin receptor kinase assays for various monomer and dimer peptides. FIG. 19A shows the substrate phosphorylation curve.

[0106] FIG. 19B shows the $EC_{50}$ values.

[0107] FIGS. **20**A-**20**B: Results of human insulin receptor kinase assays for Site 1—Site 2 and Site 2-Site 1 dimer peptides. FIG. **20**A shows the substrate phosphorylation curve. FIG. **20**B shows the $EC_{50}$ values.

[0108] FIGS. **21**A-**21**B: Results of human insulin receptor kinase assays for Site 1-Site 2 and Site 2-Site 1 peptides. FIG. **21**A shows the substrate phosphorylation curve. FIG. **21**B shows the $EC_{50}$ values.

[0109] FIGS. **22**A-**22**B: Results of time-resolved fluorescence resonance transfer assays for assessing the ability of various monomer and dimer peptides to compete with biotinylated RP9 monomer peptide for binding to soluble human insulin receptor-immunoglobulin heavy chain chimera. FIG. **22**A shows the binding curve. FIG. **22**B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

[0110] FIGS. **23**A-**23**C: Results of time-resolved fluorescence resonance transfer assays indicating the ability of various monomer and dimer peptide to compete with biotinylated S175 monomer peptide or biotinylated RP9 monomer peptide for binding to soluble human insulin receptor-immunoglobulin heavy chain chimera. FIGS. **23**A-**23**B show the binding curves. FIG. **23**C shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

[0111] FIGS. **24**A-**24**B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptide to compete with fluoroscein labeled RP9 monomer peptide for binding to soluble human insulin receptor ectodomain. FIG. **24**A shows the binding curve. FIG. **24**B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560 and 2001-2002, respectively in order of appearance).

[0112] FIGS. **25**A-**25**B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptides to compete with fluoroscein labeled RP9 monomer peptide for binding to soluble human insulin mini-receptor. FIG. **25**A shows the binding curve. FIG. **25**B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

[0113] FIGS. **26**A-**26**B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptides to compete with fluorescein labeled insulin for binding to soluble human insulin receptor ectodomain. FIG. **26**A shows the binding curve. FIG. **26**B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

[0114] FIGS. **27**A-**27**B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptides to compete with fluorescein labeled insulin for binding to soluble human insulin mini-receptor. FIG. **27**A shows the binding curve. FIG. **27**B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

[0115] FIG. **28**: A schematic drawing for the construction of protein fusions of the maltose binding protein.

[0116] FIG. **29**: BIAcore analysis of competition binding between IR and maltose binding protein fusion peptides H2C-9aa-H2C, H2C, and H2C-3aa-H2C.

[0117] FIG. **30**: Stimulation of IR autophosphorylation in vivo by maltose binding protein fusion peptides.

[0118] FIGS. **31**A-**31**C: Results of free fat cell assays for insulin and Site 2-Site 1 peptides, S519 and S520. FIG. **31**A shows the results for S519. FIG. **31**B shows the results for S520. FIG. **31**C shows the $EC_{50}$ values.

[0119] FIGS. **32**A-**32**B: Results of human insulin receptor kinase assays for insulin and Site 2-Site 1 peptides S519 and S520. FIG. **32**A shows the substrate phosphorylation curve. FIG. **32**B shows the calculated Bestfit values.

[0120] FIG. **33**: Results of in vivo experiments showing the effect of intravenous administration of Site 2-Site 1 peptide S519 in Wistar rats:

[0121] FIGS. **34**A-**34**E: Results of phage competition studies with IGF-1 surrogates RP9 (Site 1) and D815 (Site 2) peptides. Phage: RP9 (A6-like); RP6 (B6-like); D8B12 (Site 2); and D815 (Site 2); Peptides: RP9 and D815. FIGS. **34**A-**34**B show the competition curves. FIGS. **34**C-**34**E show the symbol keys and peptide groups.

[0122] FIG. **35**A-**35**E: Phage competition studies with Site 2-Site 1 dimer peptides containing 6- or 12-amino acid linkers. Phage: RP9, RP6, D8B12, and D815; Peptides: D815-6L-RP9 and D815-12L-RP9. FIGS. **35**A-**35**B show the competition curves. FIGS. **35**C-**35**E show the symbol keys and peptide groups.

[0123] FIG. **36**: Results of IGF-1 agonist assay using FDCP-2 cells. Site 1 peptides RP6, RP9, G33, and Site 2 peptide D815 were tested in the agonist assay.

[0124] FIG. **37**: Results of IGF-1 antagonist assay using FDCP-2 cells. Site 1 peptides RP6, RP9, G33, and Site 2 peptide D815 were tested in the antagonist assay.

[0125] FIG. **38**: Results of IGF-1 agonist assay using FDCP-2 cells. Site 1 peptides 20E2, S175, and RP9 were tested in the agonist assay.

[0126] FIGS. **39**: Results of agonist and antagonist studies with surrogate monomers and dimers. Monomers: D815 and RP9; Dimers: D815-6aa-RP9 and D815-12aa-RP9.

[0127] FIG. **40**: Results of agonist and antagonist studies with surrogate monomers and dimers. Monomers: G33 and D815; Dimer: D815-6aa-G33.

[0128] FIG. **41**: Results of agonist and antagonist studies with surrogate peptides and dimers. Monomers: G33, D815 and RP9; Dimers: D815-6aa-RP9 and D815-12aa-RP9.

[0129] FIG. **42**: IGF-1 standard curve using FDCP-2 cells.

[0130] FIGS. **43**A-**43**B: Peptide monomers identified from G33 and RP6 secondary libraries panned against

IGF-1R (SEQ ID NOS1262-1432). FIG. **43**A shows peptides from G33 secondary library; FIG. **43**B shows peptides from RP6 secondary library.

[**0131**] FIGS. **44**A-**44**B: Peptide dimers identified from libraries panned against IR or IGF-1R (SEQ ID NOS1433-1540). FIG. **44**A shows dimer peptides panned against IR; FIG. **44**B shows dimer peptides panned against IGF-1R.

[**0132**] FIG. **45**: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide surrogate G33 (rG33) on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

[**0133**] FIG. **46**: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide surrogate D815 (rD815) on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

[**0134**] FIG. **47**: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide surrogate RP9 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

[**0135**] FIG. **48**: Results of heterogeneous time-resolved fluorometric assay showing the effect of recombinant peptide surrogate D815-6-G33 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

[**0136**] FIG. **49**: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide surrogate D815-6-RP9 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

[**0137**] FIG. **50**: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide surrogate D815-12-RP9 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

[**0138**] FIG. **51**: Results of heterogeneous time-resolved fluorometric assays showing the effect of IGF-1 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

[**0139**] FIG. **52**: Results of time-resolved fluorescence resonance energy transfer assays showing the effect of Site 1 peptide surrogates, Site 2 peptide surrogates, and rhIGF-1 on the dissociation of biotinylated-20E2 (b-20E2, Site 1) from recombinant human IGF-1R.

[**0140**] FIG. **53**: Results of time-resolved fluorescence resonance energy transfer assays showing the effect of various peptide monomers and dimers on the dissociation of biotinylated-20E2 (b-20E2, Site 1) from recombinant human IGF-1R.

[**0141**] FIG. **54**: Results of glucose uptake assays in SGBS cells showing the potency of peptide S597 relative to human insulin.

[**0142**] FIG. **55**: Results of glucose-lowering assays in rats showing the potency of peptide S557 and S597 relative to human insulin.

[**0143**] FIG. **56**: Results of glucose-lowering assays in fasted Goettingen minipigs showing the potency of peptide S597 relative to human insulin.

[**0144**] FIG. **57**: Results of studies of disappearance of $I^{125}$-labelled peptides from site of injection.

## DETAILED DESCRIPTION OF THE INVENTION

[**0145**] This invention relates to amino acid sequences comprising motifs that bind to the insulin receptor (IR) and/or insulin-like growth factor receptor (IGF-1R). In addition to binding to IR and/or IGF-1R, the amino acid sequences also possess either agonist, partial agonist or antagonist activity at IR or IGF-1R. In addition, the amino acid sequences bind to separate binding sites (Sites 1 or 2) on IR or IGF-1R.

[**0146**] Although capable of binding to IR or IGF-1R at sites which participate in conferring agonist or antagonist activity, the amino acid sequences are not based on the native insulin or IGF-1 sequences, nor do they reflect an obvious homology to any such sequences.

[**0147**] The amino acid sequences of the invention may be peptides, polypeptides, or proteins. These terms as used herein should not be considered limiting with respect to the size of the various amino acid sequences referred to herein and which are encompassed within this invention. Thus, any amino acid sequence comprising at least one of the IR or IGF-1R binding motifs disclosed herein, and which binds to IR or IGF-1R is within the scope of this invention. In preferred embodiments, the amino acid sequences confer insulin or IGF-1 agonist or antagonist activity. The amino acid sequences of the invention are typically artificial, i.e., non-naturally occurring, peptides, or polypeptides. Amino acid sequences useful in the invention may be obtained through various means such as chemical synthesis, phage display, cleavage of proteins or polypeptides into fragments, or by any means which amino acid sequences of sufficient length to possess binding ability may be made or obtained.

[**0148**] The amino acid sequences provided by this invention should have an affinity for IR sufficient to provide adequate binding for the intended purpose. Thus, for use as a therapeutic, the peptide, polypeptide, or protein provided by this invention should have an affinity ($K_d$) of between about $10^{-7}$ to about $10^{-15}$ M. More preferably the affinity is $10^{-8}$ to about $10^{-12}$ M. Most preferably, the affinity is $10^{-10}$ to about $10^{-12}$ M. For use as a reagent in a competitive binding assay to identify other ligands, the amino acid sequence preferably has affinity for the receptor of between about $10^{-5}$ to about $10^{-12}$ M.

[**0149**] The present invention describes several different binding motifs, which bind to active sites on IR or IGF-1R. The binding motifs are defined based on the analysis of several different amino acid sequences and analyzing the frequency that particular amino acids or types of amino acids occur at a particular position of the amino acid sequence as described in the related applications of Beasley et al. International Application PCT/US00/08528, filed Mar. 29, 2000, and Beasley et al., U.S. application Ser. No. 09/538,038, filed Mar. 29, 2000.

[**0150**] Also included within the scope of this invention are amino acid sequences containing substitutions, additions, or

deletions based on the teachings disclosed herein and which bind to IR or IGF-1R with the same or altered affinity. For example, sequence tags (e.g., FLAG® tags) or amino acids, such as one or more lysines, can be added to the peptide sequences of the invention (e.g., at the N-terminal or C-terminal ends) as described in detail herein. Sequence tags can be used for peptide purification or localization. Lysines can be used to increase peptide solubility or to allow for biotinylation. Alternatively, amino acid residues located at the carboxy and amino terminal regions of the consensus motifs described below, which comprise sequence tags (e.g., FLAG® tags), or which contain amino acid residues that are not associated with a strong preference for a particular amino acid, may optionally be deleted providing for truncated sequences. Certain amino acids (e.g., C-terminal or N-terminal residues) such as lysine which promote the stability or biotinylation of the amino acids sequences may be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger sequence which is soluble, or linked to a solid support.

[0151] Peptides that bind to IR or IGF-1R, and methods and kits for identifying such peptides, have been disclosed by Beasley et al., International Application PCT/US00/08528 filed Mar. 29, 2000 and Beasley et al., U.S. application Ser. No. 09/538,038 filed Mar. 29, 2000, which are incorporated by reference in their entirety.

[0152] Consensus Motifs

[0153] The following motifs have been identified as conferring binding activity to IR and/or IGF-1R:

[0154] 1. $X_1X_2X_3X_4X_5$ (Formula 1; Group 1; the A6 motif) wherein $X_1$, $X_2$, $X_4$ and $X_5$ are aromatic amino acids, preferably, phenylalanine or tyrosine. Most preferably, $X_1$ and $X_5$ are phenylalanine and $X_2$ is tyrosine. $X_3$ may be any small polar amino acid, but is preferably selected from aspartic acid, glutamic acid, glycine, or serine, and is most preferably aspartic acid or glutamic acid. $X_4$ is most preferably tryptophan, tyrosine, or phenylalanine and most preferably tryptophan. Particularly preferred embodiments of the A6 motif are FYDWF (SEQ ID NO:1554) and FYEWF (SEQ ID NO:1555). The A6 motif possesses agonist activity at IGF-1R, but agonist or antagonist activity at IR depending on the identity of amino acids flanking A6. See FIG. **5**A.

[0155] Amino acid sequences that comprise the A6 motif and possess agonist activity at IR, include but are not limited to, D117/H2C: FHENFYDWFVRQVSKK (SEQ ID NO:1556); D117/H2 minus terminal lysines: FHENFYDWFVRQVS (SEQ ID NO:1557); RP9: GSLDESFYDWFERQLGKK (SEQ ID NO:1558); RP9 minus terminal lysines: GSLDESFYDWFERQLG (SEQ ID NO:1559); and S175: GRVDWLQRNANFYDWFVAELG (SEQ ID NO:1560). Preferred RP9 sequences include GLADEDFYEWFERQLR (SEQ ID NO:1561), GLADELFYEWFDRQLS (SEQ ID NO:1562), GQLDEDFYEWFDRQLS (SEQ ID NO:1563), GQLDEDFYAWFDRQLS (SEQ ID NO:1564), GFMDESFYEWFERQLR (SEQ ID NO:1565), GFWDESFYAWFERQLR (SEQ ID NO:1566), GFMDESFYAWFERQLR (SEQ ID NO:1567), and GFWDESFYEWFERQLR (SEQ ID NO:1568). Nonlimiting examples of Group 1 (Formula 1; A6) amino acid sequences are shown in FIGS. **1**A-**1**O.

[0156] 2. $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ (Formula 2, Group 3; the B6 motif) wherein $X_6$ and $X_7$ are aromatic amino acids, preferably, phenylalanine or tyrosine. Most preferably, $X_6$ is phenylalanine and $X_7$ is tyrosine. $X_8$, Xg, $X_{11}$, and $X_{12}$ may be any amino acid. $X_{10}$ and $X_{13}$ are hydrophobic amino acids, preferably leucine, isoleucine, phenylalanine, tryptophan or methionine, but more preferably leucine or isoleucine. $X_{10}$ is most preferably isoleucine for binding to IR and leucine for binding to IGF-1R. $X_{13}$ is most preferably leucine. Amino acid sequences of Formula 2 may function as an antagonist at the IGF-1R, or as an agonist at the IR. Preferred consensus sequences of the Formula 2 motif are FYX$_8$ $X_9$ I $X_{11}$ $X_{12}$ L (SEQ ID NO:1569), F Y $X_8$ $X_9$ I $X_{11}$ $X_{12}$ L (SEQ ID NO:1570), F Y $X_8$A I $X_{11}$ $X_{12}$ L (SEQ ID NO:1571), and F Y $X_8$Y F $X_{11}$ $X_{12}$ L (SEQ ID NO:1572).

[0157] Another Formula 2 motif for use with this invention comprises F Y $X_8$Y F $X_{11}$ $X_{12}$ L (SEQ ID NO:1573) and is shown as Formula 2A ("NNRP") below: $X_{115}$ $X_{116}$ $X_{117}$ $X_{118}$ F Y $X_8$ Y F $X_{11}$ $X_{12}$ L $X_{119}$ $X_{120}$ $X_{121}$ $X_{122}$, (SEQ ID NO:1574) wherein $X_{115}$-$X_{118}$ and $X_{118}$-$X_{122}$ may be any amino acid which allows for binding to IR or IGF-1R. $X_{115}$ is preferably selected from the group consisting of tryptophan, glycine, aspartic acid, glutamic acid, and arginine. Aspartic acid, glutamic acid, glycine, and arginine are more preferred. Tryptophan is most preferred. The preference for tryptophan is based on its presence in clones at a frequency three to five fold higher than that expected over chance for a random substitution, whereas aspartic acid, glutamic acid and arginine are present about two fold over the frequency expected for random substitution.

[0158] $X_{116}$ preferably is an amino acid selected from the group consisting of aspartic acid, histidine, glycine, and asparagine. $X_{117}$ and $X_{118}$ are preferably glycine, aspartic acid, glutamic acid, asparagine, or alanine. More preferably $X_{117}$ is glycine, aspartic acid, glutamic acid and asparagine whereas $X_{118}$ is more preferably glycine, aspartic acid, glutamic acid or alanine. $X_8$ when present in the Formula 2A motif is preferably arginine, glycine, glutamic acid, or serine. $X_{11}$ when present in the Formula 2A motif is preferably glutamic acid, asparagine, glutamine, or tryptophan, but most preferably glutamic acid. $X_{12}$ when present in the Formula 2A motif is preferably aspartic acid, glutamic acid, glycine, lysine or glutamine, but most preferably aspartic acid. $X_{119}$ is preferably glutamic acid, glycine, glutamine, aspartic acid or alanine, but most preferably glutamic acid. $X_{120}$ is preferably glutamic acid, aspartic acid, glycine or glutamine, but most preferably glutamic acid. $X_{121}$ is preferably tryptophan, tyrosine, glutamic acid, phenylalanine, histidine, or aspartic acid, but most preferably tryptophan or tyrosine. $X_{122}$ is preferably glutamic acid, aspartic acid or glycine; but most preferably glutamic acid. Preferred amino acid residue are identified based on their frequency in clones over two fold over that expected for a random event, whereas the more preferred sequences occur about 3-5 times as frequently as expected.

[0159] 3. $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ (Formula 3, reverse B6, revB6), wherein $X_{14}$ and $X_{17}$ are hydrophobic amino acids; $X_{14}$, $X_{17}$ are preferably leucine, isoleucine, and valine, but most preferably leucine; $X_{15}$, $X_{16}$, $X_{18}$ and $X_{19}$ may be any amino acid; $X_{20}$ is an aromatic amino acid, preferably tyrosine or histidine, but most preferably tyrosine; and $X_{21}$ is an aromatic amino acid, but preferably phenylalanine or tyrosine, and most preferably phenylalanine. For use as an IGF-1R binding ligand, an aromatic amino acid is strongly preferred at $X_{18}$.

[0160] 4. $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$ (Formula 4; Group 7; the F8 motif) wherein $X_{22}$, $X_{25}$, $X_{26}$, $X_{28}$, $X_{29}$, $X_{30}$, $X_{33}$, $X_{34}$, $X_{35}$, $X_{36}$, $X_{37}$, $X_{38}$, $X_{40}$, and $X_{41}$ are any amino acid. $X_{35}$ and $X_{37}$ may be any amino acid when the F8 motif is used as an IR binding ligand or as a component of an IR binding ligand, however for use as an IGF-1R binding ligand, glycine is strongly preferred at $X_{37}$ and a hydrophobic amino acid, particularly, leucine, is preferred at $X_{35}$ $X_{23}$ is a hydrophobic amino acid. Methionine, valine, leucine or isoleucine are preferred amino acids for $X_{23}$, however, leucine which is most preferred for preparation of an IGF-1R binding ligand is especially preferred for preparation of an IR binding ligand. At least one cysteine is located at $X_{24}$ through $X_{27}$, and one at $X_{39}$ or $X_{40}$. Together the cysteines are capable of forming a cysteine cross-link to create a looped amino acid sequence. In addition, although a spacing of 14 amino acids in between the two cysteine residues is preferred, other spacings may also be used provided binding to IGF-1R or IR is maintained. Accordingly, other amino acids may be substituted for the cysteines at positions $X_{24}$ and $X_{39}$ if the cysteines occupy other positions.

[0161] In one embodiment, for example, the cysteine at position $X_{24}$ may occur at position $X_{27}$ which will produce a smaller loop provided that the cysteine is maintained at position $X_{39}$. These smaller looped peptides are described herein as Formula 5, infra. $X_{27}$ is any polar amino acid, but is preferably selected from glutamic acid, glutamine, aspartic acid, asparagine, or as discussed above cysteine. The presence of glutamic acid at position $X_{27}$ decreases binding to IR but has less of an effect on binding to IGF-1R. $X_{31}$ is any aromatic amino acid and $X_{32}$ is any small amino acid. For binding to IGF-1R, glycine or serine is preferred at position $X_{31}$, however, tryptophan is highly preferred for binding to IR. At position $X_{32}$, glycine is preferred for both IGF-1R and IR binding. $X_{36}$ is an aromatic amino acid. A preferred consensus sequence for F8 is $X_{22}$ LC $X_{25}$ $X_{26}$ E $X_{28}X_{29}X_{30}$ WG $X_{33}$ $X_{34}$ $X_{35}$ $X_{36}$ $X_{37}$ $X_{38}$ C $X_{40}$ $X_{41}$ (SEQ ID NO:1575) whereas the amino acids are defined above. A more preferred F8 sequence is HLCVLEELFWGASLFGYCSG ("F8"; SEQ ID NO:1576). Amino acid sequences comprising the F8 sequence motif preferably bind to IR over IGF-1R. FIGS. **2**A-**2**E list nonlimiting examples of Formula 4 amino acid sequences.

[0162] 5.) $X_{42}$ $X_{43}$ $X_{44}$ $X_{45}$ $X_{46}$ $X_{47}$ $X_{48}$ $X_{49}$ $X_{50}$ $X_{51}$ $X_{52}$ $X_{53}$ $X_{54}$ $X_{55}$ $X_{56}$ $X_{57}$ $X_{58}$ $X_{59}$ $X_{60}$ $X_{61}$ (Formula 5; mini F8 motif) wherein $X_{42}$, $X_{43}$, $X_{44}$, $X_{45}$, $X_{53}$, $X_{55}$, $X_{56}$, $X_{58}$, $X_{60}$ and $X_{61}$ are any amino acid. $X_{43}$, $X_{46}$, $X_{49}$, $X_{50}$ and $X_{54}$ are hydrophobic amino acids, however, $X_{43}$ and $X_{46}$ are preferably leucine, whereas $X_{50}$ is preferably phenylalanine or tyrosine but most preferably phenylalanine. $X_{47}$ and $X_{59}$ are cysteines. $X_{48}$ is preferably a polar amino acid, i.e., aspartic acid or glutamic acid, but most preferably glutamic acid. Use of the small amino acid at position 54 may confer IGF-1R specificity. $X_{51}$, $X_{52}$, and $X_{57}$ are small amino acids, preferably glycine. A preferred consensus sequence for mini F8 is $X_{42}$ $X_{43}$ $X_{44}$ $X_{45}$ L C E $X_{49}$ F G G $X_{53}$ $X_{54}$ $X_{55}$ $X_{56}$ G $X_{58}$ C $X_{60}$ $X_{61}$ (SEQ ID NO:1577). Amino acid sequences comprising the sequence of Formula 5 preferably bind to IGF-1R or IR.

[0163] 6. $X_{62}$ $X_{63}$ $X_{64}$ $X_{65}$ $X_{66}$ $X_{67}$ $X_{68}$ $X_{69}$ $X_{70}$ $X_{71}$ $X_{72}$ $X_{73}$ $X_{74}$ $X_{75}$ $X_{76}$ $X_{77}$ $X_{78}$ $X_{79}$ $X_{80}$ $X_{81}$ (Formula 6; Group 2; the D8 motif) wherein $X_{62}$, $X_{65}$, $X_{68}$, $X_{69}$, $X_{71}$, $X_{73}$, $X_{76}$, $X_{77}$, $X_{78}$, $X_{80}$ and $X_{81}$ may be any amino acid. $X_{66}$ may also be any amino acid, however, there is a strong preference for glutamic acid. Substitution of $X_{66}$ with glutamine or valine may result in attenuation of binding. $X_{63}$, $X_{70}$, and $X_{74}$ are hydrophobic amino acids. $X_{63}$ is preferably leucine, isoleucine, methionine, or valine, but most preferably leucine. $X_{70}$ and $X_{74}$ are preferably valine, isoleucine, leucine, or methionine. $X_{74}$ is most preferably valine. $X_{64}$ is a polar amino acid, more preferably aspartic acid or glutamic acid, and most preferably glutamic acid. $X_{67}$ and $X_{75}$ are aromatic amino acids. Whereas tryptophan is highly preferred at $X_{67}$, $X_{75}$ is preferably tyrosine or tryptophan but most preferably tyrosine. $X_{72}$ and $X_{79}$ are cysteines that again are believed to form a loop which position amino acid may be altered by shifting the cysteines in the amino acid sequence.

[0164] D8 is most useful as an amino acid sequence having a preference for binding to IR as only a few D8 sequences capable of binding to IGF-1R over background have been detected. A preferred sequence for binding to IR is $X_{62}$ L $X_{64}$ $X_{65}$ $X_{66}$ W $X_{68}$ $X_{69}$ $X_{70}$ $X_{71}$ C $X_{73}$ $X_{74}$ $X_{75}$ $X_{76}$ $X_{77}$ $X_{78}$ C $X_{80}$ $X_{81}$ (SEQ ID NO:1578). Examples of specific peptide sequences comprising this motif include D8: KWLDQEWAWVQCEVYGRGCPSKK (SEQ ID NO:1579); and D8 minus terminal lysines: KWLDQEWAWVQCEVYGRGCPS (SEQ ID NO:1580). Preferred D8 monomer sequences include SLEEEWAQIQCEIYGRGCRY (SEQ ID NO:1581) and SLEEEWAQIQCEIWGRGCRY (SEQ ID NO:1582). Preferred D8 dimer sequences include SLEEEWAQIECEVYGRGCPS (SEQ ID NO:1583), and SLEEEWAQIECEVWGRGCPS (SEQ ID NO:1584). Nonlimiting examples of Group 2 (Formula 6; D8) amino acid sequences are shown in FIGS. **3**A-**3**E.

[0165] 7. H $X_{82}$, $X_{83}$, $X_{84}$ $X_{85}$ $X_{86}$ $X_{87}$ $X_{88}$ $X_{89}$ $X_{90}$ $X_{91}$ $X_{92}$ (Formula 7) wherein $X_{82}$ is proline or alanine but most preferably proline; $X_{83}$ is a small amino acid more preferably proline, serine or threonine and most preferably proline; $X_{84}$ is selected from leucine, serine or threonine but most preferably leucine; $X_{85}$ is a polar amino acid preferably glutamic acid, serine, lysine or asparagine but more preferably serine; $X_{86}$ may be any amino acid but is preferably a polar amino acid such as histidine, glutamic acid, aspartic acid, or glutamine; $X_{87}$ is an aliphatic amino acid preferably leucine, methionine or isoleucine and most preferably leucine; amino acid $X_{88}$, $X_{89}$ and $X_{90}$ may be any amino acids; $X_{91}$ is an aliphatic amino acid with a strong preference for leucine as is $X_{92}$. Phenylalanine may also be used at position 92. A preferred consensus sequence of Formula 7 is H P P L S $X_{86}$ L $X_{88}X_{89}X_{90}$ L L (SEQ ID NO:1585). The Formula 7 motif binds to IR with little or no binding to IGF-1R.

[0166] 8. Another sequence is $X_{104}$ $X_{105}$ $X_{106}$ $X_{107}$ $X_{108}$ $X_{109}$ $X_{110}$ $X_{111}$ $X_{112}$ $X_{113}$ $X_{114}$ (Formula 8) which comprises eleven amino acids wherein at least one, and preferably two of the amino acids of $X_{106}$ through $X_{111}$ are tryptophan. In addition, it is also preferred that when two tryptophan amino acids are present in the sequence they are separated by three amino acids, which are preferably, in sequential order proline, threonine and tyrosine with proline being adjacent to the tryptophan at the amino terminal end. Accordingly, the most preferred sequence for $X_{107}$ $X_{108}$ $X_{109}$ $X_{110}$ $X_{111}$ is WPTYW (SEQ ID NO:1586). At least one of the three amino acids on the amino terminal ($X_{104}$, $X_{105}$ $X_{106}$) and at least one of the amino acids carboxy terminal ($X_{112}$ $X_{113}$

$X_{114}$) ends immediately flanking $X_{107}$-$X_{111}$ are preferably a cysteine residue, most preferably at $X_{105}$ and $X_{113}$ respectively. Without being bound by theory, the cysteines are preferably spaced so as to allow for the formation of a loop structure. $X_{104}$ and $X_{114}$ are both small amino acids such as, for example, alanine and glycine. Most preferably, $X_{104}$ is alanine and $X_{114}$ is glycine. $X_{105}$ may be any amino acid but is preferably valine. $X_{112}$ is preferably asparagine. Thus, the most preferred sequence is ACVWPTYWNCG (SEQ ID NO:1587).

[0167] 9. An amino acid sequence comprising JBA5: DYKDLCQSWGVRIGWLAGLCPKK (SEQ ID NO:1541); or JBA5 without terminal lysines: LCQSWGVRIGWLAGLCP (SEQ ID NO:1542) (Formula 9). The Formula 9 motif is another motif believed to form a cysteine loop that possesses agonist activity at both IR and IGF-1R. Although IR binding is not detectable by ELISA, binding of Formula 9 to IR is competed by insulin and is agonistic.

[0168] 10. W $X_{123}$ G Y $X_{124}$ W $X_{125}$ $X_{126}$ (SEQ ID NO:1543) (Formula 10; Group 6) wherein $X_{123}$ is selected from proline, glycine, serine, arginine, alanine or leucine, but more preferably proline; $X_{124}$ is any amino acid, but preferably a charged or aromatic amino acid; $X_{125}$ is a hydrophobic amino acid preferably leucine or phenylalanine, and most preferably leucine. $X_{126}$ is any amino acid, but preferably a small amino acid. In one embodiment of the present invention, the Formula 10, Group 6 motif is WPGY (SEQ ID NO: 1588). Examples of specific peptide sequences comprising this motif include E8: KVRGFQGGTVWPGYEWLRNAAKK (SEQ ID NO:1589); and E8 minus terminal lysines: KVRGFQGGTVWPGYEWLRNAA (SEQ ID NO:1590). Preferred Group 6 sequences include WAGYEWF (SEQ ID NO:1591), WEGYEWL (SEQ ID NO:1592), WAGYEWL (SEQ ID NO:1593), WEGYEWF (SEQ ID NO:1594), and DSDWAGYEWFEEQLD (SEQ ID NO:1595). Nonlimiting examples of Group 6 amino acid sequences are shown in FIGS. **4**A-**4**B.

[0169] The IR and IGF-1R binding activities of representative Group 1 (Formula 1; A6); Group 2 (Formula 6; D8); and Group 6 (Formula 10); and Group 7 (Formula 4; F8) amino acid sequences are summarized in FIGS. **8** and **9**A-**9**B. Group 1 (Formula 1; A6) amino acid sequences contain the consensus sequence FyxWF (SEQ ID NO:1596), which is typically agonistic in cell-based assays. Group 2 (Formula 6; D8) amino acid sequences are composed of two internal sequences having a consensus sequence VYGR (SEQ ID NO:1597) and two cysteine residues each. Thus, Group 2 peptides are capable of forming a cyclic peptide bridged with a disulfide bond. Neither of these consensus sequences have any significant linear sequence similarities greater than 2 or 3 amino acids with mature insulin. Group 7 (Formula 4; F8) amino acid sequences are composed of two internal exemplary sequences which do not have any significant sequence homology, but have two cysteine residues 13-14 residues apart, thus being capable of forming a cyclic peptide with a long loop anchored by a disulfide bridge.

[0170] Amino and Carboxyl Terminal Extensions Modulate Activity of Motifs

[0171] In addition to the motifs stated above, the invention also provides preferred sequences at the amino terminal or carboxyl terminal ends which are capable of enhancing binding of the motifs to either IR, IGF-1R, or both. In addition, the use of the extensions described below does not preclude the possible use of the motifs with other substitutions, additions or deletions that allow for binding to IR, IGF-1R, or both.

[0172] Formula 1

[0173] Any amino acid sequence may be used for extensions of the amino terminal end of A6, although certain amino acids in amino terminal extensions may be identified which modulate activity. Preferred carboxy terminal extensions for A6 are A6 $X_{93}$ $X_{94}$ $X_{95}$ $X_{96}$ $X_{97}$ wherein $X_{93}$ may be any amino acid, but is preferably selected from the group consisting of alanine, valine, aspartic acid, glutamic acid, and arginine, and $X_{94}$ and $X_{97}$ are any amino acid; $X_{95}$ is preferably glutamine, glutamic acid, alanine or lysine but most preferably glutamine. The presence of glutamic acid at $X_{95}$ however may confer some IR selectivity. Further, the failure to obtain sequences having an asparagine or aspartic acid at position $X_{95}$ may indicate that these amino acids should be avoided to maintain or enhance sufficient binding to IR and IGF-1R. $X_{96}$ is preferably a hydrophobic or aliphatic amino acid, more preferably leucine, isoleucine, valine, or tryptophan but most preferably leucine. Hydrophobic residues, especially tryptophan at $X_{96}$ may be used to enhance IR selectivity.

[0174] Formula 2

[0175] B6 with amino terminal and carboxy terminal extensions may be represented as $X_{98}$ $X_{99}$ B6 $X_{100}$. $X_{98}$ is optionally aspartic acid and $X_{99}$ is independently an amino acid selected from the group consisting of glycine, glutamine, and proline. The presence of an aspartic acid at $X_{98}$ and a proline at $X_{99}$ is associated with an enhancement of binding for both IR and IGF-1R. A hydrophobic amino acid is preferred for the amino acid at $X_{100}$, an aliphatic amino acid is more preferred. Most preferably leucine, for IR and valine for IGF-1R. Negatively charged amino acids are preferred at both the amino and carboxy terminals of Formula 2A.

[0176] Formula 3

[0177] An amino terminal extension of Formula 3 defined as $X_{101}$ $X_{102}$ $X_{103}$ revB6 wherein $X_{103}$ is a hydrophobic amino acid, preferably leucine, isoleucine or valine, and $X_{102}$ and $X_{101}$ are preferably polar amino acids, more preferably aspartic acid or glutamic acid may be useful for enhancing binding to IR and IGF-1R. No preference is apparent for the amino acids at the carboxy terminal end of Formula 3.

[0178] Formula 10

[0179] In one preferred embodiment, Formula 10 sequences W $X_{123}$ G Y $X_{124}$ W $X_{125}$ $X_{126}$ (SEQ ID NO:1543) can include an amino terminal extension comprising the sequence DSD and/or a carboxy terminal extension comprising the sequence EQLD (SEQ ID NO:1598).

[0180] IR Binding Preferences

[0181] As indicated above, the amino acid sequences containing the motifs of this invention may be constructed to have enhanced selectivity for either IR or IGF-1R by choosing appropriate amino acids at specific positions of the

motifs or the regions flanking them. By providing amino acid preferences for IR or IGF-1R, this invention provides the means for constructing amino acid sequences with minimized activity at the non-cognate receptor. For example, the amino acid sequences disclosed herein with high affinity and activity for IR and low affinity and activity for IGF-1R are desirable as IR agonist as their propensity to promote undesirable cell proliferation, an activity of IGF-1 agonists, is reduced. Ratios of IR binding affinity to IGF-1R binding affinity for specific sequences are provided in FIGS. **1**A-**1**O; **2**A-**2**E; **3**A-**3**E; **4**A-**4**I; **44**A-**44**B. As an insulin therapeutic, the IR/IGF-1R binding affinity ratio is preferably greater than 100. Conversely, for use as an IGF-1R therapeutic, the IR/IGF-1R ratio should be less than 0.01. Examples of peptides that selectively bind to IGF-1R are shown below.

TABLE 1

IGF-IR-SELECTIVE SEQUENCES

| Clone | SEQ ID NO: | Sequence | Ratios over Background E-Tag | IGF-1R | IR | Comparisons IGF-1R/IR | IR/IGF-1R |
|---|---|---|---|---|---|---|---|
| FORMULA I (Group I; A6-like): | | | | | | | |
| A6L-0-E6-IR | 1599 | YRGMLVLGRSSDGAGKVAFERPARIGQTVFAVN⑦ | 31.0 | 31.0 | 1.8 | 17.0 | 0.1 |
| H2CA-4-G9-IGFR | 1600 | GIISQSCPESFYDWFAGQVSDPWWCW | 8.6 | 9.5 | 0.6 | 16.0 | 0.1 |
| H2CA-4-H6-IGFR | 1601 | VGRASGFPENFYDWFGRQLSLQSGEQ | 4.9 | 10.5 | 0.7 | 14.6 | 0.1 |
| A6L-0-E4-IR | 1602 | YRGMLVLGRISDGAG#VASEPPARIGRKVFAVN⑦ | 26.0 | 16.0 | 1.3 | 13.0 | 0.1 |
| A6L-0-H3-IR | 1603 | YRGMLVLGRISGGAGKAASERPARIGQKVSAVN⑦ | 27.0 | 26.0 | 2.0 | 13.0 | 0.1 |
| H2CA-4-F5-IGFR | 1604 | VGYQGQGDENFYDWFIRQVSGRLGVQ | 5.5 | 9.7 | 0.8 | 12.3 | 0.1 |
| H2CA-4-H8-IGFR | 1605 | SACQFDCHENFYDWFARQVSGGAAYG | 5.6 | 9.2 | 1.0 | 9.4 | 0.1 |
| H2CA-4-F11-IGFR | 1606 | SAAQLFFQESFYDWFLRQVAESSQPN | 3.5 | 6.8 | 1.0 | 6.7 | 0.1 |
| H2CA-4-F6-IGFR | 1607 | AVRATRFDEAFYDWFVRQISDGQGNK | 3.9 | 7.3 | 1.1 | 6.4 | 0.2 |
| H2CA-4-F10-IGFR | 1608 | VNQSGSIHENFYDWFERQVSHQRGVR | 4.9 | 5.7 | 1.0 | 5.9 | 0.2 |
| H2CA-1-A3-IGFR | 1609 | APDPSDFQEIFYDWFVRQVSRMPGGG | 7.7 | 3.8 | 0.8 | 5.1 | 0.2 |
| H2CA-3-C8-IGFR | 1610 | SSCDGAGHESFYEWFVRQVSGCRSV | 15.1 | 5.6 | 1.2 | 4.8 | 0.2 |
| H2CA-2-B9-IGFR | 1611 | RAGSSDFHEDFYEWFVRQVSLSLKGK | 9.3 | 7.0 | 1.7 | 4.2 | 0.2 |
| H2CA-4-H4-IGFR | 1612 | QAVQPGFHEEFYDWFVRQVSTGVGGG | 3.9 | 4.1 | 1.0 | 4.2 | 0.2 |
| E4Dα-4-H2-IR | 1613 | GFREGNFYEWFQAQVT | 37.8 | 33.9 | 8.2 | 4.1 | 0.2 |
| H2CA-4-F7-IGFR | 1614 | SSIGGGFHENFYDWFSRQLSQSPPLK | 1.5 | 3.2 | 0.8 | 4.1 | 0.2 |
| H2CA-3-D6-IGFR | 1615 | QSPVGSSHEDFYDWFFRQVAQSGAHQ | 8.3 | 9.0 | 2.2 | 4.0 | 0.3 |
| H2CA-3-D8-IGFR | 1616 | NYRRQVFNGNFYDWFDRQVFSLVTPG | 10.9 | 7.2 | 1.8 | 4.0 | 0.3 |
| H2CA-4-G11-IGFR | 1617 | TLDGGSFEEQFYDWFVRQLSYRTNPD | 10.8 | 9.5 | 2.5 | 3.9 | 0.3 |
| H2CA-4-F1-IGFR | 1618 | FYVQQWGHENFYDWFDRQVSQSGGAG | 5.8 | 3.5 | 0.9 | 3.8 | 0.3 |
| H2CA-3-D7-IGFR | 1619 | LRRQAPVEENFYDWFVRQVSGDRVGG | 13.3 | 3.0 | 0.8 | 3.7 | 0.3 |
| H2CA-1-A7-IGFR | 1620 | RCGRELYHSTFYDWFDRQVAGRTCPS | 8.0 | 2.2 | 0.6 | 3.7 | 0.3 |
| H2CA-2-B4-IGFR | 1621 | CCLLCRFQQNFYDWFVCQGISRLRPL | 3.5 | 4.1 | 1.1 | 3.6 | 0.3 |
| H2CA-2-B3-IGFR | 1622 | PPLASDLDVQFYGWFVQQVSPPGRGG | 7.7 | 3.8 | 1.0 | 3.6 | 0.3 |
| H2CA-2-B2-IGFR | 1623 | GAPVDQLHEDFYDWFVRQVSQAATG | 4.1 | 3.4 | 1.0 | 3.5 | 0.3 |
| E4Dα-2-D11-IR | 1624 | GFREGSFYDWFQAQVT | 40.2 | 11.1 | 3.3 | 3.4 | 0.3 |
| 20E2Bβ-4-G6-IR | 1625 | SQAGSAFYAWFDQVLRTVHSA | 22.4 | 6.2 | 1.9 | 3.3 | 0.3 |
| H2CA-4-H9-IGFR | 1626 | RGAVAGFHDQFYDWFDRQVSRVHKFG | 8.7 | 5.6 | 1.9 | 3.0 | 0.3 |

TABLE 1-continued

IGF-IR-SELECTIVE SEQUENCES

| Clone | SEQ ID NO: | Sequence | Ratios over Background E-Tag | IGF-1R | IR | Comparisons IGF-1R/IR | IR/IGF-1R |
|---|---|---|---|---|---|---|---|
| H2CA-2-B11-IGFR | 1627 | AICDAGFHEHFYDWFALQVSDCGRQS | 11.9 | 4.6 | 1.6 | 3.0 | 0.3 |
| H2CA-3-E8-IGFR | 1628 | LGYQEPFQQNFYDWFVRQVSGAENAG | 13.2 | 6.3 | 2.2 | 2.9 | 0.3 |
| A6S-2-D11-IR | 1629 | EAASLGSQDRNFYDWFVRQVV | 48.4 | 37.4 | 13.5 | 2.8 | 0.4 |
| A6S-2-D1-IR | 1630 | VERSASSQDGNEYDWFWQIR | 37.8 | 30.6 | 12.0 | 2.6 | 0.4 |
| A6S-3-E2-IR | 1631 | TSEVQRRSQDNFYDWFVAQVA | 33.1 | 24.7 | 9.8 | 2.5 | 0.4 |
| H2CA-3-E11-IGFR | 1632 | HLADGQFHEKFYDWFERQISSRCNDC | 4.7 | 2.2 | 1.0 | 2.2 | 0.5 |
| H2CA-3-C11-IGFR | 1633 | FRTLAAQHDSFYDWFDRQVSGAAGER | 9.3 | 3.3 | 1.6 | 2.1 | 0.5 |
| A6-PD1-IGFR | 1634 | SFHEDFYDWFDRQVSGSLKK | | | | | |
| H2C-PD1-IGFR(R | 1558 | GSLDESFYDWFERQLGKK | | | | | |
| FORMULA 2 (Group 2; B6-like): | | | | | | | |
| 20C-3-G3-IGFR | 1635 | TFYSCLASLLTGTPQPNRGPWERCR | 33.1 | 32.3 | 1.2 | 27.0 | <0.1 |
| 20C-4-C7-IGFR | 1636 | FFYDCLAALLQGVARYHDLCAVEIT | 35.3 | 28.0 | 1.3 | 21.8 | <0.1 |
| B6Hα-1-B5-IR | 1637 | CCTTEMVVMDARDDPFYHKLSELVTGG | 41.5 | 20.5 | 1.0 | 20.5 | 0.0 |
| R20β-4-A6-IR | 1638 | RGQSDAFYSGLWALIGLSDG | 9.3 | 25.9 | 1.5 | 17.3 | 0.1 |
| 20E2B-1-A6-IGFR | 1639 | GVRAMSFYDALVSVLGLGPSG | 18.6 | 18.1 | 1.1 | 16.8 | 0.1 |
| R20α-4-20A12-IR | 1640 | RLFYCGIQALGANLGYSGCV | 48.6 | 39.9 | 2.4 | 16.6 | 0.1 |
| 20E2Bβ-4-G7-IR | 1641 | LQPCSGFYECIERLIGVKLSG | 19.9 | 25.2 | 1.6 | 15.8 | 0.1 |
| NNRPγ-4-B11-IR | 1642 | LKDGFYDYFWQRLHLGS | 4.1 | 18.7 | 1.2 | 15.5 | 0.1 |
| 20E2B-3-C6-IGFR | 1643 | VEGRGLFYDLLRQLLARRQNG | 17.9 | 16.8 | 1.1 | 14.8 | 0.1 |
| B6Hα-1-A2-IR | 1644 | RGCNDDGGKGWSDDPFYHKLSELICGG | 22.3 | 14.6 | 1.0 | 14.6 | 0.1 |
| 20E2A-4-F11-IGFR | 1645 | QGGSASFYDAIDRLLRMRIGG | 21.3 | 18.8 | 1.3 | 14.6 | 0.1 |
| B6Hα-3-E9-IR | 1646 | RCEEKQAEVGPSSDPFYHKMSELLGCR | 44.6 | 24.2 | 1.7 | 14.2 | 0.1 |
| 20C-3-F6-IGFR | 1647 | DRDFCRFYERLTALVGGQVDGGWPC | 33.5 | 26.1 | 1.9 | 14.1 | 0.1 |
| 20E2B-4-H3-IGFR | 1648 | KLHNLMFYYGLQRLVWGAGLG | 11.2 | 14.8 | 1.1 | 13.9 | 0.1 |
| 20E2B-3-02-IGFR | 1649 | GNGDGMFYQLLSLLVGRDMHV | 13.1 | 8.9 | 0.6 | 13.8 | 0.1 |
| 20C-3-A1-IGFR | 1650 | SSYGCDGFYLMLFSLGLVASQELEC | 26.5 | 20.8 | 1.5 | 13.7 | 0.1 |
| 20E2B-3-E3-IGFR | 1651 | PDLHKGFYAQLAQLIRGQLLS | 22.4 | 16.3 | 1.3 | 13.1 | 0.1 |
| R20α-3-20E2-IR | 1652 | FYDAIDQLVRGSARAGGTRD | 46.3 | 39.9 | 3.1 | 12.9 | 0.1 |
| 20E2B-4-H12-IGFR | 1653 | YSCGDGFYSLLSDLLGGQFRC | 6.5 | 9.7 | 0.8 | 12.8 | 0.1 |
| B6Hα-3-F11-IR | 1654 | RGMKEEVLVGGSTDPFYHKLSELLQGS | 49.5 | 18.7 | 1.6 | 11.7 | 0.1 |
| 20E2B-3-D2-IGFR | 1655 | IQQELTFYDLLHRLVRSELGS | 20.7 | 12.4 | 1.1 | 11.7 | 0.1 |
| 20E2B-3-D8-IGFR | 1656 | GGTEVDFYRALERLVRGQLGL | 20.4 | 17.7 | 1.6 | 11.3 | 0.1 |
| 20E2B-3-E8-IGFR | 1657 | LRIANLFYQRLWDLAFGGGG | 15.7 | 16.7 | 1.5 | 11.1 | 0.1 |
| B6Hα-2-C4-IR | 1658 | RCGRW*AEMGAGDDPFYHKLSELVCG | 20.7 | 9.9 | 0.9 | 11.0 | 0.1 |
| R20α-4-20C11-IR | 1659 | DRAFYNGLRDLVGAVYGAWD | 43.7 | 30.8 | 3.0 | 10.3 | 0.1 |

TABLE 1-continued

IGF-IR-SELECTIVE SEQUENCES

| Clone | SEQ ID NO: | Sequence | Ratios over Background E-Tag | IGF-1R | IR | Comparisons IGF-1R/IR | IR/IGF-1R |
|---|---|---|---|---|---|---|---|
| 20E2B-4-F8-IGFR | 1660 | PVGVQGFYEGLSRLVLGRGGW | 12.3 | 7.3 | 0.8 | 9.7 | 0.1 |
| 20E2B-1-A11-IGFR | 1661 | RFSTDGFYQYLLALVGGGPVG | 15.0 | 9.5 | 1.0 | 9.7 | 0.1 |
| 20E2B-3-D4-IGFR | 1662 | NSRDGGFYLQLERLLGFPVTG | 8.1 | 7.9 | 0.8 | 9.6 | 0.1 |
| 20E2B-2-B11-IGFR | 1663 | VVTPVNFYRALEALVRG.RLG | 13.9 | 10.6 | 1.1 | 9.4 | 0.1 |
| 20E2B-3-C8-IGFR | 1664 | QPAPDGFYSALMKLIGRGGVS | 18.5 | 15.6 | 1.8 | 8.9 | 0.1 |
| 20E2B-2-B2-IGFR | 1665 | PGTDLGFYQALRCVVIQGACD | 11.7 | 4.9 | 0.6 | 8.1 | 0.1 |
| 20E2B-4-F10-IGFR | 1666 | AQPCGGFYGLLEQLVGRSVCD | 19.0 | 17.3 | 2.2 | 7.8 | 0.1 |
| 20E2B-4-F9-IGFR | 1667 | QPDHSYFYSLLQELVGSEERL | 11.9 | 14.7 | 1.9 | 7.7 | 0.1 |
| 20C-3-A4-IGFR | 1668 | QFYGCLLDLSLGVPSFGWRRRCITA | 17.7 | 8.8 | 1.2 | 7.6 | 0.1 |
| 20E2B-3-D11-IGFR | 1669 | LGVTDGFYAALGYLIHGVGQF | 14.3 | 12.2 | 1.6 | 7.6 | 0.1 |
| 20E2B-3-C11-IGFR | 1670 | CMM.DGFYAGLGCLLTAGEGR | 15.3 | 15.4 | 2.1 | 7.5 | 0.1 |
| 20E2B-2-B3-IGFR | 1671 | ICTGQGFYQVLCGLLRGTSAR | 9.1 | 5.3 | 0.7 | 7.4 | 0.1 |
| 20E2B-3-D12-IGFR | 1672 | QGNVLDFYGWIGRLLAKQGSD | 10.3 | 6.2 | 0.9 | 7.3 | 0.1 |
| 20E2B-3-E12-IGFR | 1673 | VATSQGFYSGLSELLQGGGNV | 13.9 | 6.0 | 0.8 | 7.3 | 0.1 |
| 20E2B-2-B8-IGFR | 1674 | IWATGDFYRLLSQLVMGRVGT | 17.4 | 5.7 | 0.8 | 7.2 | 0.1 |
| NNRPγ-4-A9-IR | 1675 | EGSGFYGYFFSLLGLQG | 3.0 | 10.0 | 1.4 | 7.1 | 0.1 |
| 20E2B-4-G11-IGFR | 1676 | RQGTGSFYLMLEQLLVGARGP | 8.9 | 4.5 | 0.6 | 7.0 | 0.1 |
| 20E2B-3-D6-IGFR | 1677 | DSVGDNFYQLLESLVGGHGVG | 20.7 | 17.8 | 2.6 | 6.9 | 0.1 |
| B6Hα-2-C7-IR | 1678 | RGIVAMVEATEVGSDHDPFYHKLSELVQGS | 45.1 | 6.7 | 1.0 | 6.7 | 0.1 |
| 20E2B-2-B7-IGFR | 1679 | LSSDGQFYRALNLLLQGSAGR | 18.0 | 6.1 | 0.9 | 6.7 | 0.1 |
| 20E2B-3-C4-IGFR | 1680 | ASSASGFYELLQRLAGLGLEV | 23.4 | 20.4 | 3.3 | 6.2 | 0.2 |
| 20C-3-E4-IGFR | 1681 | FFYRCLSRLLGGQLGSRLGLSCIGD | 37.7 | 7.7 | 1.3 | 6.0 | 0.2 |
| NNRPγ-4-A1-IR | 1682 | IIGGFYSYFNSVLRLGT | 9.7 | 10.9 | 1.8 | 6.0 | 0.2 |
| 20E2B-4-H8-IGFR | 1683 | PAGPCGFYCGLGLLLHGDQSP | 7.2 | 5.3 | 0.9 | 5.9 | 0.2 |
| 20E2B-4-H9-IGFR | 1684 | RCQGTGFYTCIQELIGFGDPD | 4.5 | 5.2 | 0.9 | 5.6 | 0.2 |
| B6Hα-2-C10-IR | 1685 | SGAKVIWTGDSGDPFYHKLSELLQGS | 46.9 | 5.8 | 1.1 | 5.3 | 0.2 |
| 20E2A-3-C7-IGFR | 1686 | VGTVAGFYDAIAQLVARASRV | 17.6 | 5.4 | 1.1 | 5.1 | 0.2 |
| 20E2B-1-A8-IGFR | 1687 | TLRSPTFYDWLEMVLTHGQGG | 16.1 | 4.4 | 0.9 | 5.0 | 0.2 |
| NNRPγ-4-A7-IR | 1688 | RFDPFYSYFVNLLGASA | 2.5 | 6.3 | 1.3 | 4.9 | 0.2 |
| B6Hα-3-E8-IR | 1689 | RGKTAAVIVGRPADPFYHKLSELLQGG | 47.6 | 5.3 | 1.1 | 4.8 | 0.2 |
| B6Hα-3-F10-IR | 1690 | GCVVEWQKWHGASDPFYHKLSELGGCS | 47.2 | 8.8 | 1.9 | 4.6 | 0.2 |
| B6Hα-2-D6-IR | 1691 | GRTMAVMAAGGPDDPFYHKLSELVQGG | 33.5 | 4.4 | 1.0 | 4.4 | 0.2 |

TABLE 1-continued

IGF-IR-SELECTIVE SEQUENCES

| Clone | SEQ ID NO: | Sequence | Ratios over Background E-Tag | IGF-1R | IR | Comparisons IGF-1R/IR | IR/IGF-1R |
|---|---|---|---|---|---|---|---|
| B6Hα-3-E7-IR | 1692 | GCAVVEEAERSRGDPFYHKLSELIQGC | 47.0 | 5.6 | 1.3 | 4.3 | 0.2 |
| B6Hα-2-D1-IR | 1693 | GCEVIVEEGDSADPFYHKLSELCQGS | 11.7 | 5.4 | 1.3 | 4.2 | 0.2 |
| 20E2A-3-D10-IGFR | 1694 | MMVVDGFYDALHQLVVAQSLG | 20.6 | 6.9 | 1.8 | 3.9 | 0.3 |
| 20E2A-3-A12-IGFR | 1695 | LSVALSFYDALGQLVAGEGRW | 16.1 | 4.3 | 1.1 | 3.9 | 0.3 |
| B6Hα-4-G8-IR | 1696 | GGTKAVAKVGTRDDPFYHKLSELLQGS | 32.3 | 6.1 | 1.7 | 3.6 | 0.3 |
| B6L-4-D7-IR | 1697 | AETSVQVGWIRLQSVWPGEHWNTVDPFY HKLSELLRGSGA | 14.3 | 4.8 | 1.4 | 3.4 | 0.3 |
| B6Hα-1-A3-IR | 1698 | SRAKVEAEMPDSGDPFYHKLSELLASG | 37.4 | 2.6 | 0.8 | 3.3 | 0.3 |
| B6Hα-3-F7-IR | 1699 | SRVAATKEKRPSDDPFYHKLSELLQGS | 41.5 | 3.1 | 1.0 | 3.1 | 0.3 |
| B6Hα-2-D8-IR | 1700 | SSETAKMVTGTRDDPFYHKLSELVQGS | 19.3 | 3.0 | 1.0 | 3.0 | 0.3 |
| B6Hα-1-B3-IR | 1701 | GCITAENGAGDPFYHKLSELGGCS | 33.1 | 3.2 | 1.1 | 2.9 | 0.3 |
| B6Hα-3-E5-IR | 1702 | RCGDEEGWQENRRDDPFYHKLSELFGGC | 28.8 | 2.9 | 1.0 | 2.9 | 0.3 |
| 20E2A-4-G11-IGFR | 1703 | MNVFVSFYDAIDQLVCQRIGC | 20.7 | 3.3 | 1.3 | 2.6 | 0.4 |
| 20E2Bβ-3-C7-IR | 1704 | QSGSGDFYDWLSRLIRGNGDG | 1.5 | 3.1 | 1.5 | 2.0 | 0.5 |
| B6Hα-3-E6-IR | 1705 | CGAKMTGTPNDPFYHKLSELLQRG | 18.2 | 2.3 | 1.2 | 1.9 | 0.5 |
| 20E2A-3-A3-IGFR | 1706 | GHYFGSFYDAIDQLVAGMLPG | 5.2 | 3.0 | 1.5 | 1.9 | 0.5 |
| B6L-4-A7-IR | 1707 | AGTPAQVG*NRLWSVWPGEHWNTVDPFY NKLSELLRESGA | 11.6 | 3.4 | 1.9 | 1.8 | 0.6 |
| B6Hα-3-F1-IR | 1708 | CSMAAVAEAGDDDDPFYHKLSELCQGS | 22.5 | 2.4 | 1.3 | 1.8 | 0.5 |
| B6L-3-G6-IR | 1709 | VDTPAQVGWNRLWSVGPGEHWYTDDPFY H*LSELLRESGA | 7.6 | 2.5 | 1.8 | 1.4 | 0.7 |
| B6L-3-G5-IR | 1710 | AETSAQVGWQRLWSVWPGDHWSTLDPFY HKLSELLRESGA | 11.5 | 2.0 | 1.4 | 1.4 | 0.7 |
| 20E2A-3-A4-IGFR | 1711 | AGSVTSFYDAMEQLVATGTSA | 16.8 | 2.5 | 1.8 | 1.4 | 0.7 |
| B6-PD1-IGFR | 1712 | TDDGFYDALEQLVQGSKK | | | | | |
| 20E2-PD1-IGFR (RP10) | 1713 | GSFYEALQRLVGGEQGKK | | | | | |
| FORMULA 10 (Group 6): | | | | | | | |
| R20β-4-E8-IR | 1714 | VRGFQGGTVWPGYEWLRNAA | 41.0 | 34.9 | 3.6 | 9.7 | 0.1 |
| 40F-4-D1-IGFR | 1715 | LSCLAYSRHGIWRPSTDLGLGRSVGEGSVS TRWRGYDWFE | 4.9 | 4.6 | 0.3 | 13.1 | 0.1 |
| 40F-4-B1-IGFR | 1716 | GLDHSDAVGVHLGFAWPAQARGRWEAGGLE DTWAGYDWL | 4.1 | 3.0 | 0.2 | 13.1 | 0.1 |
| 40F-4-D10-IGFR | 1717 | W.GYAWLS | 4.9 | 4.5 | 0.4 | 11.7 | 0.1 |

⑦ indicates text missing or illegible when filed

[0182] Besides relative binding at IR or IGF-1R, relative efficacy at the cognate receptor is another important consideration for choosing a potential therapeutic. Thus, a sequence that is efficacious at IR but has little or no significant activity at IGF-1R may also be considered as an important IR therapeutic, irrespective of the relative binding affinities at IR and IGF-1R. For example, A6 selectivity for IR may be enhanced by including glutamic acid in a carboxyl terminal extension at position $X_{95}$. IR selectivity of the B6 motif may be enhanced by having a tryptophan or

phenylalanine at $X_{11}$. Tryptophan at $X_{13}$ also favors selectivity of IR. A tryptophan amino acid at $X_{13}$ rather than leucine at that position also may be used to enhance selectivity for IR. In the reverse B6 motif, a large amino acid at $X_{15}$ favors IR selectivity. Conversely, small amino acids may confer specificity for IGF-1R. In the F8 motif, an L in position $X_{23}$ is essentially required for IR binding. In addition, tryptophan at $X_{31}$ is also highly preferred. At $X_{32}$, glycine is preferred for IR selectivity.

[0183] Multiple Binding Sites on IR and IGF-1R

[0184] The competition data disclosed herein reveals that at least two separate binding sites are present on IR and IGF-1R which recognize the different sequence motifs provided by this invention.

[0185] As shown in FIG. **6**, competition data indicate that peptides comprising the A6 motifs compete for binding to the same site on IR (Site 1) whereas the D8 motifs compete for a second site (Site 2). The identification of peptides that bind to separate binding sites on IR and IGF-1R provides for various schemes of binding to IR or IGF-1R to increase or decrease its activity. Examples of such schemes for IR are illustrated in FIG. **7**.

[0186] The table below shows sequences based on their groups, which bind to Site 1 or Site 2.

TABLE 2

| Clone | Sequence | SEQ ID NO: |
|---|---|---|
| REPRESENTATIVE SITE 1 PEPTIDES | | |
| A6-like (FYxWF) (SEQ ID NO: 1596): | | |
| G3 | KRGGGTFYEWFESALRKHGAGKK | 1718 |
| H2 | VTFTSAVFHENFYDWFVRQVSKK | 1719 |
| H2C | **FHENFYDWFVRQVSKK** | 1556 |
| A6S-IR3-E12 | **GRVDWLQRNANFYDWFVAELG** | 1560 |
| A6S-IR4-G1 | **NGVERAGTGDNFYDWFVAQLH** | 1720 |
| H2CB-R3-B12 | **QSDSGTVHDRFYGWFRDTWAS** | 1721 |
| 20E2A-R3-B11 | GRFYGWFQDAIDQLMPWGFDP | 1722 |
| rB6-F6 | RYGRWGLAQQFYDWFDR | 1723 |
| E4D□-1-B8-IR~ | GFREGQRWYWFVAQVT | 1724 |
| H2CA-4-F11-IR | TYKARFLHENFYDWFNRQVSQYFGRV | 1725 |
| H2CB-R3-D2 | WTDVDGFHSGFYRWFQNQWER | 1726 |
| H2CB-R3-D12 | VASGHVLHGQFYRWFVDQFAL | 1727 |
| H2CB-R4-H5 | QARVGNVHQQFYEWFREVMQG | 1728 |
| H2C-B-E8* | TGHRLGLDEQFYWWFRDALSG | 1729 |
| H2CB-3-B6-IR~ | VGDFCVSHDCFYGWFLRESMQ | 1730 |
| A6S-IR2-C1 | RMYFSTGAPQNFYDWFVQEWD | 1731 |
| B6-like (FYxxLxxL) (SEQ ID NO: 1732): | | |
| 20C11 | **KDRAFYNGLRDLVGAVYGAWDKK** | 1733 |
| 20E2 | **DYKDFYDAIDQLVRGSARAGGTRDKK** | 1734 |
| B62-R3-C7 | **EHWNTVDPFYFTLFEWLRESG** | 1735 |
| B62-R3-C10 | **EHWNTVDPFYQYFSELLRESG** | 1736 |
| 20E2B-3-B3-IR | AGVNAGFYRYFSTLLDWWDQG | 1737 |
| 20E2-B-E3* | IQGWEPFYGWFDDWAQMFEE | 1738 |
| 20E2A-R4-F9 | PPWGARFYDAIEQLVFDNLCC | 1739 |
| RPNN-4-G6-HOLO* | RWPNFYGYFESLLTHFS | 1740 |
| RPNN-4-F3-HOLO* | HYNAFYEYFQVLLAETW | 1741 |
| 20E2A-R4-E2 | IGRVRSFYDAIDKLFQSDWER | 1742 |
| RPNN-2-C1-IR* | EGWDFYSYFSGLLASVT | 1743 |
| 20E2B-4-F12-IR | SVKEVQFYRYFYDLLQSEESG | 1744 |
| 20E2-B-E12 | GNSGGSFYRYFQLLLDSDGMS | 1745 |
| 20E2A-R3-B6 | RDAGSSFYDAIDQLVCLTYFC | 1746 |

TABLE 2-continued

| Clone | Sequence | SEQ ID NO: |
|---|---|---|
| Reverse B6-like (LxxLxxYF) (SEQ ID NO: 1747): | | |
| rB6-A12 | **LDALDRLMRYFEERPSL** | 1748 |
| rB6-F9 | **PLAELWAYFEHSEQGRSSAH** | 1749 |
| rB6-4-E7-IR | LDPLDALLQYFWSVPGH | 1750 |
| rB6-4-F9-IR | RGRLGSLSTQFYNWFAE | 1751 |
| rB6-E6 | ADELEWLLDYFMHQPRP | 1752 |
| rB6-4-F12-IR | DGVLEELFSYFSATVGP | 1753 |
| Group 6 (WPxYxWL) (SEQ ID NO: 1754): | | |
| R20□-4-A4-IR | WPGYLFFEEALQDWRGSTED | 1755 |
| Peptides by design**: | | |
| H2C-PD1-IR~ | AAVHEQFYDWFADQYKK | 1756 |
| A6W-PD1-1R~ | QAPSNFYDWFVREWDKK | 1757 |
| 20E2-PD1-IR~ | QSFYDYIEELLGGEWKK | 1758 |
| B6C-PD1-IR~ | DPFYQGLWEWLRESGKK | 1759 |
| REPRESENTATIVE SITE 2 PEPTIDES (C-C LOOPS) | | |
| F8-derived (Long C-C loop): | | |
| F8 | HLCVLEELFWGASLFGYCSG | 1760 |
| F8-C12 | **FQSLLEELVWGAPLFRYGTG** | 1761 |
| F8-Des2 | **PLCVLEELFWGASLFGYCSG** | 1762 |
| F8-F12 | PLCVLEELFWGASLFGQCSG | 1763 |
| F8-B9 | HLCVLEELFWGASLFGQCSG | 1764 |
| F8-B12 | DLRVLCELFGGAYVLGYCSE | 1765 |
| NNKH-2B3 | HRSVLKQLSWGASLFGQWAG | 1766 |
| NNKH-2F9~ | HLSVGEELSWWVALLGQWAR | 1767 |
| NNKH-4H4~ | APVSTEELRWGALLFGQWAG | 1768 |
| D8-derived (Small C-C loop): | | |
| D8 | **KWLDQEWAWVQCEVYGRGCPSKK** | 1769 |
| D8-G1 | **QLEEEWAGVQCEVYGRECPS** | 1770 |
| D8-B5~ | ALEEEWAWVQVRSIRSGLPL | 1771 |
| D8-A7 | SLDQEWAWVQCEVYGRGCLS | 1772 |
| D8-F1~ | WLEHEWAQIQCELYGRGCTY | 1773 |
| Midi C-C loop: | | |
| D8-F10 | GLEQGCPWVGLEVQCRGCPS | 1774 |
| F8-B12~ | DLRVLCELFGGAYVLGYCSE | 1775 |
| F8-A9 | PLWGLCELFGGASLFGYCSS | 1776 |

**Based on analysis of entire panning data, amino acid preferences at each position were calculated to define these "idealized" peptides;
*Peptides synthesized and currently being purified;
~ Peptides planned.

[0187] In various aspects of the present invention, amino acid sequences comprising Site 1 motifs may bind to Site 1 of IR or Site 1 of IGF-1R. Similarly, amino acids sequences comprising Site 2 motifs may bind to Site 2 of IR or Site 2 of IGF-1R. However, specific peptides may show higher binding affinity for IR than for IGF-1R, while other peptides may show higher binding affinity for IGF-1R than for IR. In addition, Site 1 and Site 2 on IR do not "crosstalk", i.e., Site 1-binding sequences do not compete with Site 2-binding sequences at IR. In contrast, Site 1 and Site 2 on IGF-1R do show some crosstalk, suggesting an allosteric effect. These aspects are illustrated in the Examples described hereinbelow.

[0188] Multivalent Ligands

[0189] This invention provides ligands that preferentially bind different sites on IR and IGF-1R. The A6 amino acid sequence motif confers binding to IR at Site 1 (FIG. **6**). The D8 amino acid sequence motif confers binding to IR at Site 2 (FIG. **6**). Accordingly, multimeric ligands may be prepared according to the invention by covalently linking amino acid sequences. Depending on the purpose intended for the multivalent ligand, amino acid sequences that bind the same or different sites may be combined to form a single molecule. Where the multivalent ligand is constructed to bind to the same corresponding site on different receptors, or different subunits of a receptor, the amino acid sequences of the ligand for binding to the receptors may be the same or different, provided that if different amino acid sequences are used, they both bind to the same site.

[0190] Multivalent ligands may be prepared by either expressing amino acid sequences which bind to the individual sites separately and then covalently linking them together, or by expressing the multivalent ligand as a single amino acid sequence which comprises within it the combination of specific amino acid sequences for binding.

[0191] Various combinations of amino acid sequences may be combined to produce multivalent ligands having specific desirable properties. Thus, agonists may be combined with agonists, antagonists combined with antagonists, and agonists combined with antagonists. Combining amino acid sequences that bind to the same site to form a multivalent ligand may be useful to produce molecules that are capable of cross-linking together multiple receptor units. Multivalent ligands may also be constructed to combine amino acid sequences which bind to different sites (FIG. **7**).

[0192] In view of the discovery disclosed herein of monomers having agonist properties at IR or IGF-1R, preparation of multivalent ligands may be useful to prepare ligands having more desirable pharmacokinetic properties due to the presence of multiple bind sites on a single molecule. In addition, combining amino acid sequences that bind to different sites with different affinities provides a means for modulating the overall potency and affinity of the ligand for IR or IGF-1R.

[0193] Construction of Hybrids

[0194] In one embodiment, hybrids of at least two peptides (e.g., dimer peptides) may be produced as recombinant fusion polypeptides, which are expressed in any suitable expression system. The polypeptides may bind the receptor as either fusion constructs containing amino acid sequences besides the ligand binding sequences or as cleaved proteins from which signal sequences or other sequences unrelated to ligand binding are removed. Sequences for facilitating purification of the fusion protein may also be expressed as part of the construct. Such sequences optionally may be subsequently removed to produce the mature binding ligand. Recombinant expression also provides means for producing large quantities of ligand. In addition, recombinant expression may be used to express different combinations of amino acid sequences and to vary the orientation of their combination, i.e., amino to carboxyl terminal orientation.

[0195] In one embodiment shown below (FIG. **28**), MBP-FLAG®-PEPTIDE-$(GGS)_n$ (SEQ ID NO: 1777)-PEPTIDE-E-TAG, a fusion construct producing a peptide dimer comprises a maltose binding protein amino acid sequence (MBP) or similar sequence useful for enabling the affinity chromatography purification of the expressed peptide sequences. This purification facilitating sequence may then be attached to a FLAG® sequence to provide a cleavage site to remove the initial sequence. The dimer then follows which includes the intervening linker and a tag sequence may be included at the carboxyl terminal portion to facilitate identification/purification of the expression of peptide. In the representative construct illustrated above, G and S are glycine and serine residues, which make up the linker sequence. As non-limiting examples, n can be 1, 2, 3, or 4 to yield a linker sequence of 3, 6, 9, and 12 amino acids, respectively.

[0196] In addition to producing the dimer peptides by recombinant protein expression, dimer peptides may also be produced by peptide synthesis whereby a synthetic technique such as Merrifield synthesis (Merrifield, 1997), may be used to construct the entire peptide.

[0197] Other methods of constructing dimer peptides include introducing a linker molecule that activates the terminal end of a peptide so that it can covalently bind to a second peptide. Examples of such linkers include, but are not limited to, diaminoproprionic acid activated with an oxyamino function. A preferred linker is a dialdehyde having the formula $O{=}CH{-}(CH_2)_n{-}CH{=}O$, wherein n is at least 2 to 6, but is preferably 6 to produce a linker of about 25 to 30 angstroms in length. Other preferred linkers are shown in Table 3. Linkers may be used, for example, to couple monomers at either the carboxyl terminal or the amino terminal ends to form dimer peptides. Also, the chemistry can be inverted, i.e., the peptides to be coupled can be equipped with aldehyde functions, either by oxidation with sodium periodate of an N-terminal serine, or by oxidation of any other vicinal hydroxy- or amino-groups, and the linker can comprise two oxyamino functions (e.g., at end of a polyethylene glycol linker) or amino groups which are coupled by reductive amination.

[0198] In specific embodiments, Site 1-Site 2 and Site 2-Site 1 orientations are possible. In addition, N-terminal to N-terminal (N—N); C-terminal to C-terminal (C—C); N-terminal to C-terminal (N—C); and C-terminal to N-terminal (C—N) linkages are possible. Accordingly, peptides may be oriented Site 1 to Site 2, or Site 2 to Site 1, and may be linked N-terminus to N-terminus, C-terminus to C-terminus, N-terminus to C-terminus, or C-terminus to N-terminus. In certain cases, a specific orientation may be preferable to others, for example, for maximal agonist or antagonist activity.

[0199] In an unexpected and surprising result, the orientation and linkage of the monomer subunits has been found to dramatically alter dimer activity (see Examples, below). In particular, certain Site 1/Site 2 heterodimer sequences show agonist or antagonist activity at IR, depending on orientation and linkage of the constituent monomer subunits. For example, a Site 1-Site 2 orientation (C—N linkage), e.g., the S453 heterodimer, shows antagonist activity at IR (FIG. **18**A; Table 7). In contrast, a Site 2-Site 1 orientation (C—N linkage), e.g., the S455 heterodimer, shows potent agonist activity at IR (FIG. **18**D; Table 7). Similarly, Site 1-Site 2 (C—N linkage) heterodimers, e.g., S425 and S459, show antagonist activity at IR (Table 7), while Site 1-Site 2 (C—C or N—N linkage) heterodimers, e.g., S432-S438, S454, and S456, show agonist activity (Table 7).

[0200] Whether produced by recombinant gene expression or by conventional chemical linkage technology, the various amino acid sequences may be coupled through linkers of various lengths. Where linked sequences are expressed recombinantly, and based on an average amino acid length of about 4 angstroms, the linkers for connecting the two amino acid sequences would typically range from about 3 to about 12 amino acids corresponding to from about 12 to about 48 Å. Accordingly, the preferred distance between binding sequences is from about 2 to about 50 Å. More preferred is 4 to about 40. The degree of flexibility of the linker between the amino acid sequences may be modulated by the choice of amino acids used to construct the linker. The combination of glycine and serine is useful for producing a flexible, relatively unrestrictive linker. A more rigid linker may be constructed by using amino acids with more complex side chains within the linkage sequence.

[0201] Characterization Of Specific Dimers

[0202] Specific dimers which are comprised of monomer subunits that both bind with high affinity to the same site on IR (i.e., homodimers), or monomer subunits that bind to different sites on IR (i.e., heterodimers) are disclosed herein.

[0203] Other combinations of peptides are within the scope of this invention and may be determined as demonstrated in the examples described herein.

[0204] Peptide Synthesis

[0205] Many conventional techniques in molecular biology, protein biochemistry, and immunology may be used to produce the amino acid sequences for use with this invention. The present invention encompasses the specific amino acid sequences shown in FIGS. **1-4**, **8**, and **9** and Table 7, inter alia, without additions (e.g., linker or spacer sequences) deletions, alterations, or modification. The present invention further encompasses variants that include additional sequences, altered sequences, and functional fragments thereof. In a preferred embodiment, the amino acid sequence variant or fragment shares at least one function characteristic (e.g., binding, agonist, or antagonist activity) of the reference sequence. Variant peptides include, for example, genetically engineered mutants, and may differ from the amino acid sequences shown in the figures and tables of the application by the addition, deletion, or substitution of one or more amino acid residues. Alterations may occur at the amino- or carboxy-terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. In addition, variants may comprise synthetic or non-naturally occurring amino acids in accordance with this invention.

[0206] Variant amino acid sequences can have conservative changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. More infrequently, a variant peptide can have non-conservative changes, e.g., substitution of a glycine with a tryptophan. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing binding or biological activity can be found using computer programs well known in the art, for example, DNASTAR software (DNASTAR, Inc., Madison, Wis.). Guidance is also provided by the data disclosed herein. In particular, FIGS. **1-4**, **8**, **9**, **43**, **44**, and Table 7, inter alia, teach which amino acid residues can be deleted, added, substituted, or modified, while maintaining the IR- or IGF-1R-related function(s) (e.g., binding, agonist, or antagonist activity) of the amino acid sequences.

[0207] For the purposes of this invention, the amino acids are grouped as follows: amino acids possessing alcohol groups are serine (S) and threonine (T). Aliphatic amino acids are isoleucine (I), leucine (L), valine (V), and methionine (M). Aromatic amino acids are phenylalanine (F), histidine (H), tryptophan (W), and tyrosine (Y). Hydrophobic amino acids are alanine (A), cysteine (C), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), leucine (L), methionine (M), arginine (R), threonine (T), valine (V), tryptophan (W), and tyrosine (Y). Negative amino acids are aspartic acid (D) and glutamic acid (E). The following amino acids are polar amino acids: cysteine (C), aspartic acid (D), glutamic acid (E), histidine (H), lysine (K), asparagine (N), glutamine (Q), arginine (R), serine (S), and threonine (T). Positive amino acids are histidine (H), lysine (K), and arginine (R). Small amino acids are alanine (A), cysteine (C), aspartic acid (D), glycine (G), asparagine (N), proline (P), serine (S), threonine (T), and valine (V). Very small amino acids are alanine (A), glycine (G) and serine (S). Amino acids likely to be involved in a turn formation are alanine (A), cysteine (C), aspartic acid (D), glutamic acid (E), glycine (G), histidine (H), lysine (K), asparagine (N), glutamine (Q), arginine (R), serine (S), proline (P), and threonine (T). As non-limiting examples, the amino acids within each of these defined groups may be substituted for each other in the formulas described above, as conservative substitutions, subject to the specific preferences stated herein.

[0208] Substantial changes in function can be made by selecting substitutions that are less conservative than those shown in the defined groups, above. For example, non-conservative substitutions can be made which more significantly affect the structure of the peptide in the area of the alteration, for example, the alpha-helical, or beta-sheet structure; the charge or hydrophobicity of the molecule at the target site; or the bulk of the side chain. The substitutions which generally are expected to produce the greatest changes in the peptide's properties are those where 1) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl, or alanyl; 2) a cysteine or proline is substituted for (or by) any other residue; 3) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or 4) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) a residue that does not have a side chain, e.g., glycine.

[0209] Amino acid preferences have been identified for certain peptides and peptide groups of the present invention. For example, amino acid preferences for the RP9, D8, and Group 6 (Formula 10) peptides are shown in Tables 17-19, below.

[0210] Variants also include amino acid sequences in which one or more residues are modified (i.e., by phosphorylation, sulfation, acylation, PEGylation, etc.), and mutants comprising one or more modified residues. Amino acid sequences may also be modified with a label capable of

providing a detectable signal, either directly or indirectly, including, but not limited to, radioisotope, fluorescent, and enzyme labels. Fluorescent labels include, for example, Cy3, Cy5, Alexa, BODIPY, fluorescein (e.g., FluorX, DTAF, and FITC), rhodamine (e.g., TRITC), auramine, Texas Red, AMCA blue, and Lucifer Yellow. Preferred isotope labels include $^{3}H$, $^{14}C$, $^{32}P$, $^{35}S$, $^{36}Cl$, $^{51}Cr$, $^{57}Co$, $^{58}Co$, $^{59}Fe$, $^{90}Y$, $^{125}I$, $^{131}I$, and $^{186}Re$. Preferred enzyme labels include peroxidase, β-glucuronidase, β-D-glucosidase, β-D-galactosidase, urease, glucose oxidase plus peroxidase, and alkaline phosphatase (see, e.g., U.S. Pat. Nos. 3,654,090; 3,850,752 and 4,016,043). Enzymes can be conjugated by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde, and the like. Enzyme labels can be detected visually, or measured by calorimetric, spectrophotometric, fluorospectrophotometric, amperometric, or gasometric techniques. Other labeling systems, such as avidin/biotin, Tyramide Signal Amplification (TSA™), are known in the art, and are commercially available (see, e.g., ABC kit, Vector Laboratories, Inc., Burlingame, Calif.; NEN® Life Science Products, Inc., Boston, Mass.).

[0211] Recombinant Synthesis of Peptides

[0212] To obtain recombinant peptides, DNA sequences encoding these peptides may be cloned into any suitable vectors for expression in intact host cells or in cell-free translation systems by methods well known in the art (see Sambrook et al., 1989). The particular choice of the vector, host, or translation system is not critical to the practice of the invention.

[0213] A large number of vectors, including bacterial, yeast, and mammalian vectors, have been described for replication and/or expression in various host cells or cell-free systems, and may be used for gene therapy as well as for simple cloning or protein expression. In one aspect of the present invention, an expression vector comprises a nucleic acid encoding a IR or IGF-1R agonist or antagonist peptide, as described herein, operably linked to at least one regulatory sequence. Regulatory sequences are known in the art and are selected to direct expression of the desired protein in an appropriate host cell. Accordingly, the term regulatory sequence includes promoters, enhancers and other expression control elements (see D. V. Goeddel (1990) *Methods Enzymol.* 185:3-7). Enhancer and other expression control sequences are described in *Enhancers and Eukaryotic Gene Expression*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1983). It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transfected and/or the type of peptide desired to be expressed.

[0214] Several regulatory elements (e.g., promoters) have been isolated and shown to be effective in the transcription and translation of heterologous proteins in the various hosts. Such regulatory regions, methods of isolation, manner of manipulation, etc. are known in the art. Non-limiting examples of bacterial promoters include the β-lactamase (penicillinase) promoter; lactose promoter; tryptophan (trp) promoter; araBAD (arabinose) operon promoter; lambda-derived $P_1$ promoter and N gene ribosome binding site; and the hybrid tac promoter derived from sequences of the trp and lac UV5 promoters. Non-limiting examples of yeast promoters include the 3-phosphoglycerate kinase promoter, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter, galactokinase (GAL1) promoter, galactoepimerase promoter, and alcohol dehydrogenase (ADH1) promoter. Suitable promoters for mammalian cells include, without limitation, viral promoters, such as those from Simian Virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus (ADV), and bovine papilloma virus (BPV). Preferred replication and inheritance systems include M13, ColE1, SV40, baculovirus, lambda, adenovirus, CEN ARS, 2 μm ARS and the like. While expression vectors may replicate autonomously, they may also replicate by being inserted into the genome of the host cell, by methods well known in the art.

[0215] To obtain expression in eukaryotic cells, terminator sequences, polyadenylation sequences, and enhancer sequences that modulate gene expression may be required. Sequences that cause amplification of the gene may also be desirable. Furthermore, sequences that facilitate secretion of the recombinant product from cells, including, but not limited to, bacteria, yeast, and animal cells, such as secretory signal sequences and/or preprotein or proprotein sequences, may also be included. These sequences are well described in the art. DNA sequences can be optimized, if desired, for more efficient expression in a given host organism or expression system. For example, codons can be altered to conform to the preferred codon usage in a given host cell or cell-free translation system using well-established techniques.

[0216] Codon usage data can be obtained from publicly-available sources, for example, the Codon Usage Database at http://www.kazusa.or.jp/codon/. In addition, computer programs that translate amino acid sequence information into nucleotide sequence information in accordance with codon preferences (i.e., backtranslation programs) are widely available. See, for example, Backtranslate program from Genetics Computer Group (GCG), Accelrys, Inc., Madison, Wis.; and Backtranslation Applet from Entelechon GmbH, Regensburg, Germany. Thus, using the peptide sequences disclosed herein, one of ordinary skill in the art can design nucleic acids to yield optimal expression levels in the translation system or host cell of choice.

[0217] Expression and cloning vectors will likely contain a selectable marker, a gene encoding a protein necessary for survival or growth of a host cell transformed with the vector. The presence of this gene ensures growth of only those host cells that express the inserts. Typical selection genes encode proteins that 1) confer resistance to antibiotics or other toxic substances, e.g., ampicillin, neomycin, methotrexate, etc.; 2) complement auxotrophic deficiencies, or 3) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for Bacilli. Markers may be an inducible or non-inducible gene and will generally allow for positive selection. Non-limiting examples of markers include the ampicillin resistance marker (i.e., beta-lactamase), tetracycline resistance marker, neomycin/kanamycin resistance marker (i.e., neomycin phosphotransferase), dihydrofolate reductase, glutamine synthetase, and the like. The choice of the proper selectable marker will depend on the host cell, and appropriate markers for different hosts as understood by those of skill in the art.

[0218] Suitable expression vectors for use with the present invention include, but are not limited to, pUC, pBluescript (Stratagene), pET (Novagen, Inc., Madison, Wis.), and pREP (Invitrogen) plasmids. Vectors can contain one or more replication and inheritance systems for cloning or

expression, one or more markers for selection in the host, e.g., antibiotic resistance, and one or more expression cassettes. The inserted coding sequences can be synthesized by standard methods, isolated from natural sources, or prepared as hybrids. Ligation of the coding sequences to transcriptional regulatory elements (e.g., promoters, enhancers, and/or insulators) and/or to other amino acid encoding sequences can be carried out using established methods.

[0219] Suitable cell-free expression systems for use with the present invention include, without limitation, rabbit reticulocyte lysate, wheat germ extract, canine pancreatic microsomal membranes, *E. coli* S30 extract, and coupled transcription/translation systems (Promega Corp., Madison, Wis.). These systems allow the expression of recombinant peptides upon the addition of cloning vectors, DNA fragments, or RNA sequences containing protein-coding regions and appropriate promoter elements.

[0220] Non-limiting examples of suitable host cells include bacteria, archea, insect, fungi (e.g., yeast), plant, and animal cells (e.g., mammalian, especially human). Of particular interest are *Escherichia coli, Bacillus subtilis, Saccharomyces cerevisiae*, SF9 cells, C129 cells, 293 cells, *Neurospora*, and immortalized mammalian myeloid and lymphoid cell lines. Techniques for the propagation of mammalian cells in culture are well-known (see, Jakoby and Pastan (Eds), 1979, *Cell Culture. Methods in Enzymology*, volume 58, Academic Press, Inc., Harcourt Brace Jovanovich, N.Y.). Examples of commonly used mammalian host cell lines are VERO and HeLa cells, CHO cells, and WI38, BHK, and COS cell lines, although it will be appreciated by the skilled practitioner that other cell lines may be used, e.g., to provide higher expression, or other features.

[0221] Host cells can be transformed, transfected, or infected as appropriate by any suitable method including electroporation, calcium chloride-, lithium chloride-, lithium acetate/polyethylene glycol-, calcium phosphate-, DEAE-dextran-, liposome-mediated DNA uptake, spheroplasting, injection, microinjection, microprojectile bombardment, phage infection, viral infection, or other established methods. Alternatively, vectors containing the nucleic acids of interest can be transcribed in vitro, and the resulting RNA introduced into the host cell by well-known methods, e.g., by injection (see, Kubo et al., 1988, *FEBS Letts.* 241:119). The cells into which have been introduced nucleic acids described above are meant to also include the progeny of such cells.

[0222] Nucleic acids encoding the peptides of the invention may be isolated directly from recombinant phage libraries (e.g., RAPIDLIB® or GRABLIB® libraries) described herein. Alternatively, the polymerase chain reaction (PCR) method can be used to produce nucleic acids of the invention, using the recombinant phage libraries as templates. Primers used for PCR can be synthesized using the sequence information provided herein and can further be designed to introduce appropriate new restriction sites, if desirable, to facilitate incorporation into a given vector for recombinant expression.

[0223] Nucleic acids encoding the peptides of the present invention can also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage et al., 1981, *Tetra. Letts.* 22:1859-1862, or the triester method according to Matteucci et al., 1981, *J. Am. Chem. Soc.*, 103:3185, and can performed on commercial, automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single-stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strands together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

[0224] The nucleic acids encoding the peptides of the invention can be produced in large quantities by replication in a suitable host cell. Natural or synthetic nucleic acid fragments, comprising at least ten contiguous bases coding for a desired amino acid sequence can be incorporated into recombinant nucleic acid constructs, usually DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the nucleic acid constructs will be suitable for replication in a unicellular host, such as yeast or bacteria, but may also be intended for introduction to (with and without integration within the genome) cultured mammalian or plant or other eukaryotic cells, cell lines, tissues, or organisms. The purification of nucleic acids produced by the methods of the present invention is described, for example, in Sambrook et al., 1989; F. M. Ausubel et al., 1992, *Current Protocols in Molecular Biology*, J. Wiley and Sons, New York, N.Y.

[0225] These nucleic acids can encode variant or truncated forms of the peptides as well as the reference peptides shown in FIGS. **1-4**, **8**, and **9** and Table 7, inter alia. Large quantities of the nucleic acids and peptides of the present invention may be prepared by expressing the nucleic acids or portions thereof in vectors or other expression vehicles in compatible prokaryotic or eukaryotic host cells. The most commonly used prokaryotic hosts are strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or *Pseudomonas* may also be used. Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant, insect, or amphibian or avian species, may also be useful for production of the proteins of the present invention. For example, insect cell systems (i.e., lepidopteran host cells and baculovirus expression vectors) are particularly suited for large-scale protein production.

[0226] Host cells carrying an expression vector (i.e., transformants or clones) are selected using markers depending on the mode of the vector construction. The marker may be on the same or a different DNA molecule, preferably the same DNA molecule. In prokaryotic hosts, the transformant may be selected, e.g., by resistance to ampicillin, tetracycline or other antibiotics. Production of a particular product based on temperature sensitivity may also serve as an appropriate marker.

[0227] For some purposes, it is preferable to produce the peptide in a recombinant system in which the peptide contains an additional sequence (e.g., epitope or protein) tag that facilitates purification. Non-limiting examples of epitope tags include c-myc, haemagglutinin (HA), polyhistidine (6X-HIS) (SEQ ID NO: 1778), GLU-GLU, and DYKDDDDK (SEQ ID NO:1779) or DYKD (SEQ ID NO:1545; FLAG®) epitope tags. Non-limiting examples of protein tags include glutathione-S-transferase (GST), green fluorescent protein (GFP), and maltose binding protein (MBP). In one approach, the coding sequence of a peptide can be cloned into a vector that creates a fusion with a sequence tag of interest. Suitable vectors include, without

limitation, PRSET (Invitrogen Corp., San Diego, Calif.), PGEX (Amersham Pharmacia Biotech, Inc., Piscataway, N.J.), PEGFP (CLONTECH Laboratories, Inc., Palo Alto, Calif.), and PMAL™ (New England BioLabs, Inc., Beverly, Mass.) plasmids. Following expression, the epitope or protein tagged peptide can be purified from a crude lysate of the translation system or host cell by chromatography on an appropriate solid-phase matrix. In some cases, it may be preferable to remove the epitope or protein tag (i.e., via protease cleavage) following purification.

[0228] Methods for directly purifying peptides from sources such as cellular or extracellular lysates are well known in the art (see Harris and Angal, 1989). Such methods include, without limitation, sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), preparative disc-gel electrophoresis, isoelectric focusing, high-performance liquid chromatography (HPLC), reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, countercurrent distribution, and combinations thereof. Peptides can be purified from many possible sources, for example, plasma, body tissues, or body fluid lysates derived from human or animal, including mammalian, bird, fish, and insect sources.

[0229] Antibody-based methods may also be used to purify peptides. Antibodies that recognize these peptides or fragments derived therefrom can be produced and isolated. The peptide can then be purified from a crude lysate by chromatography on an antibody-conjugated solid-phase matrix (see Harlow and Lane, 1998).

[0230] Chemical Synthesis of Peptides

[0231] Alternately, peptides may be chemically synthesized by commercially available automated procedures, including, without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. The peptides are preferably prepared by solid-phase peptide synthesis; for example, as described by Merrifield (1965; 1997).

[0232] According to methods known in the art, peptides can be chemically synthesized by commercially available automated procedures, including, without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation, classical solution synthesis. In addition, recombinant and synthetic methods of peptide production can be combined to produce semi-synthetic peptides. The peptides of the invention are preferably prepared by solid phase peptide synthesis as described by Merrifield, 1963, *J. Am. Chem. Soc.* 85:2149; 1997. In one embodiment, synthesis is carried out with amino acids that are protected at the alpha-amino terminus. Trifunctional amino acids with labile side-chains are also protected with suitable groups to prevent undesired chemical reactions from occurring during the assembly of the peptides. The alpha-amino protecting group is selectively removed to allow subsequent reaction to take place at the amino-terminus. The conditions for the removal of the alpha-amino protecting group do not remove the side-chain protecting groups.

[0233] The alpha-amino protecting groups are those known to be useful in the art of stepwise peptide synthesis. Included are acyl type protecting groups, e.g., formyl, trifluoroacetyl, acetyl, aromatic urethane type protecting groups, e.g., benzyloxycarbonyl (Cbz), substituted benzyloxycarbonyl and 9-fluorenylmethyloxycarbonyl (Fmoc), aliphatic urethane protecting groups, e.g., t-butyloxycarbonyl (Boc), isopropyloxycarbonyl, cyclohexyloxycarbonyl, and alkyl type protecting groups, e.g., benzyl, triphenylmethyl. The preferred protecting group is Boc. The side-chain protecting groups for Tyr include tetrahydropyranyl, tert-butyl, trityl, benzyl, Cbz, 4-Br-Cbz and 2,6-dichlorobenzyl. The preferred side-chain protecting group for Tyr is 2,6-dichlorobenzyl. The side-chain protecting groups for Asp include benzyl, 2,6-dichlorobenzyl, methyl, ethyl, and cyclohexyl. The preferred side-chain protecting group for Asp is cyclohexyl. The side-chain protecting groups for Thr and Ser include acetyl, benzoyl, trityl, tetrahydropyranyl, benzyl, 2,6-dichlorobenzyl, and Cbz. The preferred protecting group for Thr and Ser is benzyl. The side-chain protecting groups for Arg include nitro, Tos, Cbz, adamantyloxycarbonyl, and Boc. The preferred protecting group for Arg is Tos. The side-chain amino group of Lys can be protected with Cbz, 2-Cl-Cbz, Tos, or Boc. The 2-Cl-Cbz group is the preferred protecting group for Lys.

[0234] The side-chain protecting groups selected must remain intact during coupling and not be removed during the deprotection of the amino-terminus protecting group or during coupling conditions. The side-chain protecting groups must also be removable upon the completion of synthesis, using reaction conditions that will not alter the finished peptide.

[0235] Solid phase synthesis is usually carried out from the carboxy-terminus by coupling the alpha-amino protected (side-chain protected) amino acid to a suitable solid support. An ester linkage is formed when the attachment is made to a chloromethyl or hydroxymethyl resin, and the resulting peptide will have a free carboxyl group at the C-terminus. Alternatively, when a benzhydrylamine or p-methylbenzhydrylamine resin is used, an amide bond is formed and the resulting peptide will have a carboxamide group at the C-terminus. These resins are commercially available, and their preparation has described by Stewart et al., 1984, Solid Phase Peptide Synthesis (2nd Edition), Pierce Chemical Co., Rockford, Ill.

[0236] The C-terminal amino acid, protected at the side chain if necessary and at the alpha-amino group, is coupled to the benzhydrylamine resin using various activating agents including dicyclohexylcarbodiimide (DCC), N,N'-diisopropyl-carbodiimide and carbonyldiimidazole. Following the attachment to the resin support, the alpha-amino protecting group is removed using trifluoroacetic acid (TFA) or HCl in dioxane at a temperature between 0 and 25° C. Dimethylsulfide is added to the TFA after the introduction of methionine (Met) to suppress possible S-alkylation. After removal of the alpha-amino protecting group, the remaining protected amino acids are coupled stepwise in the required order to obtain the desired sequence. Various activating agents can be used for the coupling reactions including DCC,N,N'-diisopropyl-carbodiimide, benzotriazol-1-yl-oxy-tris-(dimethylamino) phosphonium hexa-fluorophosphate (BOP) and DCC-hydroxybenzotriazole (HOBt). Each protected amino acid is used in excess (>2.0 equivalents), and the couplings are usually carried out in N-methylpyrrolidone (NMP) or in DMF, $CH_2Cl_2$ or mixtures thereof. The extent of completion of the coupling reaction is monitored at each stage, e.g., by the ninhydrin reaction as described by Kaiser et al., 1970, *Anal. Biochem.* 34:595. In cases where incomplete coupling

is found, the coupling reaction is repeated. The coupling reactions can be performed automatically with commercially available instruments.

[0237] After the entire assembly of the desired peptide, the peptide-resin is cleaved with a reagent such as liquid HF for 1-2 hours at 0° C., which cleaves the peptide from the resin and removes all side-chain protecting groups. A scavenger such as anisole is usually used with the liquid HF to prevent cations formed during the cleavage from alkylating the amino acid residues present in the peptide. The peptide-resin can be deprotected with TFA/dithioethane prior to cleavage if desired.

[0238] Side-chain to side-chain cyclization on the solid support requires the use of an orthogonal protection scheme which enables selective cleavage of the side-chain functions of acidic amino acids (e.g., Asp) and the basic amino acids (e.g., Lys). The 9-fluorenylmethyl (Fm) protecting group for the side-chain of Asp and the 9-fluorenylmethyloxycarbonyl (Fmoc) protecting group for the side-chain of Lys can be used for this purpose. In these cases, the side-chain protecting groups of the Boc-protected peptide-resin are selectively removed with piperidine in DMF. Cyclization is achieved on the solid support using various activating agents including DCC, DCC/HOBt, or BOP. The HF reaction is carried out on the cyclized peptide-resin as described above.

[0239] Peptide Libraries

[0240] Peptide libraries produced and screened according to the present invention are useful in providing new ligands for IR and IGF-1R. Peptide libraries can be designed and panned according to methods described in detail herein, and methods generally available to those in the art (see, e.g., U.S. Pat. No. 5,723,286 issued Mar. 3, 1998 to Dower et al.). In one aspect, commercially available phage display libraries can be used (e.g., RAPIDLIB® or GRABLIB®, DGI Biotechnologies, Inc., Edison, N.J.; Ph.D. C7C Disulfide Constrained Peptide Library, New England Biolabs). In another aspect, an oligonucleotide library can be prepared according to methods known in the art, and inserted into an appropriate vector for peptide expression. For example, vectors encoding a bacteriophage structural protein, preferably an accessible phage protein, such as a bacteriophage coat protein, can be used. Although one skilled in the art will appreciate that a variety of bacteriophage may be employed in the present invention, in preferred embodiments the vector is, or is derived from, a filamentous bacteriophage, such as, for example, f1, fd, Pf1, M13, etc. In particular, the fd-tet vector has been extensively described in the literature (see, e.g., Zacher et al., 1980, *Gene* 9:127-140; Smith et al., 1985, *Science* 228:1315-1317; Parmley and Smith, 1988, *Gene* 73:305-318).

[0241] The phage vector is chosen to contain or is constructed to contain a cloning site located in the 5' region of the gene encoding the bacteriophage structural protein, so that the peptide is accessible to receptors in an affinity enrichment procedure as described hereinbelow. The structural phage protein is preferably a coat protein. An example of an appropriate coat protein is pill. A suitable vector may allow oriented cloning of the oligonucleotide sequences that encode the peptide so that the peptide is expressed at or within a distance of about 100 amino acid residues of the N-terminus of the mature coat protein. The coat protein is typically expressed as a preprotein, having a leader sequence.

[0242] Thus, desirably the oligonucleotide library is inserted so that the N-terminus of the processed bacteriophage outer protein is the first residue of the peptide, i.e., between the 3'-terminus of the sequence encoding the leader protein and the 5'-terminus of the sequence encoding the mature protein or a portion of the 5' terminus. The library is constructed by cloning an oligonucleotide which contains the variable region of library members (and any spacers, as discussed below) into the selected cloning site. Using known recombinant DNA techniques (see generally, Sambrook et al., 1989, *Molecular Cloning, A Laboratory Manual,* 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), an oligonucleotide may be constructed which, inter alia; 1) removes unwanted restriction sites and adds desired ones; 2) reconstructs the correct portions of any sequences which have been removed (such as a correct signal peptidase site, for example); 3) inserts the spacer residues, if any; and/or 4) corrects the translation frame (if necessary) to produce active, infective phage.

[0243] The central portion of the oligonucleotide will generally contain one or more IR and/or IGF-1R binding sequences and, optionally, spacer sequences. The sequences are ultimately expressed as peptides (with or without spacers) fused to or in the N-terminus of the mature coat protein on the outer, accessible surface of the assembled bacteriophage particles. The size of the library will vary according to the number of variable codons, and hence the size of the peptides, which are desired. Generally the library will be at least about $10^6$ members, usually at least $10^7$, and typically $10^8$ or more members. To generate the collection of oligonucleotides which forms a series of codons encoding a random collection of amino acids and which is ultimately cloned into the vector, a codon motif is used, such as $(NNK)_x$, where N may be A, C, G, or T (nominally equimolar), K is G or T (nominally equimolar), and x is typically up to about 5, 6, 7, 8, or more, thereby producing libraries of penta-, hexa-, hepta-, and octa-peptides or larger. The third position may also be G or C, designated "S". Thus, NNK or NNS 1) code for all the amino acids; 2) code for only one stop codon; and 3) reduce the range of codon bias from 6:1 to 3:1.

[0244] It should be understood that, with longer peptides, the size of the library that is generated may become a constraint in the cloning process. The expression of peptides from randomly generated mixtures of oligonucleotides in appropriate recombinant vectors is known in the art (see, e.g., Oliphant et al., *Gene* 44:177-183). For example, the codon motif $(NNK)_6$ produces 32 codons, one for each of 12 amino acids, two for each of five amino acids, three for each of three amino acids and one (amber) stop codon. Although this motif produces a codon distribution as equitable as available with standard methods of oligonucleotide synthesis, it results in a bias against peptides containing one-codon residues. In particular, a complete collection of hexacodons contains one sequence encoding each peptide made up of only one-codon amino acids, but contains 729 ($3^6$) sequences encoding each peptide with only three-codon amino acids.

[0245] An alternative approach to minimize the bias against one-codon residues involves the synthesis of 20 activated trinucleotides, each representing the codon for one of the 20 genetically encoded amino acids. These are synthesized by conventional means, removed from the support

while maintaining the base and 5-OH-protecting groups, and activated by the addition of 3'O-phosphoramidite (and phosphate protection with b-cyanoethyl groups) by the method used for the activation of mononucleosides (see, generally, McBride and Caruthers, 1983, *Tetrahedron Letters* 22:245). Degenerate oligocodons are prepared using these trimers as building blocks. The trimers are mixed at the desired molar ratios and installed in the synthesizer. The ratios will usually be approximately equimolar, but may be a controlled unequal ratio to obtain the over- to under-representation of certain amino acids coded for by the degenerate oligonucleotide collection. The condensation of the trimers to form the oligocodons is done essentially as described for conventional synthesis employing activated mononucleosides as building blocks (see, e.g., Atkinson and Smith, 1984, *Oligonucleotide Synthesis*, M. J. Gait, Ed., p. 35-82). This procedure generates a population of oligonucleotides for cloning that is capable of encoding an equal distribution (or a controlled unequal distribution) of the possible peptide sequences. Advantageously, this approach may be employed in generating longer peptide sequences, since the range of bias produced by the $(NNK)_6$ motif increases by three-fold with each additional amino acid residue.

**[0246]** When the codon motif is $(NNK)_x$, as defined above, and when x equals 8, there are $2.6.\times10^{10}$ possible octa-peptides. A library containing most of the octa-peptides may be difficult to produce. Thus, a sampling of the octa-peptides may be accomplished by constructing a subset library using up to about 10% of the possible sequences, which subset of recombinant bacteriophage particles is then screened. If desired, to extend the diversity of a subset library, the recovered phage subset may be subjected to mutagenesis and then subjected to subsequent rounds of screening. This mutagenesis step may be accomplished in two general ways: the variable region of the recovered phage may be mutagenized, or additional variable amino acids may be added to the regions adjoining the initial variable sequences.

**[0247]** To diversify around active peptides (i.e., binders) found in early rounds of panning, the positive phage can sequenced to determine the identity of the active peptides. Oligonucleotides can then be synthesized based on these peptide sequences. The syntheses are done with a low level of all bases incorporated at each step to produce slight variations of the primary oligonucleotide sequences. This mixture of (slightly) degenerate oligonucleotides can then be cloned into the affinity phage by methods known to those in the art. This method produces systematic, controlled variations of the starting peptide sequences as part of a secondary library. It requires, however, that individual positive phage be sequenced before mutagenesis, and thus is useful for expanding the diversity of small numbers of recovered phage.

**[0248]** An alternate approach to diversify the selected phage allows the mutagenesis of a pool, or subset, of recovered phage. In accordance with this approach, phage recovered from panning are pooled and single stranded DNA is isolated. The DNA is mutagenized by treatment with, e.g., nitrous acid, formic acid, or hydrazine. These treatments produce a variety of damage to the DNA. The damaged DNA is then copied with reverse transcriptase, which misincorporates bases when it encounters a site of damage. The segment containing the sequence encoding the receptor-binding peptide is then isolated by cutting with restriction nuclease(s) specific for sites flanking the peptide coding sequence. This mutagenized segment is then recloned into undamaged vector DNA, the DNA is transformed into cells, and a secondary library according to known methods. General mutagenesis methods are known in the art (see Myers et al., 1985, *Nucl. Acids Res.* 13:3131-3145; Myers et al., 1985, *Science* 229:242-246; Myers, 1989, *Current Protocols in Molecular Biology Vol. I,* 8.3.1-8.3.6, F. Ausubel et al., eds, J. Wiley and Sons, New York).

**[0249]** In another general approach, the addition of amino acids to a peptide or peptides found to be active, can be carried out using various methods. In one, the sequences of peptides selected in early panning are determined individually and new oligonucleotides, incorporating the determined sequence and an adjoining degenerate sequence, are synthesized. These are then cloned to produce a secondary library. Alternatively, methods can be used to add a second IR or IGF-1R binding sequence to a pool of peptide-bearing phage. In accordance with one method, a restriction site is installed next to the first IR or IGF-1R binding sequence. Preferably, the enzyme should cut outside of its recognition sequence. The recognition site may be placed several bases from the first binding sequence. To insert a second IR or IGF-1R binding sequence, the pool of phage DNA is digested and blunt-ended by filling in the overhang with Klenow fragment. Double-stranded, blunt-ended, degenerately synthesized oligonucleotides are then ligated into this site to produce a second binding sequence juxtaposed to the first binding sequence. This secondary library is then amplified and screened as before.

**[0250]** While in some instances it may be appropriate to synthesize longer peptides to bind certain receptors, in other cases it may be desirable to provide peptides having two or more IR or IGF-1R binding sequences separated by spacer (e.g., linker) residues. For example, the binding sequences may be separated by spacers that allow the regions of the peptides to be presented to the receptor in different ways. The distance between binding regions may be as little as 1 residue, or at least 2-20 residues, or up to at least 100 residues. Preferred spacers are 3, 6, 9, 12, 15, or 18 residues in length. For probing large binding sites or tandem binding sites (e.g., Site 1 and Site 2 of IR), the binding regions may be separated by a spacer of residues of up to 20 to 30 amino acids. The number of spacer residues when present will typically be at least 2 residues, and often will be less than 20 residues.

**[0251]** The oligonucleotide library may have binding sequences which are separated by spacers (e.g., linkers), and thus may be represented by the formula: $(NNK)_y-(abc)_n-(NNK)_z$ where N and K are as defined previously (note that S as defined previously may be substituted for K), and y+z is equal to about 5, 6, 7, 8, or more, a, b and c represent the same or different nucleotides comprising a codon encoding spacer amino acids, n is up to about 3, 6, 9, or 12 amino acids, or more. The spacer residues may be somewhat flexible, comprising oligo-glycine, or oligo-glycine-glycine-serine, for example, to provide the diversity domains of the library with the ability to interact with sites in a large binding site relatively unconstrained by attachment to the phage protein. Rigid spacers, such as, e.g., oligo-proline, may also be inserted separately or in combination with other spacers, including glycine spacers. It may be desired to have

the IR or IGF-1R binding sequences close to one another and use a spacer to orient the binding sequences with respect to each other, such as by employing a turn between the two sequences, as might be provided by a spacer of the sequence glycine-proline-glycine, for example. To add stability to such a turn, it may be desirable or necessary to add cysteine residues at either or both ends of each variable region. The cysteine residues would then form disulfide bridges to hold the variable regions together in a loop, and in this fashion may also serve to mimic a cyclic peptide. Of course, those skilled in the art will appreciate that various other types of covalent linkages for cyclization may also be used.

[0252] Spacer residues as described above may also be situated on either or both ends of the IR or IGF-1R binding sequences. For instance, a cyclic peptide may be designed without an intervening spacer, by having a cysteine residue on both ends of the peptide. As described above, flexible spacers, e.g., oligo-glycine, may facilitate interaction of the peptide with the selected receptors. Alternatively, rigid spacers may allow the peptide to be presented as if on the end of a rigid arm, where the number of residues, e.g., proline residues, determines not only the length of the arm but also the direction for the arm in which the peptide is oriented. Hydrophilic spacers, made up of charged and/or uncharged hydrophilic amino acids, (e.g., Thr, His, Asn, Gln, Arg, Glu, Asp, Met, Lys, etc.), or hydrophobic spacers of hydrophobic amino acids (e.g., Phe, Leu, Ile, Gly, Val, Ala, etc.) may be used to present the peptides to receptor binding sites with a variety of local environments.

[0253] Notably, some peptides, because of their size and/or sequence, may cause severe defects in the infectivity of their carrier phage. This causes a loss of phage from the population during reinfection and amplification following each cycle of panning. To minimize problems associated with defective infectivity, DNA prepared from the eluted phage can be transformed into appropriate host cells, such as, e.g., *E. coli*, preferably by electroporation (see, e.g., Dower et al., *Nucl. Acids Res.* 16:6127-6145), or well known chemical means. The cells are cultivated for a period of time sufficient for marker expression, and selection is applied as typically done for DNA transformation. The colonies are amplified, and phage harvested for affinity enrichment in accordance with established methods. Phage identified in the affinity enrichment may be re-amplified by infection into the host cells. The successful transformants are selected by growth in an appropriate antibiotic(s), e.g., tetracycline or ampicillin. This may be done on solid or in liquid growth medium.

[0254] For growth on solid medium, the cells are grown at a high density (about $10^8$ to $10^9$ transformants per $m^2$) on a large surface of, for example, L-agar containing the selective antibiotic to form essentially a confluent lawn. The cells and extruded phage are scraped from the surface and phage are prepared for the first round of panning (see, e.g., Parmley and Smith, 1988, *Gene* 73:305-318). For growth in liquid culture, cells may be grown in L-broth and antibiotic through about 10 or more doublings. The phage are harvested by standard procedures (see Sambrook et al., 1989, *Molecular Cloning*, 2nd ed.). Growth in liquid culture may be more convenient because of the size of the libraries, while growth on solid media likely provides less chance of bias during the amplification process.

[0255] For affinity enrichment of desired clones, generally about $10^3$ to $10^4$ library equivalents (a library equivalent is one of each recombinant; $10^4$ equivalents of a library of $10^9$ members is $10^9 \times 10^4 = 10^{13}$ phage), but typically at least $10^2$ library equivalents, up to about $10^5$ to $10^6$, are incubated with a receptor (or portion thereof) to which the desired peptide is sought. The receptor is in one of several forms appropriate for affinity enrichment schemes. In one example the receptor is immobilized on a surface or particle, and the library of phage bearing peptides is then panned on the immobilized receptor generally according to procedures known in the art. In an alternate scheme, a receptor is attached to a recognizable ligand (which may be attached via a tether). A specific example of such a ligand is biotin. The receptor, so modified, is incubated with the library of phage and binding occurs with both reactants in solution. The resulting complexes are then bound to streptavidin (or avidin) through the biotin moiety. The streptavidin may be immobilized on a surface such as a plastic plate or on particles, in which case the complexes (phage/peptide/receptor/biotin/streptavidin) are physically retained; or the streptavidin may be labeled, with a fluorophor, for example, to tag the active phage/peptide for detection and/or isolation by sorting procedures, e.g., on a fluorescence-activated cell sorter.

[0256] Phage that associate with IR or IGF-1R via non-specific interactions are removed by washing. The degree and stringency of washing required will be determined for each receptor/peptide of interest. A certain degree of control can be exerted over the binding characteristics of the peptides recovered by adjusting the conditions of the binding incubation and the subsequent washing. The temperature, pH, ionic strength, divalent cation concentration, and the volume and duration of the washing will select for peptides within particular ranges of affinity for the receptor. Selection based on slow dissociation rate, which is usually predictive of high affinity, is the most practical route. This may be done either by continued incubation in the presence of a saturating amount of free ligand, or by increasing the volume, number, and length of the washes. In each case, the rebinding of dissociated peptide-phage is prevented, and with increasing time, peptide-phage of higher and higher affinity are recovered. Additional modifications of the binding and washing procedures may be applied to find peptides that bind receptors under special conditions. Once a peptide sequence that imparts some affinity and specificity for the receptor molecule is known, the diversity around this binding motif may be embellished. For instance, variable peptide regions may be placed on one or both ends of the identified sequence. The known sequence may be identified from the literature, or may be derived from early rounds of panning in the context of the present invention.

[0257] Screening Assays

[0258] In another embodiment of this invention, screening assays to identify pharmacologically active ligands at IR and/or IGF-1R are provided. Ligands may encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 daltons. Such ligands can comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably

at least two of the functional chemical groups. Ligands often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Ligands can also comprise biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs, or combinations thereof.

[0259] Ligands may include, for example, 1) peptides such as soluble peptides, including Ig-tailed fusion peptides and members of random peptide libraries (see, e.g., Lam et al, 1991, *Nature* 354:82-84; Houghten et al, 1991, *Nature* 354:84-86) and combinatorial chemistry-derived molecular libraries made of D- and/or L-configuration amino acids; 2) phosphopeptides (e.g., members of random and partially degenerate, directed phosphopeptide libraries, see, e.g., Songyang et al, 1993, *Cell* 72:767-778); 3) antibodies (e.g., polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, and single chain antibodies as well as Fab, $F(ab')_2$, Fab expression library fragments, and epitope-binding fragments of antibodies); and 4) small organic and inorganic molecules.

[0260] Ligands can be obtained from a wide variety of sources including libraries of synthetic or natural compounds. Synthetic compound libraries are commercially available from, for example, Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, N.J.), Brandon Associates (Merrimack, N.H.), and Microsource (New Milford, Conn.). A rare chemical library is available from Aldrich Chemical Company, Inc. (Milwaukee, Wis.). Natural compound libraries comprising bacterial, fungal, plant or animal extracts are available from, for example, Pan Laboratories (Bothell, Wash.). In addition, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides.

[0261] Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts can be readily produced. Methods for the synthesis of molecular libraries are readily available (see, e.g., DeWitt et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:6909; Erb et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann et al., 1994, *J. Med. Chem.* 37:2678; Cho et al., 1993, *Science* 261:1303; Carell et al., 1994, *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell et al., 1994, *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop et al., 1994, *J. Med. Chem.* 37:1233). In addition, natural or synthetic compound libraries and compounds can be readily modified through conventional chemical, physical and biochemical means (see, e.g., Blondelle et al., 1996, *Trends in Biotech.* 14:60), and may be used to produce combinatorial libraries. In another approach, previously identified pharmacological agents can be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, and the analogs can be screened for IR-modulating activity.

[0262] Numerous methods for producing combinatorial libraries are known in the art, including those involving biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide or peptide libraries, while the other four approaches are applicable to polypeptide, peptide, non-peptide oligomer, or small molecule libraries of compounds (K. S. Lam, 1997, *Anticancer Drug Des.* 12:145).

[0263] Libraries may be screened in solution by methods generally known in the art for determining whether ligands competitively bind at a common binding site. Such methods may including screening libraries in solution (e.g., Houghten, 1992, *Biotechniques* 13:412-421), or on beads (Lam, 1991, *Nature* 354:82-84), chips (Fodor, 1993, *Nature* 364:555-556), bacteria or spores (Ladner U.S. Pat. No. 5,223,409), plasmids (Cull et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:1865-1869), or on phage (Scott and Smith, 1990, *Science* 249:386-390; Devlin, 1990, *Science* 249:404-406; Cwirla et al., 1990, *Proc. Natl. Acad. Sci. USA* 97:6378-6382; Felici, 1991, *J. Mol. Biol.* 222:301-310; Ladner, supra).

[0264] Where the screening assay is a binding assay, IR, or one of the IR-binding peptides disclosed herein, may be joined to a label, where the label can directly or indirectly provide a detectable signal. Various labels include radioisotopes, fluorescent molecules, chemiluminescent molecules, enzymes, specific binding molecules, particles, e.g., magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin, etc. For the specific binding members, the complementary member would normally be labeled with a molecule that provides for detection, in accordance with known procedures.

[0265] A variety of other reagents may be included in the screening assay. These include reagents like salts, neutral proteins, e.g., albumin, detergents, etc., which are used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used. The components are added in any order that produces the requisite binding. Incubations are performed at any temperature that facilitates optimal activity, typically between 4° and 40° C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Normally, between 0.1 and 1 hr will be sufficient. In general, a plurality of assay mixtures is run in parallel with different test agent concentrations to obtain a differential response to these concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration or below the level of detection.

[0266] The screening assays provided in accordance with this invention are based on those disclosed in International application WO 96/04557, which is incorporated herein in its entirety. Briefly, WO 96/04557 discloses the use of reporter peptides that bind to active sites on targets and possess agonist or antagonist activity at the target. These reporters are identified from recombinant libraries and are either peptides with random amino acid sequences or variable antibody regions with at least one CDR region that has been randomized (rVab). The reporter peptides may be expressed in cell recombinant expression systems, such as for example in *E. coli*, or by phage display (see WO 96/04557 and Kay et al. 1996, *Mol. Divers.* 1(2):139-40, both of which are incorporated herein by reference). The reporters identified from the libraries may then be used in accordance with this invention either as therapeutics them-

selves, or in competition binding assays to screen for other molecules, preferably small, active molecules, which possess similar properties to the reporters and may be developed as drug candidates to provide agonist or antagonist activity. Preferably, these small organic molecules are orally active.

[0267] The basic format of an in vitro competitive receptor binding assay as the basis of a heterogeneous screen for small organic molecular replacements for insulin may be as follows: occupation of the active site of IR is quantified by time-resolved fluorometric detection (TRFD) with streptavidin-labeled europium (saEu) complexed to biotinylated peptides (bP). In this assay, saEu forms a ternary complex with bP and IR (i.e., IR:bP:saEu complex). The TRFD assay format is well established, sensitive, and quantitative (Tompkins et al., 1993, *J. Immunol. Methods* 163:209-216). The assay can use a single-chain antibody or a biotinylated peptide. Furthermore, both assay formats faithfully report the competition of the biotinylated ligands binding to the active site of IR by insulin.

[0268] In these assays, soluble IR is coated on the surface of microtiter wells, blocked by a solution of 0.5% bovine serum albumin (BSA) and 2% non-fat milk in PBS, and then incubated with biotinylated peptide or rVab. Unbound bP is then washed away and saEu is added to complex with receptor-bound bP. Upon addition of the acidic enhancement solution, the bound europium is released as free $Eu^{3+}$ which rapidly forms a highly fluorescent and stable complex with components of the enhancement solution. The IR:bP bound saEu is then converted into its highly fluorescent state and detected by a detector such as Wallac Victor II (EG&G Wallac, Inc.)

[0269] Phage display libraries can also be screened for ligands that bind to IR or IGF-1R, as described above. Details of the construction and analyses of these libraries, as well as the basic procedures for biopanning and selection of binders, have been published (see, e.g., WO 96/04557; Mandecki et al., 1997, *Display Technologies—Novel Targets and Strategies*, P. Guttry (ed), International Business Communications, Inc. Southborogh, Mass., pp. 231-254; Ravera et al., 1998, *Oncogene* 16:1993-1999; Scott and Smith, 1990, *Science* 249:386-390); Grihalde et al., 1995, *Gene* 166:187-195; Chen et al., 1996, *Proc. Natl. Acad. Sci. USA* 93:1997-2001; Kay et al., 1993, *Gene* 128:59-65; Carcamo et al., 1998, *Proc. Natl. Acad. Sci. USA* 95:11146-11151; Hoogenboom, 1997, *Trends Biotechnol.* 15:62-70; Rader and Barbas, 1997, *Curr. Opin. Biotechnol.* 8:503-508; all of which are incorporated herein by reference).

[0270] The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g., peptides are generally unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis, and testing are generally used to avoid large-scale screening of molecules for a target property.

[0271] There are several steps commonly taken in the design of a mimetic from a compound having a given target property. First, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide (e.g., by substituting each residue in turn). These parts or residues constituting the active region of the compound are known as its "pharmacophore".

[0272] Once the pharmacophore has been found, its structure is modeled according to its physical properties (e.g., stereochemistry, bonding, size, and/or charge), using data from a range of sources (e.g., spectroscopic techniques, X-ray diffraction data, and NMR). Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms), and other techniques can be used in this modeling process.

[0273] In a variant of this approach, the three dimensional structure of the ligand and its binding partner are modeled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this in the design of the mimetic.

[0274] A template molecule is then selected, and chemical groups that mimic the pharmacophore can be grafted onto the template. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the mimetic is easy to synthesize, is likely to be pharmacologically acceptable, does not degrade in vivo, and retains the biological activity of the lead compound. The mimetics found are then screened to ascertain the extent they exhibit the target property, or to what extent they inhibit it. Further optimization or modification can then be carried out to arrive at one or more final mimetics for in vivo or clinical testing.

[0275] This invention provides specific IR and IGF-1R amino acid sequences that function as either agonists or antagonists at IR and/or IGF-1R. Additional sequences may be obtained in accordance with the procedures described herein.

[0276] Use of the Peptides Provided by this Invention

[0277] The IR and IGF-1R agonist and antagonist peptides provided by this invention are useful as lead compounds for identifying other more potent or selective therapeutics, assay reagents for identifying other useful ligands by, for example, competition screening assays, as research tools for further analysis of IR and IGF-1R, and as therapeutics in pharmaceutical compositions. In one embodiment, one or more of the disclosed peptides can be provided as components in a kit for identifying other ligands (e.g., small, organic molecules) that bind to IR or IGF-1R. Such kits may also comprise IR or IGF-1R, or functional fragments thereof. The peptide and receptor components of the kit may be labeled (e.g., by radioisotopes, fluorescent molecules, chemiluminescent molecules, enzymes or other labels), or may be unlabeled and labeling reagents may be provided. The kits may also contain peripheral reagents such as buffers, stabilizers, etc. Instructions for use can also be provided.

[0278] In another embodiment, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which are derived from the peptide sequences, and include members that bind to Site 1 and/or Site 2 of IR or IGF-1R. Such libraries can be used to identify sequence variants that increase or modulate the binding and/or activity of the original peptide at IR or IGF-1R, as

described in the related applications of Beasley et al. International Application PCT/US00/08528, filed Mar. 29, 2000, and Beasley et al., U.S. application Ser. No. 09/538,038, filed Mar. 29, 2000, in accordance with well-established techniques.

[0279] IR agonist amino acid sequences provided by this invention are useful as insulin analogs and may therefore be developed as treatments for diabetes or other diseases associated with a decreased response or production of insulin. For use as an insulin supplement or replacement, non-limiting examples of amino acid sequences include D117/H2C: FHENFYDWFVRQVSK (SEQ ID NO:1780); D117/H2C minus terminal lysine: FHENFYDWFVRQVS (SEQ ID NO:1557); D118: DYKDFYDAIQLVRSARAGGTRDKK (SEQ ID NO:1781); D118 minus FLAG® tag and terminal lysines: FYDAIQLVRSARAGGTRD (SEQ ID NO:1782); D119: KDRAFYNGLRDLVGAVYGAWDKK (SEQ ID NO:1733); D119 minus terminal lysines: KDRAFYNGLRDLVGAVYGAWD (SEQ ID NO:residues 1-21 of SEQ ID NO: 1733); D116/JBA5: DYKDLCQSWGVRIGWLAGLCPKK (SEQ ID NO:1541); D116/JBA5 minus FLAG® tag and terminal lysines: LCQSWGVRIGWLAGLCP (SEQ ID NO:1542); D113/H2: DYKDVTFTSAVFHENFYDWFVRQVSKK (SEQ ID NO:1783); D113/H2 minus FLAG® tag and terminal lysines: VTFTSAVFHENFYDWFVRQVS (SEQ ID NO:1784); and S175: GRVDWLQRNANFYDWFVAELG (SEQ ID NO:1560). Preferred peptide dimer sequences are represented by S325, S332, S333, S335, S337, S353, S374-S376, S378, S379, S381, S414, S415, and S418 (see Table 7). Other preferred dimers sequences are represented by S455, S457, S458, S467, S468, S471, S499, S510, S518, S519, and S520 sequences (see Table 7). Especially preferred are the S519 dimer sequence, which shows in vitro and in vivo activity comparable to insulin (see FIGS. **31**A-C, 32A-B, and 33), S557 (see, e.g., FIG. **55**) and S597 (see, e.g., FIGS. **54-56**).

[0280] IGF-1R antagonist amino acid sequences provided by this invention are useful as treatments for cancers, including, but not limited to, breast, prostate, colorectal, and ovarian cancers. Human and breast cancers are responsible for over 40,000 deaths per year, as present treatments such as surgery, chemotherapy, radiation therapy, and immunotherapy show limited success. The IGF-1R antagonist amino acid sequences disclosed herein are also useful for the treatment or prevention of diabetic retinopathy. Recent reports have shown that a previously identified IGF-1R antagonist can suppress retinal neovascularization, which causes diabetic retinopathy (Smith et al., 1999, *Nat. Med.* 5:1390-1395).

[0281] IGF-1R agonist amino acid sequences provided by this invention are useful for development as treatments for neurological disorders, including stroke and diabetic neuropathy. Reports of several different groups implicate IGF-1R in the reduction of global brain ischemia, and support the use of IGF-1 for the treatment of diabetic neuropathy (reviewed in Auer et al., 1998, *Neurology* 51:S39-S43; Apfel, 1999, *Am. J. Med.* 107:34 S-42S).

[0282] I. Modification of Peptides

[0283] The peptides of the invention may be subjected to one or more modifications known in the art, which may be useful for manipulating storage stability, pharmacokinetics, and/or any aspect of the bioactivity of the peptide, such as, e.g., potency, selectivity, and drug interaction. Chemical modification to which the peptides may be subjected includes, without limitation, the conjugation to a peptide of one or more of polyethylene glycol (PEG), monomethoxy-polyethylene glycol, dextran, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polypropylene glycol, polyoxyethylated polyols (e.g., glycerol) and polyvinyl alcohol, colominic acids or other carbohydrate based polymers, polymers of amino acids, and biotin derivatives. PEG conjugation of proteins at Cys residues is disclosed, e.g., in Goodson, R. J. & Katre, N. V. (1990) Bio/Technology 8, 343 and Kogan, T. P. (1992) Synthetic Comm. 22, 2417.

[0284] Other useful modifications include, without limitation, acylation, using methods and compositions such as described in, e.g., U.S. Pat. No. 6,251,856, and WO 00/55119.

[0285] J. Therapeutic Administration

[0286] The peptides of the present invention may be administered individually or in combination with other pharmacologically active agents. It will be understood that such combination therapy encompasses different therapeutic regimens, including, without limitation, administration of multiple agents together in a single dosage form or in distinct, individual dosage forms. If the agents are present in different dosage forms, administration may be simultaneous or near-simultaneous or may follow any predetermined regimen that encompasses administration of the different agents.

[0287] For example, when used to treat diabetes or other diseases or syndromes associated with a decreased response or production of insulin, hyperlipidemia, obesity, appetite-related syndromes, and the like, the peptides of the invention may be advantageously administered in a combination treatment regimen with one or more agents, including, without limitation, insulin, insulin analogues, insulin derivatives, glucagon-like peptide-1 or -2 (GLP-1, GLP-2), derivatives or analogues of GLP-1 or GLP-2 (such as are disclosed, e.g., in WO 00/55119). It will be understood that an "analogue" of insulin, GLP-1, or GLP-2 as used herein refers to a peptide containing one or more amino acid substitutions relative to the native sequence of insulin, GLP-1, or GLP-2, as applicable; and "derivative" of insulin, GLP-1, or GLP-2 as used herein refers to a native or analogue insulin, GLP-1, or GLP-2 peptide that has undergone one or more additional chemical modifications of the amino acid sequence, in particular relative to the natural sequence. Insulin derivatives and analogues are disclosed, e.g., in U.S. Pat. Nos. 5,656,722, 5,750,497, 6,251,856, and 6,268,335. In some embodiments, the combination agent is one of $LyS^{B29}$(ε-myristoyl)des(B30) human insulin, $LyS^{B29}$(ε-tetradecanoyl)des(B30) human insulin and $B^{29}$-$N^{\epsilon}$-(N-lithocolyl-γ-glutamyl)-des(B30) human insulin. Also suitable for combination therapy are non-peptide antihyperglycemic agents, antihyperlipidemic agents, and the like such as those well-known in the art.

[0288] In one embodiment, the invention encompasses methods of treating diabetes or related syndromes comprising administering a first amount of peptide S597 or peptide S557 and a second amount of a long-acting insulin analogue,

such as, e.g., $Lys^{B29}$(ε-myristoyl)des(B30) human insulin, $LyS^{B29}$(ε-tetradecanoyl)des(B30) human insulin. or $B^{29}$-$N^{\epsilon}$-(N-lithocolyl-γ-glutamyl)-des(B30) human insulin, wherein the first and second amounts together are effective for treating the syndrome. As used herein, a long-acting insulin analogue is one that exhibits a protracted profile of action relative to native human insulin, as disclosed, e.g., in U.S. Pat. No. 6,451,970.

[0289] K. Methods of Administration

[0290] The amino acid sequences of this invention may be administered as pharmaceutical compositions comprising standard carriers known in the art for delivering proteins and peptides and by gene therapy. Preferably, a pharmaceutical composition includes, in admixture, a pharmaceutically (i.e., physiologically) acceptable carrier, excipient, or diluent, and one or more of an IR or IGF-1R agonist or antagonist peptide, as an active ingredient. The preparation of pharmaceutical compositions that contain peptides as active ingredients is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients that are pharmaceutically (i.e., physiologically) acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH-buffering agents, which enhance the effectiveness of the active ingredient.

[0291] An IR or IGF-1R agonist or antagonist peptide can be formulated into a pharmaceutical composition as neutralized physiologically acceptable salt forms. Suitable salts include the acid addition salts (i.e., formed with the free amino groups of the peptide molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

[0292] The pharmaceutical compositions can be administered systemically by oral or parenteral routes. Non-limiting parenteral routes of administration include subcutaneous, intramuscular, intraperitoneal, intravenous, transdermal, inhalation, intranasal, intra-arterial, intrathecal, enteral, sublingual, or rectal. Due to the labile nature of the amino acid sequences parenteral administration is preferred. Preferred modes of administration include aerosols for nasal or bronchial absorption; suspensions for intravenous, intramuscular, intrasternal or subcutaneous, injection; and compounds for oral administration.

[0293] Intravenous administration, for example, can be performed by injection of a unit dose. The term "unit dose" when used in reference to a pharmaceutical composition of the present invention refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e., liquid used to dilute a concentrated or pure substance (either liquid or solid), making that substance the correct (diluted) concentration for use. For injectable administration, the composition is in sterile solution or suspension or may be emulsified in pharmaceutically- and physiologically-acceptable aqueous or oleaginous vehicles, which may contain preservatives, stabilizers, and material for rendering the solution or suspension isotonic with body fluids (i.e., blood) of the recipient.

[0294] Excipients suitable for use are water, phosphate buffered saline, pH 7.4, 0.15 M aqueous sodium chloride solution, dextrose, glycerol, dilute ethanol, and the like, and mixtures thereof. Illustrative stabilizers are polyethylene glycol, proteins, saccharides, amino acids, inorganic acids, and organic acids, which may be used either on their own or as admixtures. The amounts or quantities, as well as routes of administration, used are determined on an individual basis, and correspond to the amounts used in similar types of applications or indications known to those of skill in the art.

[0295] Pharmaceutical compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of modulation of IR or IGF-1R activity desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are specific for each individual. However, suitable dosages may range from about 10 to 200 nmol active peptide per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusions sufficient to maintain picomolar concentrations (e.g., approximately 1 μM to approximately 10 nM) in the blood are contemplated. An exemplary formulation comprises the IR or IGF-1R agonist or antagonist peptide in a mixture with sodium busulfite USP (3.2 mg/ml); disodium edetate USP (0.1 mg/ml); and water for injection q.s.a.d. (1 ml).

[0296] Further guidance in preparing pharmaceutical formulations can be found in, e.g., Gilman et al. (eds), 1990, Goodman and Gilman's: *The Pharmacological Basis of Therapeutics,* 8th ed., Pergamon Press; and *Remington's Pharmaceutical Sciences,* 17th ed., 1990, Mack Publishing Co., Easton, Pa.; Avis et al. (eds), 1993, *Pharmaceutical Dosage Forms: Parenteral Medications*, Dekker, New York; Lieberman et al. (eds), 1990, Pharmaceutical Dosage Forms: Disperse Systems, Dekker, New York.

[0297] The present invention further contemplates compositions comprising an IR or IGF-1R agonist or antagonist peptide, and a physiologically acceptable carrier, excipient, or diluent as described in detail herein.

[0298] The constructs as described herein may also be used in gene transfer and gene therapy methods to allow the expression of one or more amino acid sequences of the present invention. The amino acid sequences of the present invention can be used for gene therapy and thereby provide an alternative method of treating diabetes which does not

rely on the administration or expression of insulin. Expressing insulin for use in gene therapy requires the expression of a precursor product, which must then undergo processing including cleavage and disulfide bond formation to form the active product. The amino acid sequences of this invention, which possess activity, are relatively small, and thus do not require the complex processing steps to become active. Accordingly, these sequences provide a more suitable product for gene therapy.

[0299] Gene transfer systems known in the art may be useful in the practice of the gene therapy methods of the present invention. These include viral and non-viral transfer methods. A number of viruses have been used as gene transfer vectors, including polyoma, i.e., SV40 (Madzak et al., 1992, *J. Gen. Virol.*, 73:1533-1536), adenovirus (Berkner, 1992, *Curr. Top. Microbiol. Immunol.*, 158:39-6; Berkner et al., 1988, *Bio Techniques*, 6:616-629; Gorziglia et al., 1992, *J. Virol.*, 66:4407-4412; Quantin et al., 1992, *Proc. Natl. Acad. Sci. USA*, 89:2581-2584; Rosenfeld et al., 1992, *Cell*, 68:143-155; Wilkinson et al., 1992, *Nucl. Acids Res.*, 20:2233-2239; Stratford-Perricaudet et al., 1990, *Hum. Gene Ther.*, 1:241-256), vaccinia virus (Mackett et al., 1992, *Biotechnology*, 24:495-499), adeno-associated virus (Muzyczka, 1992, *Curr. Top. Microbiol. Immunol.* 158:91-123; Ohi et al., 1990, *Gene*, 89:279-282), herpes viruses including HSV and EBV (Margolskee, 1992, *Curr. Top. Microbiol. Immunol.* 158:67-90; Johnson et al., 1992, *J. Virol.*, 66:2952-2965; Fink et al., 1992, *Hum. Gene Ther.* 3:11-19; Breakfield et al., 1987, *Mol. Neurobiol.*, 1:337-371; Fresse et al., 1990, *Biochem. Pharmacol.* 40:2189-2199), and retroviruses of avian (Brandyopadhyay et al., 1984, *Mol. Cell. Biol.*, 4:749-754; Petropouplos et al., 1992, *J. Virol.*, 66:3391-3397), murine (Miller, 1992, *Curr. Top. Microbiol. Immunol.* 158:1-24; Miller et al., 1985, *Mol. Cell. Biol.*, 5:431-437; Sorge et al., 1984, *Mol. Cell. Biol.*, 4:1730-1737; Mann et al., 1985, *J. Virol.*, 54:401-407), and human origin (Page et al., 1990, *J. Virol.*, 64:5370-5276; Buchschalcher et al., 1992, *J. Virol.*, 66:2731-2739). Most human gene therapy protocols have been based on disabled murine retroviruses.

[0300] Non-viral gene transfer methods known in the art include chemical techniques such as calcium phosphate coprecipitation (Graham et al., 1973, *Virology*, 52:456-467; Pellicer et al., 1980, *Science*, 209:1414-1422), mechanical techniques, for example microinjection (Anderson et al., 1980, *Proc. Natl. Acad. Sci. USA*, 77:5399-5403; Gordon et al., 1980, *Proc. Natl. Acad. Sci. USA*, 77:7380-7384; Brinster et al., 1981, *Cell*, 27:223-231; Constantini et al., 1981, *Nature*, 294:92-94), membrane fusion-mediated transfer via liposomes (Felgner et al., 1987, *Proc. Natl. Acad. Sci. USA*, 84:7413-7417; Wang et al., 1989, *Biochemistry*, 28:9508-9514; Kaneda et al., 1989, *J. Biol. Chem.*, 264:12126-12129; Stewart et al., 1992, *Hum. Gene Ther.* 3:267-275; Nabel et al., 1990, *Science*, 249:1285-1288; Lim et al., 1992, *Circulation*, 83:2007-2011; U.S. Pat. Nos. 5,283,185 and 5,795,587), and direct DNA uptake and receptor-mediated DNA transfer (Wolff et al., 1990, *Science*, 247:1465-1468; Wu et al., 1991, *BioTechniques*, 11:474-485; Zenke et al., 1990, *Proc. Natl. Acad. Sci. USA*, 87:3655-3659; Wu et al., 1989, *J. Biol. Chem.*, 264:16985-16987; Wolff et al., 1991, *BioTechniques*, 11:474-485; Wagner et al., 1991, *Proc. Natl. Acad. Sci. USA*, **88**:**4255**-4259; Cotten et al., 1990, *Proc. Natl. Acad. Sci. USA*, 87:4033-4037; Curiel et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:8850-8854; Curiel et al., 1991, *Hum. Gene Ther.* 3:147-154).

[0301] Many types of cells and cell lines (e.g., primary cell lines or established cell lines) and tissues are capable of being stably transfected by or receiving the constructs of the invention. Examples of cells that may be used include, but are not limited to, stem cells, B lymphocytes, T lymphocytes, macrophages, other white blood lymphocytes (e.g., myelocytes, macrophages, or monocytes), immune system cells of different developmental stages, erythroid lineage cells, pancreatic cells, lung cells, muscle cells, liver cells, fat cells, neuronal cells, glial cells, other brain cells, transformed cells of various cell lineages corresponding to normal cell counterparts (e.g., K562, HEL, HL60, and MEL cells), and established or otherwise transformed cells lines derived from all of the foregoing. In addition, the constructs of the present invention may be transferred by various means directly into tissues, where they would stably integrate into the cells comprising the tissues. Further, the constructs containing the DNA sequences of the peptides of the invention can be introduced into primary cells at various stages of development, including the embryonic and fetal stages, so as to effect gene therapy at early stages of development.

[0302] In one approach, plasmid DNA is complexed with a polylysine-conjugated antibody specific to the adenovirus hexon protein, and the resulting complex is bound to an adenovirus vector. The trimolecular complex is then used to infect cells. The adenovirus vector permits efficient binding, internalization, and degradation of the endosome before the coupled DNA is damaged.

[0303] In another approach, liposome/DNA is used to mediate direct in vivo gene transfer. While in standard liposome preparations the gene transfer process is non-specific, localized in vivo uptake and expression have been reported in tumor deposits, for example, following direct in situ administration (Nabel, 1992, *Hum. Gene Ther.* 3:399-410).

[0304] Suitable gene transfer vectors possess a promoter sequence, preferably a promoter that is cell-specific and placed upstream of the sequence to be expressed. The vectors may also contain, optionally, one or more expressible marker genes for expression as an indication of successful transfection and expression of the nucleic acid sequences contained in the vector. In addition, vectors can be optimized to minimize undesired immunogenicity and maximize long-term expression of the desired gene product(s) (see Nabe, 1999, *Proc. Natl. Acad. Sci. USA* 96:324-326). Moreover, vectors can be chosen based on cell-type that is targeted for treatment.

[0305] Illustrative examples of vehicles or vector constructs for transfection or infection of the host cells include replication-defective viral vectors, DNA virus or RNA virus (retrovirus) vectors, such as adenovirus, herpes simplex virus and adeno-associated viral vectors. Adeno-associated virus vectors are single stranded and allow the efficient delivery of multiple copies of nucleic acid to the cell's nucleus. Preferred are adenovirus vectors. The vectors will normally be substantially free of any prokaryotic DNA and may comprise a number of different functional nucleic acid sequences. An example of such functional sequences may be a DNA region comprising transcriptional and translational

initiation and termination regulatory sequences, including promoters (e.g., strong promoters, inducible promoters, and the like) and enhancers which are active in the host cells. Also included as part of the functional sequences is an open reading frame (polynucleotide sequence) encoding a protein of interest. Flanking sequences may also be included for site-directed integration. In some situations, the 5'-flanking sequence will allow homologous recombination, thus changing the nature of the transcriptional initiation region, so as to provide for inducible or non-inducible transcription to increase or decrease the level of transcription, as an example.

[0306] In general, the encoded and expressed peptide may be intracellular, i.e., retained in the cytoplasm, nucleus, or in an organelle, or may be secreted by the cell. For secretion, a signal sequence may be fused to the peptide sequence. As previously mentioned, a marker may be present for selection of cells containing the vector construct. The marker may be an inducible or non-inducible gene and will generally allow for positive selection under induction, or without induction, respectively. Examples of marker genes include neomycin, dihydrofolate reductase, glutamine synthetase, and the like. The vector employed will generally also include an origin of replication and other genes that are necessary for replication in the host cells, as routinely employed by those having skill in the art. As an example, the replication system comprising the origin of replication and any proteins associated with replication encoded by a particular virus may be included as part of the construct. The replication system must be selected so that the genes encoding products necessary for replication do not ultimately transform the cells. Such replication systems are represented by replication-defective adenovirus (see G. Acsadi et al., 1994, *Hum. Mol. Genet.* 3:579-584) and by Epstein-Barr virus. Examples of replication defective vectors, particularly, retroviral vectors that are replication defective, are BAG, (see Price et al., 1987, *Proc. Natl. Acad. Sci. USA,* 84:156; Sanes et al., 1986, *EMBO J.,* 5:3133). It will be understood that the final gene construct may contain one or more genes of interest, for example, a gene encoding a bioactive metabolic molecule. In addition, cDNA, synthetically produced DNA or chromosomal DNA may be employed utilizing methods and protocols known and practiced by those having skill in the art.

[0307] According to one approach for gene therapy, a vector encoding an IR or IGF-1R agonist or antagonist peptide is directly injected into the recipient cells (in vivo gene therapy). Alternatively, cells from the intended recipients are explanted, genetically modified to encode an IR or IGF-1R agonist or antagonist peptide, and reimplanted into the donor (ex vivo gene therapy). An ex vivo approach provides the advantage of efficient viral gene transfer, which is superior to in vivo gene transfer approaches. In accordance with ex vivo gene therapy, the host cells are first transfected with engineered vectors containing at least one gene encoding an IR or IGF-1R agonist or antagonist peptide, suspended in a physiologically acceptable carrier or excipient such as saline or phosphate buffered saline, and the like, and then administered to the host or host cells. The desired gene product is expressed by the injected cells, which thus introduce the gene product into the host. The introduced gene products can thereby be utilized to treat or ameliorate a disorder that is related to altered insulin or IGF-1 levels (e.g., diabetes).

[0308] The described constructs may be administered in the form of a pharmaceutical preparation or composition containing a pharmaceutically acceptable carrier and a physiological excipient, in which preparation the vector may be a viral vector construct, or the like, to target the cells, tissues, or organs of the recipient organism of interest, including human and non-human mammals. The composition may be formed by dispersing the components in a suitable pharmaceutically acceptable liquid or solution such as sterile physiological saline or other injectable aqueous liquids. The amounts of the components to be used in such compositions may be routinely determined by those having skill in the art. The compositions may be administered by parenteral routes of injection, including subcutaneous, intravenous, intramuscular, and intrasternal.

## EXAMPLES

[0309] The examples as set forth herein are meant to exemplify the various aspects of the present invention and are not intended to limit the invention in any way.

[0310] The following materials were used in the examples described below. Soluble IGF-1R was obtained from R&D Systems (Minneapolis, Minn.; Cat. # 391-GR/CF). Insulin receptor was prepared according to Bass et al., 1996. The insulin was either from Sigma (St. Louis, Mo.; Cat. # I-0259) or Boehringer. The IGF-1 was from PeproTech (Cat. # 100-11). All synthetic peptides were synthesized by Novo Nordisk, AnaSpec, Inc. (San Jose, Calif.), PeptioGenics (Livermore, Calif.), or Research Genetics (Huntsville, Ala.) at >80% purity. The Maxisorb Plates were from NUNC via Fisher (Cat. # 12565347). The HRP/Anti-M13 conjugate was from Pharmacia (Cat. # 27-9421-01). The ABTS solution was from BioF/X (Cat. # ABTS-0100-04).

### Example 1

### Monomer and Dimer Peptides

[0311] A. Cloning

[0312] Monomer and dimer peptides were constructed and expressed as protein fusions to a chitin binding domain (CBD) using the pTYB2 vector from the IMPACT™-CN system (New England Biolabs (NEB), Beverly, Mass.). The pTYB2 vector encodes a protein-splicing element (termed intein), which initiates self-cleavage upon the addition of DTT. The intein self-cleavage separates the dimer from the affinity tag, to allow purification.

[0313] In the pTYB2 construct, the C-terminus of the peptide sequence was fused to the N-terminus of the intein/CBD sequence. Two peptide-flanking epitope tags were included: a shortened-FLAG® at the N-terminus and E-Tag at the C-terminus. This fusion was generated by ligating a vector fragment encoding the intein/CBD with a PCR product encoding the peptide of interest.

[0314] The vector fragment was obtained by digesting at appropriate restriction sites the pTBY2 vector. The digested DNA fragment was resolved on a 1% agarose gel, excised, and purified by QIAEXII (QIAGEN, Valencia, Calif.). To obtain the PCR product of the target proteins, primers were synthesized which anneal to appropriate sequences. The vector and insert were ligated overnight at 15° C. The ligation product was purified using QIAquick spin columns (QIAGEN) and electroporations were performed at 1500 V in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 10 ng of DNA and 40 μl of *E. coli* strain BL21.

[0315] Immediately following electroporation, 1 ml of pre-warmed (40° C.) 2xYT medium containing 2% glucose (2xYT-G) was added to the transformants. The transfor-

mants were grown at 37° C. for 1 h, and then plated onto 2xYT-AG plates and incubated overnight at 37° C. Individual colonies were isolated and used to innoculate 2xYT-G. The cultures were grown overnight at 37° C. Plasmid DNA was isolated from the cultures and sequencing was performed to confirm that the correct construct was obtained.

[0316] Small-Scale Expression of Peptide-CBD Fusion Proteins

[0317] *E. coli* ER2566 (New England Biolabs) containing plasmids encoding peptide-CBD fusion proteins were grown in 2xYT-AG at 37° C. overnight, with agitation (250 rpm). The following day, the cultures were used to inoculate media (2×YT-G) to obtain an $OD_{600}$ of 0.1. Upon reaching an $OD_{600}$ of 0.6, expression of the fusion protein was induced by the addition of IPTG (isopropyl-β-D-thiogalactopyranoside) to a final concentration of 0.3 mM. Cells were grown for 3 h. Following this, cells were pelleted by centrifugation and the cell pellets were analyzed by SDS-PAGE electrophoresis. Production of the correct molecular weight fusion proteins was confirmed by Western blot analysis using the monoclonal antibody anti-E-Tag-HRP conjugate (Amersham Pharmacia).

[0318] Large-Scale Expression and Purification of Soluble Peptide-CBD Fusion Proteins

[0319] *E. coli* ER2566 carrying plasmids encoding the fusion proteins were grown in 2xYT-AG media at 37° C. for 8 h, with agitation (250 rpm). The cultures were back-diluted into to 2 L volumes of 2xYT-A to achieve an $OD_{600}$ of 0.1. Upon reaching an $OD_{600}$ of 0.5, IPTG was added to a final concentration of 0.3 mM. Cells were grown at 30° C. overnight. The next day cells were isolated by centrifugation. Samples of the cell pellet were analyzed by SDS-PAGE followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product.

[0320] Purification

[0321] The cell pellets were disrupted mechanically by sonication or chemically by treatment with the mild detergent. After removal of cell debris by centrifugation, the soluble proteins in the clarified lysate were prepared for chromatographic purification by dilution or dialysis into the appropriate starting buffer. The CBD fusions were purified by chitin affinity chromatography according to the manufacturer's instructions (New England Biolabs). The lysate was loaded onto a chitin affinity column and the column was washed with 10 volumes of column buffer. Three bed volumes of the DTT containing cleavage buffer were loaded onto the column and the column was incubated overnight. The next day, the target protein was eluted by continuing the flow of the cleavage buffer without DTT. The purified proteins were analyzed for purity and integrity by SDS-PAGE and Western blot analysis according to standard protocols.

Example 2

PEG-Based Dimer Peptides

[0322] A. Synthesis of the aldehyde containing peptide

[0323] The peptide was synthesized by stepwise solid phase synthesis on Rink amide Tentagel (0.21 mmol/g). Three equivalents of Fmoc-amino acids were used. The serine residue was introduced into the peptide by either coupling Fmoc-Ser(tBu)-OH to the N-terminal peptide or coupling Boc-Ser(tBu) to a selectively protected lysine side-chain. The peptide was then deprotected and cleaved from the resin by treatment with 95% TFA (trifluoroacetic acid; aq) containing TIS (triisopropylsilan). Periodate oxidation, using 2 equivalent of $NaIO_4$ in 20% DMSO (dimethyl sulfoxide)-80% phosphate buffer pH 7.5 (45 μl/μmol peptide) for 5 min at room temperature (RT), converted the 2-amino alcohol moiety in an F-oxoacyl group. The peptide was purified immediately following oxidation.

[0324] B. Synthesis of the PEG-Based Dimer

[0325] The unprotected and oxidized peptide (4.2 equivalent) was dimerized on the dioxyamino-PEG (polyethylene glycol)-linker (1 equivalent) in 90% DMSO-10% 20 mM NaOAc buffer, pH 5.1 (4.2 μl/μmol peptide). The solution was left for 1 hr at 38° C. and the progress of the reaction was monitored by MALDI-MS (matrix-assisted laser desorption/ionization mass spectrometry). Following this, the crude dimer was purified by semi-preparative HPLC (high performance liquid chromatography).

[0326] The molecular weights and inter peptide distance of various linkers is shown in Table 3, below.

TABLE 3

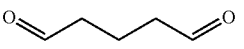
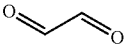
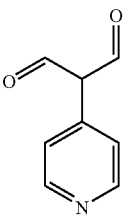
| Structure | Number | MW | MW (—$2H_2O$) |
|---|---|---|---|
|  | 1 | 100.1 | 64.1 |
|  | 2 | 58.04 | 22.04 |
|  | 3 | 149.15 | 113.15 |

TABLE 3-continued

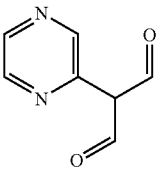
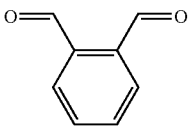
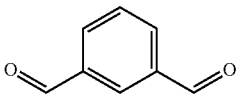
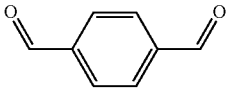
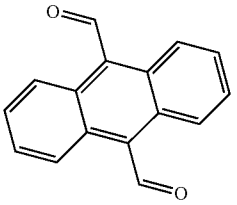
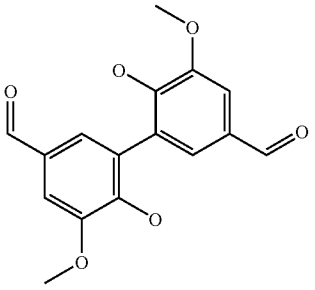
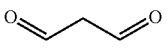
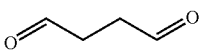
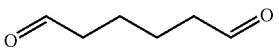
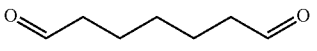
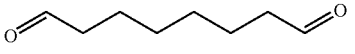
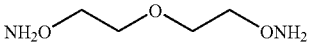
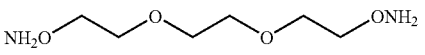
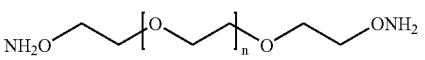
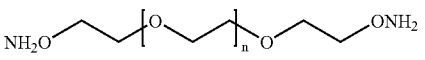
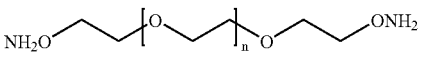
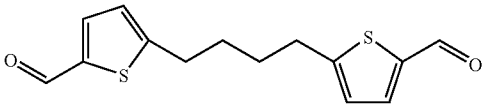
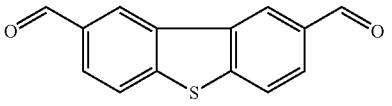
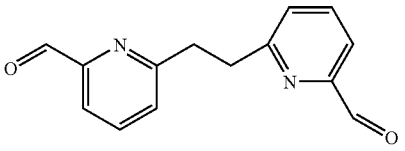
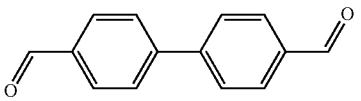
| Structure | Number | MW | MW (—$2H_2O$) |
|---|---|---|---|
|  | 4 | 150.14 | 114.14 |
|  | 5 | 134.13 | 98.13 |
|  | 6 | 134.13 | 98.13 |
|  | 7 | 134.13 | 98.13 |
|  | 8 | 234.25 | 198.25 |
|  | 9 | 302.3 | 266.3 |
|  | 10 | 72.06 | 36.06 |
|  | 11 | 86.09 | 50.09 |
|  | 12 | 114.14 | 78.14 |
|  | 13 | 128.08 | 92.08 |
|  | 14 | 142.19 | 106.19 |
| $(HCO)_4$-$(Lys)_2$-Lys-Gly-$NH_2$ | 15 | | |
|  | 16 | 136.2 | 100.2 |

TABLE 3-continued

| Structure | Number | MW | MW (—$2H_2O$) |
|---|---|---|---|
|  | 17 | 180.2 | 144.2 |
|  n = 2 | 18 | 224.3 | 188.3 |
|  n = 3 | 19 | 268.3 | 232.3 |
|  n = 4 | 20 | 312.4 | 276.4 |
|  | 21 | 278.4 | 242.4 |
|  | 22 | 240.3 | 204.3 |
|  | 23 | 240.3 | 204.3 |
|  | 24 | 210.2 | 192.2 |

Example 3

Determination of Insulin Receptor Binding

[0327] IR was incubated with $^{125}$I-labeled insulin at various concentrations of test substance and the $K_d$ was calculated. According to this method, human insulin receptor (HIR) or human IGF-1 receptor (HIGF-1R) was purified from transfected cells after solubilization with Triton X-100. The assay buffer contained 100 mM HEPES (pH 7.8), 100 mM NaCl, 10 mM $MgCl_2$, 0.5% human serum albumin (HSA), 0.2% gammaglobulin and 0.025% Triton X-100. The receptor concentration was chosen to give 30-60% binding of 2000 cpm (3 pM) of its $^{125}$I-labeled ligand (TyrA14-$^{125}$I-HI or Tyr31-$^{125}$I-IGF1) and a dilution series of the substances to be tested was added. After equilibration for 2 days at 4° C. each sample (200 μl) was precipitated by addition of 400 μl 25% PEG 6000, centrifuged, washed with 1 ml 15% PEG 6000, and counted in a gamma-counter.

[0328] The insulin/IGF-1 competition curve was fitted to a one-site binding model and the calculated parameters for receptor concentration, insulin affinity, and non-specific binding were used in calculating the binding constants of the test substances. Representative curves for insulin competition are shown in FIGS. **10**A-**10**C; **11**A-**11**D. Qualitative data are provided in Table 4, below.

[0329] Table 4 illustrates IR affinities for the RP9 monomer peptide and various RP9 monomer truncations. The results demonstrate that RP9 N-terminal sequence (GSLD; SEQ ID NO:1785) and C-terminal sequence (LGKK; SEQ ID NO:1786) can be deleted without substantially affecting HIR binding affinity (Table 4).

TABLE 4

| Peptide | SEQ ID NO: | Formula | Site IR | Sequence | HIR Kd (mol/l) |
|---|---|---|---|---|---|
| S386 | 1559 | 1 | 1 | GSLDESFYDWFERQLG | $3.2*10^{-7}$ |
| S395 | 1787 | 1 | 1 | GSLDESFYDWFERQL | $9.1 * 10^{-8}$ |
| S394 | 1788 | 1 | 1 | GSLDESFYDWFERQ | $8.1 * 10^{-8}$ |
| S396 | 1789 | 1 | 1 | GSLDESFYDWFER | $>2 * 10^{-5}$ |

TABLE 4-continued

| Peptide | SEQ ID NO: | Formula | Site IR | Sequence | HIR Kd (mol/l) |
|---|---|---|---|---|---|
| S399 | 1790 | 1 | 1 | ESFYDWFERQL | $9.1 * 10^{-8}$ |
| S400 | 1791 | 1 | 1 | ESFYDWFERQ | $6.3 * 10^{-7}$ |

[0330] FIGS. **10**A-**10**C demonstrate that Site 1-Site 2 heterodimer peptides 537, 538, and 539 bound to IR with substantially higher (several orders of magnitude) affinity than corresponding monomer (D117 and 540) and homodimer (521 and 535) peptides. FIGS. **11**A-**11**D demonstrate that Site 1-Site 2 heterodimer peptides, 537 and 538, bound to IR with markedly higher affinity than the monomer peptide D117.

Example 4

Adipocyte Assay for Determination of Insulin Agonist Activity

[0331] Insulin increases uptake of $^3$H glucose into adipocytes and its conversion into lipid. Incorporation of $^3$H into the lipid phase was determined by partitioning of lipid phase into a scintillant mixture, which excludes water-soluble $^3$H products. The effect of compounds on the incorporation of $^3$H glucose at a sub-maximal insulin dose was determined, and the results expressed as increase relative to full insulin response. The method was adapted from Moody et al., 1974, *Horm Metab Res.* 6(1):12-6.

[0332] Mouse epididymal fat pads were dissected out, minced into digestion buffer (Krebs-Ringer 25 mM HEPES, 4% HSA, 1.1 mM glucose, 0.4 mg/ml Collagenase Type 1, pH 7.4), and digested for up to 1.5 h at 36.5° C. After filtration, washing (Krebs-Ringer HEPES, 1% HSA), and resuspension in assay buffer (Krebs-Ringer HEPES, 1% HSA), free fat cells were pipetted into 96-well Picoplates (Packard), containing test solution and approximately an $ED_{20}$ insulin.

[0333] The assay was started by addition of $^3$H glucose (Amersham TRK 239), in a final concentration of 0.45 mM glucose. The assay was incubated for 2 h, 36.5° C., in a Labshaker incubation tower, 400 rpm, then terminated by the addition of Permablend/Toluene scintillant (or equivalent), and the plates sealed, before standing for at least 1 h and detection in a Packard Top Counter or equivalent. A full insulin standard curve (8 dose) was run as control on each plate.

[0334] Data are presented graphically, as effect of compound on an (approximate) $ED_{20}$ insulin response, with data normalized to a full insulin response. The assay can also be run at basal or maximal insulin concentration. Representative dose-response curves for insulin and IGF-1 are shown in FIGS. **12**-**18**. Qualitative data are shown in Tables 5-7.

[0335] In free fat cell (FFC) assays, truncated synthetic RP9 monomer peptides S390 and S394 showed potency similar to full-length RP9 monomer peptides (FIGS. **12**A-**12**D). Truncated synthetic RP9 homodimer peptides S415 and S417 were highly potent in FFC assays, but less potent than full-length RP9 homodimer peptides (FIGS. **13**A-**13**C; compare to peptides 521 and 535, described below). The potency of recombinant RP9 homodimer peptides 521 and 535 in FFC assays is shown in FIGS. **14**A-**14**C. The curves are flattened, suggesting that the binding mechanism may not be mediated by simple intramolecular binding (FIGS. **14**A-**14**C).

[0336] Results further indicated that synthetic RP9 homodimer peptides S337 and S374 showed increased HIR biding affinity and increased potency in FFC assays compared to synthetic RP9 monomer, S371 (Table 5). Similarly, synthetic RP9 homodimer peptides S314 and S317 showed increased HIR binding affinity and increased potency in FFC assays compared to synthetic RP9 monomer, S371, and various RP9 truncations (Table 6).

TABLE 5

| Pep. | SEQ ID NO: | Formula | Site IR | Monomer or Dimer | Sequence | HIR $K_d$ (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S371 | 1558 | 1 | 1 | M (RP9) | GSLDESFYDWFERQLGKK | $6.3 * 10^{-7}$ | + |
| S337 | 1792 | 1-1 | 1-1 | D, C-Term 23 | $(GSLDESFYDWFERQLGKK\text{-}Lig)_2\text{-}23$ | $1.1 * 10^{-8}$ | +++++ |
| S374 | 1793 | 1-1 | 1-1 | D, N-Term 17 | $17\text{-}(GSLDESFYDWFERQLGKK)_2$ | $1.8 * 10^{-7}$ | ++++ |

M = monomer; D = dimer; C-Term = C-terminal linker (C-C); N-Term = N-terminal linker (N-N); 23 and 17 represent specific chemical linkers (see Table 3); For FFC: 0 is no effect, + is agonist, - is antagonist.

[0337]

TABLE 6

| Peptide | SEQ ID NO: | Form. | Site IR | Mon. or Dimer | Sequence | HIR $K_d$ (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S371 (RP9) | 1558 | 1 | 1 | M | GSLDESFYDWFERQLGKK | $6.3 * 10^{-7}$ | + |

TABLE 6-continued

| Peptide | SEQ ID NO: | Form. | Site IR | or Dimer | Mon. Sequence | HIR $K_d$ (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S395 | 1787 | 1 | 1 | M | GSLDESFYDWFERQL | $9.1 * 10^{-8}$ | + |
| S394 | 1788 | 1 | 1 | M | GSLDESFYDWFERQ | $8.1 * 10^{-8}$ | ++ |
| S396 | 1789 | 1 | 1 | M | GSLDESFYDWFER | $>2 * 10^{-5}$ | 0 |
| S390 | 1794 | 1 | 1 | M | ESFYDWFERQLG | $6.2 * 10^{-7}$ | + |
| S399 | 1790 | 1 | 1 | M | ESFYDWFERQL | $9.1 * 10^{-8}$ | ++ |
| S400 | 1791 | 1 | 1 | M | ESFYDWFERQ | $6.3 * 10^{-7}$ | 0 |
| S415 | 1795 | 1-1 | 1-1 | D; C-Term | $(ESFYDWFERQLGK)_2$-23 | $1.0 * 10^{-7}$ | ++++ |
| S417 | 1796 | 1-1 | 1-1 | D; N-Term | 23-$(ESFYDWFERQLG)_2$ | $9.2 * 10^{-7}$ | +++ |

M = monomer; D = dimer; C-Term = C-terminal linker (C-C); N-Term = N-terminal linker (N-N); 23 represents a specific chemical linker (see Table 3); For FFC: 0 is no effect, + is agonist, - is antagonist; Form. = formula; Mon. = monomer;

[0338] Site 1-Site 2 dimer peptides 537 and 538 were inactive in the FFC assays using the standard concentration of insulin (FIGS. **15**A-**15**C). However, Site 1-Site 2 dimer peptides 537 and 538 were antagonists in the FFC assay in the presence of a stimulating concentration of insulin (FIGS. **16**A-**16**C). In contrast, Site 2-Site 1 dimer peptide 539 was a full agonist in the FFC assay, with a slope similar to that of insulin (FIGS. **17**A-**17**B).

[0339] Additional experiments confirmed that FFC assay activity of Site 1-Site 2 dimer peptides was affected by the orientation of the monomer subunits (FIGS. **18**A-**18**D). In particular, dimer peptides comprising Site 1 (S372 or S373) and Site 2 (S451 or S452) monomer subunits exhibited antagonist activity in the Site 1-Site 2 orientation (C—N linkage) (dimer peptide S453); moderate levels of agonist activity in the Site 1-Site 2 orientation (N—N or C—C linkage) (dimer peptides S454 and S456); and high levels of agonist activity in the Site 2-Site 1 orientation (C—N linkage) (dimer peptide S455) (FIGS. **18**A-**18**D).

[0340] Table 7, below, shows the HIR binding affinity and FFC assay potency of various synthetic peptides, including Site 1-Site 1 dimer peptides S325, S329, S332; S333, S334, S335, S336, S337, S349, S350, S351, S352, S353, S354, S361, S362, S363, S374, S375, S376, S378, S379, S380, S381, S414, S415, S416, S417,S418, S420, and S424. These synthetic dimer peptides exhibited properties comparable to dimer peptides 521 and 535, regardless of the orientation of the monomer subunits. In particular, synthetic Site 1-Site 2 dimer peptides S425, S453, and S459 exhibited antagonist properties comparable to those of the Site 1-Site 2 dimer peptides 537 and 538. Synthetic Site 1-Site 2 dimer peptides S455, S457, and S458 exhibited agonist properties comparable to the dimer peptide 539. Synthetic Site 1-Site 2 dimer peptides S436, S437, S438, S454, S456 act as partial agonists in the FFC assay (i.e., the peptides exhibit a maximal response of less than 100% that of insulin), which is shown in the table as "++" and "+++".

[0341] Table 7 also shows properties of truncated monomer and dimer peptides, and thereby indicates which N- or C-terminal residues can be deleted without substantial loss of HIR binding affinity (e.g., see synthetic peptides S386 through S392, S394 through S403, and S436 through S445). Notably, certain Site 2-Site 1 dimers show IR affinities of $2*10^{-11}$ (see, e.g., S519 and S520). These peptides are also very potent in the fat cell assay (FIGS. **31**A-**31**B) and even more potent in the HIR kinase assay (FIGS. **32**A-**32**B) (kinase assay described below).

TABLE 7

| Peptide | SEQ ID NO: | Formula | Linkage | SiteIR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S105 | 1797 | F1 | - | 1 | FHENFYDWFVRQVAKK | 3.1 * 10-7 | ++ |
| S106 | 1798 | F1 | - | 1 | FHENFYDWFVRQASKK | 4.2 * 10-7 | ++ |
| S107 | 1799 | F1 | - | 1 | FHENFYDWFVRAVSKK | 10.0 * 10-7 | + |
| S108 | 1800 | F1 | - | 1 | FHENFYDWFVAQVSKK | 7.5 * 10-7 | + |
| S109 | 1801 | F1 | - | 1 | FHENFYDWFARQVSKK | 2.3 * 10-7 | ++ |

TABLE 7-continued

| Peptide | SEQ ID NO: | Formula | Linkage | SiteIR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S110 | 1802 | F1 | - | 1 | FHEAFYDWFVRQVSKK | 2.2 * 10-7 | ++ |
| S111 | 1803 | F1 | - | 1 | FHANFYDWFVRQVSKK | 3.3 * 10-7 | 0 |
| S112 | 1804 | F1 | - | 1 | FAENFYDWFVRQVSKK | 6.1 * 10-7 | + |
| S113 | 1805 | F1 | - | 1 | AHENFYDWFVRQVSKK | 5.9 * 10-7 | + |
| S114 | 1556 | F1 | - | | fhenfydwfvrqvskk | 8.3 * 10-6 | 0 |
| S115 | 1806 | F1 | - | 1 | EFHENFYDWFVRQVSEE | 6.5 * 10-7 | + |
| S116 | 1807 | F1 | - | 1 | FHENFYGWFVRQVSKK | 1.4 * 10-6 | ++ |
| S117 | 1808 | F2 | - | 1 | HETFYSMIRSLAK | 2.7 * 10-6 | 0 |
| S118 | 1809 | F2 | - | 1 | SDGFYNAIELLS | 2.4 * 10-6 | + |
| S119 | 1810 | F2 | - | 1 | SLNFYDALQLLAKK | 1.8 * 10-6 | 0 |
| S120 | 1811 | F2 | - | 1 | HDPFYSMMKSLLK | 2.0 * 10-6 | 0 |
| S121 | 1812 | F2 | - | 1 | NSFYEALRMLSSK | 3.1 * 10-6 | 0 |
| S122 | 1813 | F7 | - | | HPTSKEIYAKLLK | 9.3 * 10-6 | 0 |
| S123 | 1814 | F7 | - | | HPSTNQMLMKLFK | 1.6 * 10-5 | 0 |
| S124 | 1815 | F7 | - | | HPPLSELKLFLIKK | 2.3 * 10-5 | 0 |
| S127 | 1816 | F2 | - | 1 | WSDFYSYFQGLD | 1.2 * 10-6 | 0 |
| S128 | 1817 and 1818 | F1-F1 | C-C | 1-1 | $(FHENFYDWFVRQVSKK)_2$-Dap | 1.1 * 10-6 | ++ |
| S129 | 1819 | F2 | - | 1 | SSNFYQALMLLS | 2.9 * 10-6 | 0 |
| S131 | 1820 | F1 | - | 1 | FHENFYDWFVRQVSKK-Lig | 1.2 * 10-6 | + |
| S137 | 1821 | F1 | - | 1 | HENFYGWFVRQVSKK | 7.7 * 10-7 | 0 |
| S145 | 1822 and 1823 | F1-F1 | C-C | 1-1 | $(FHENFYDWFVRQVSKK)_2$-Lys | 1.5 * 10-6 | ++ |
| S158 | 1780 | F1 | - | 1 | FHENFYDWFVRQVSK | 8.1 * 10-7 | + |
| S165 | 1554 | F1 | - | 1 | FYDWF | >2 * 10-5 | 0 |
| S166 | 1824 | F1 | - | 1 | FYDWFKK | >2 * 10-5 | 0 |
| S167 | 1825 | F1 | - | 1 | AFYDWFAKK | >2 * 10-5 | - |
| S168 | 1826 | F1 | - | 1 | AAAAFYDWFAAAAAKK | 3.8 * 10-6 | 0 |
| S169 | 1827 and 1828 | F1-F1 | N-N | 1-1 | 12-$(Lig-FHENFYDWFVRQVSKK)_2$ | 5.8 * 10-7 | ++ |
| S170 | 1829 and 1830 | F1-F1 | N-N | 1-1 | $(CGFHENFYDWFVRQVSKK)_2$ (linked at cysteines) | 7.0 * 10-7 | +++ |
| S171 | 1831 | F1 | - | 1 | CGFHENFYDWFVRQVSKK | 2.9 * 10-6 | +++ |
| S172 | 1832 and 1833 | F1-F1 | N-N | 1-1 | 14-$(Lig-FHENFYDWFVRQVSKK)_2$ | 4.8 * 10-6 | +++ |
| S173 | 1834 | F3 | - | 1 | LDALDRLMRYFEERPSL | 1.2 * 10-6 | 0 |

TABLE 7-continued

| Peptide | SEQ ID NO: | Formula | Linkage | SiteIR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S174 | 1835 | F3 | - | 1 | PLAELWAYFEHSEQGRSSAH | 1.6 * 10-5 | 0 |
| S175 | 1560 | F1 | - | 1 | GRVDWLQRNANFYDWFVAELG | 2.3 * 10-7 | +++ |
| S176 | 1836 | F1 | - | 1 | NGVERAGTGDNFYDWFVAQLH | 4.7 * 10-7 | + |
| S177 | 1837 | F2 | - | 1 | EHWNTVDPFYFTLFEWLRESG | 2.7 * 10-6 | 0 |
| S178 | 1838 | F2 | - | 1 | EHWNTVDPFYQYFSELLRESG | 1.3 * 10-7 | ++ |
| S179 | 1839 | F1 | - | 1 | QSDSGTVHDRFYGWFRDTWAS | 5.4 * 10-7 | + |
| S180 | 1840 | F1 | - | 1 | AFYDWFAK | >2 * 10-5 | 0 |
| S181 | 1841 | F1 | - | 1 | AFYDWFA | >2 * 10-5 | 0 |
| S182 | 1842 | F1 | - | 1 | AFYDWF | >2 * 10-5 | 0 |
| S183 | 1843 | F1 | - | 1 | FYDWFA | >2 * 10-5 | 0 |
| S184 | 1844 | F1 | - | 1 | Ac-FYDWF | >2 * 10-5 | 0 |
| S214 | 1845 | F1 | - | 1 | AFYEWFAKK | >2 * 10-5 | 0 |
| S215 | 1846 | F1 | - | 1 | AFYGWFAKK | >2 * 10-5 | 0 |
| S216 | 1847 | F1 | - | 1 | AFYKWFAKK | >2 * 10-5 | 0 |
| S217 | 1848 and 1849 | F2-F2 | C-C | 1-1 | $(SDGFYNAIELLS\text{-}Lig)_2$-14 | 3.9 * 10-8 | ++ |
| S218 | 1850 and 1851 | F1-F1 | C-C | 1-1 | $(AFYDWFAKK\text{-}Lig)_2$-14 | 1.1 * 10-5 | 0 |
| S219 | 1852 | F1 | - | 1 | FHENAYDWFVRQVSKK | >2 * 10-5 | 0 |
| S220 | 1853 | F1 | - | 1 | FHENFADWFVRQVSKK | >2 * 10-5 | 0 |
| S221 | 1854 | F1 | - | 1 | FHENFYAWFVRQVSKK | 1.1 * 10-6 | + |
| S222 | 1855 | F1 | - | 1 | FHENFYDAFVRQVSKK | >2 * 10-5 | 0 |
| S223 | 1856 | F1 | - | 1 | FHENFYDWAVRQVSKK | >2 * 10-5 | 0 |
| S226 | 1857 | F6 | - | 2 | QLEEEWAGVQCEVYGRECPS | 1.6 * 10-6 | |
| S227 | 1858 | F1 | - | 1 | CGGFHENFYDWFVRQVSKK | 5.1 * 10-7 | ++ |
| S228 | 1859 and 1860 | F1-F1 | N-N | 1-1 | $(CGGFHENFYDWFVRQVSKK)_2$ (linked at cysteines) | 3.6 * 10-7 | ++ |
| S229 | 1861 and 1862 | F2-F4 | C-C | 1-2 | SDGFYNAIELLS-Lig 12 KHLCVLEELFWGASLFGYCSGKK-Lig | 4.4 * 10-9 | 0 |
| S231 | 1863 and 1864 | F1-F1 | C-C | 1-1 | $(FHENFYDWFVRQVSKKGGG\text{-}Lig)_2$-14 | 2.7 * 10-7 | + |
| S232 | 1865 and 1866 | F1-F1 | N-N | 1-1 | 14-$(Lig\text{-}GGGFHENFYDWFVRQVSKK)_2$ | 3.8 * 10-7 | +++ |
| S233 | 1867 and 1868 | F1-F2 | C-C | 1-1 | FHENFYDWFVRQVSKK-Lig 14 SDGFYNAIELLS-Lig | 2.6 * 10-7 | + |

TABLE 7-continued

| Pep-tide | SEQ ID NO: | Formula | Linkage | SiteIR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S234 | 1869 | F1 | - | 1 | RVDWLQRNANFYDWFVAELG | 1.3 * 10-7 | ++ |
| S235 | 1870 | F1 | - | 1 | VDWLQRNANFYDWFVAELG | 5.3 * 10-8 | ++ |
| S236 | 1871 | F1 | - | 1 | DWLQRNANFYDWFVAELG | 1.0 * 10-7 | ++ |
| S237 | 1872 | F1 | - | 1 | WLQRNANFYDWFVAELG | 8.5 * 10-7 | 0 |
| S238 | 1873 | F1 | - | 1 | LQRNANFYDWFVAELG | 8.5 * 10-7 | 0 |
| S239 | 1874 | F1 | - | 1 | QRNANFYDWFVAELG | 1.3 * 10-6 | 0 |
| S240 | 1875 | F1 | - | 1 | RNANFYDWFVAELG | 1.4 * 10-6 | |
| S241 | 1876 | F1 | - | 1 | NANFYDWFVAELG | 1.6 * 10-6 | |
| S242 | 1877 | F1 | - | 1 | ANFYDWFVAELG | 2.0 * 10-6 | |
| S243 | 1878 | F1 | - | 1 | NFYDWFVAELG | 2.0 * 10-6 | |
| S244 | 1879 | F1 | - | 1 | GRVDWLQRNANFYDWFVAELG-Lig | 2.2 * 10-7 | ++ |
| S245 | 1880 | F1 | - | 1 | Lig-GRVDWLQRNANFYDWFVAELG | 2.2 * 10-7 | + |
| S246 | 1881 and 1882 | F8-F1 | C-C | 3-1 | ACAWPTYWNCGGGG-Lig 14 FHENFYDWFVRQVSKK-Lig | 5.0 * 10-6 | |
| S248 | 1883 | F1 | - | 1 | GRVDWLQRNANFYDWFVAEL | 6.3 * 10-8 | ++ |
| S249 | 1884 | F1 | - | 1 | GRVDWLQRNANFYDWFVAE | 7.4 * 10-7 | 0 |
| S250 | 1885 | F1 | - | 1 | GRVDWLQRNANFYDWFVA | 8.9 * 10-6 | 0 |
| S251 | 1886 | F1 | - | 1 | GRVDWLQRNANFYDWFV | 5.6 * 10-6 | |
| S252 | 1887 and 1888 | F2-F2 | C-C | 1-1 | $(SDGFYNAIELLS-Lig)_2$-14 | 4.4 * 10-7 | 0 |
| S253 | 1889 and 1890 | F1-F1 | C-C | 1-1 | $(GRVDWLQRNANFYDWFVAELG-Lig)_2$-14 | 2.2 * 10-8 | ++ |
| S255 | 1891 and 1892 | F2-F2 | C-C | 1-1 | $(SDGFYNAIELLSGGG-Lig)_2$-14 | 1.6 * 10-6 | 0 |
| S256 | 1893 | F6 | - | 2 | Acy-CLEEwGASL-Tic-QCSG | 9.0 * 10-6 | - |
| S257 | 1894 | F2 | - | 1 | RWPNFYGYFESLLTHFS | 1.4 * 10-5 | 0 |
| S259 | 1895 | F2 | - | 1 | EGWDFYSYFSGLLASVT | 7.7 * 10-6 | 0 |
| S260 | 1896 | F2 | - | 1 | LDRQFYRYFQDLLVGFM | 2.3 * 10-6 | 0 |
| S261 | 1897 | F2 | - | 1 | WGRSFYRYFETLLAQGI | >2 * 10-5 | 0 |
| S262 | 1898 | F4 | - | 1 | PLCFLQELFGGASLGGYCSG | 1.9 * 10-5 | 0 |
| S263 | 1899 | F6 | - | 2 | WLEQERAWIWCEIQGSGCRA | >2 * 10-5 | 0 |
| S264 | 1900 | F1 | - | 1 | IQGWEPFYGWFDDWAQMFEE | 1.9 * 10-7 | 0 |
| S265 | 1901 | F1 | - | 1 | TGHRLGLDEQFYWWFRDALSG | 1.1 * 10-7 | 0 |
| S266 | 1902 | F6 | - | 2 | Abu-CLEEwGASL-Tic-QCSG | >2 * 10-5 | 0 |
| S268 | 1903 | F1 | - | 1 | RD-Hyp-FYDWFDDi | 4.5 * 10-7 | 0 |
| S273 | 1904 | F1-F2 | C-N | 1-1 | FHENFYDWFVRQVSKK-Lig-14-Lig-SDGFYNAIELLS | 1.5 * 10-6 | + |

TABLE 7-continued

| Peptide | SEQ ID NO: | Formula | Linkage | SiteIR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S278 | 1905 | F1-derived | - | 1 | GFREGQRWYWFVAQVT | >2 * 10-5 | 0 |
| S281 | 1906 | ES | - | | DLRVLCELFGGAYVLGYCSE | 1.1 * 10-5 | 0 |
| S282 | 1907 | F4-derived | - | | HLSVGEELSWWVALLGQWAR | >2 * 10-5 | 0 |
| S283 | 1908 | F4-derived | - | | APVSTEELRWGALLFGQWAG | >2 * 10-5 | 0 |
| S284 | 1909 | F6-derived | - | | ALEEEWAWVQVRSIRSGLPL | >2 * 10-5 | 0 |
| S285 | 1910 | F6-derived | - | | WLEHEWAQIQCELYGRGCTY | 8.3 * 10-7 | |
| S287 | 1911 | F1 | - | 1 | QAPSNFYDWFVREWDEE | 5.9 * 10-6 | 0 |
| S288 | 1912 | F2 | - | 1 | QSFYDYIEELLGGEWKK | 4.3 * 10-6 | 0 |
| S289 | 1913 | F2 | - | 1 | DPFYQGLWEWLRESGEE | >2 * 10-5 | 0 |
| S290 | 1914 and 1915 | F1-F1 | N-N | 1-1 | 7-( Lig-GGGFHENFYDWFVRQVSKK)$_2$ | 9.0 * 10-7 | ++ |
| S291 | 1916 and 1917 | F1-F1 | N-N | 1-1 | 9-(Lig-GGGFHENFYDWFVRQVSKK)$_2$ | 1.2 * 10-6 | |
| S292 | 1918 and 1919 | F1-F1 | N-N | 1-1 | 12-(Lig-GGGFHENFYDWFVRQVSKK)$_2$ | 7.5 * 10-7 | ++ |
| S293 | 1920 and 1921 | F1-F1 | N-N | 1-1 | 13-(Lig-GGGFHENFYDWFVRQVSKK)$_2$ | 1.2 * 10-7 | ++ |
| S294 | 1922 | F1 | - | 1 | DWLQRNANFYDWFVAEL-Lig | 1.3 * 10-7 | ++ |
| S295 | 1923 | F1 | - | 1 | Lig-DWLQRNANFYDWFVAEL | 4.8 * 10-7 | + |
| S300 | 1924 and 1925 | F1-F1 | C-C | 1-1 | (DWLQRNANFYDWFVAEL-Lig')$_2$-14 | 5.0 * 10-8 | +++ |
| S301 | 1926 and 1927 | F1-F1 | N-N | 1-1 | 14-(Lig'-DWLQRNANFYDWFVAEL)$_2$ | 6.4 * 10-7 | + |
| S302 | 1928 | F2 | - | 1 | SDGFYNA-Acy-ELLSG | 8.6 * 10-7 | 0 |
| S303 | 1929 | F2 | - | 1 | SGPFYEE-Acy-ELLW-Aib-G | 5.7 * 10-6 | 0 |
| S304 | 1930 | F2 | - | 1 | GGSFYDD-Acy-E-Aib-LW-Aib-G | 2.1 * 10-5 | 0 |
| S305 | 1931 | F2 | - | 1 | N-Aib-PFYDE-Acy-DE-Cha-W-Aib-G | 8.4 * 10-7 | 0 |
| S306 | 1932 | F1 | - | 1 | GRVDWLQRNANFYDWFVAE-Acy-G | 2.2 * 10-6 | +++ |
| S312 | 1933 and 1934 | F1-F1 | N-N | 1-1 | 23-(Lig'-GGGFHENFYDWFVRQVSKK)$_2$ | 2.9 * 10-6 | ++ |
| S313 | 1935 and 1936 | F2-F2 | C-C | 1-1 | (SDGFYNAIELLS-Lig')$_2$-23 | 2.4 * 10-7 | |
| S315 | 1937 | F1 | - | 1 | WFYDWFWE | 6.8 * 10-6 | 0 |

TABLE 7-continued

| Peptide | SEQ ID NO: | Formula | Linkage | SiteIR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S316 | 1938 | F10 | - | 1 | WQGYAWLS | 7.0 * 10-6 | 0 |
| S317 | 1939 | F10 | - | 1 | WPGYAWLS | >2 * 10-5 | 0 |
| S319 | 1940 | F1 | - | 1 | D-Aic-D-Aib-EFYDWFDEiPq | 8.7 * 10-7 | 0 |
| S320 | 1941 | F1 | - | 1 | KNNKEFYEWFDEiGq | 2.8 * 10-6 | 0 |
| S321 | 1942 | F1 | - | 1 | YeRD-Hyp-FYDWFDEiGq | 1.4 * 10-6 | 0 |
| S322 | 1943 | F1 | - | 1 | EWRD-Hyp-FYDWFDEi-Hyp-e | 7.2 * 10-7 | 0 |
| S325 | 1944 and 1945 | F1-F1 | N-N | 1-1 | 9-(Lig'-GSLDESFYDWFERQLGKK)$_2$ | 4.6 * 10-8 | +++++ |
| S326 | 1600 | F1 | - | 1 | GIISQSCPESFYDWFAGQVSDPWWCW | 5.9 * 10-7 | - |
| S327 | 1946 | F2 | - | 1 | TFYSCLASLLTGTPQPNRGPWERCRKK | 2.7 * 10-6 | - |
| S329 | 1947 and 1948 | F1-F1 | N-N | 1-1 | 17-(Lig'-FHENFYDWFVRQVSKK)$_2$ | 2.7 * 10-6 | ++ |
| S331 | 1949 | F4 | - | 2 | KHLCVLEELFWGASLFGYCSGKK | 1.6 * 10-6 | 0 |
| S332 | 1950 and 1951 | F1-F1 | C-C | 1-1 | (GSLDESFYDWFERQLGKK-Lig')$_2$-9 | 2.1 * 10-8 | +++++ |
| S333 | 1952 and 1953 | F1-F1 | N-N | 1-1 | 22-(Lig'-GSLDESFYDWFERQLGKK)$_2$ | 1.4 * 10-7 | ++++ |
| S334 | 1954 and 1955 | F1-F1 | N-N | 1-1 | 22-(Lig'-GGGFHENFYDWFVRQVSKK)$_2$ | 1.6 * 10-6 | +++ |
| S335 | 1956 and 1957 | F1-F1 | C-C | 1-1 | (GSLDESFYDWFERQLGKK-Lig')$_2$-22 | 9.8 * 10-8 | ++++ |
| S336 | 1958 and 1959 | F1-F1 | N-N | 1-1 | 23-(Lig'-GSLDESFYDWFERQLGKK)$_2$ | 1.5 * 10-8 | +++ |
| S337 | 1960 and 1961 | F1-F1 | C-C | 1-1 | (GSLDESFYDWFERQLGKK-Lig')$_2$-23 | 1.1 * 10-8 | +++++ |
| S342 | 1962 | F1 | - | 1 | DLWFNAKEDMNFYDWFVWQLR | 1.8 * 10-6 | 0 |
| S344 | 1963 | F2 | - | 1 | EHWNTVDPFYHWISELLRESGA | 2.0 * 10-7 | 0 |
| S345 | 1964 | F2 | - | 1 | EHWNTVDPFYQYFAELLRESGA | 2.9 * 10-6 | 0 |
| S349 | 1965 and 1966 | F1-F1 | N-N | 1-1 | 23-(Lig'-GGGFHENFYDWFVRQVSKK)$_2$ | 1.3 * 10-7 | ++++ |

TABLE 7-continued

| Peptide | SEQ ID NO: | Formula | Linkage | SiteIR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S350 | 1967 and 1968 | F1-F1 | C-C | 1-1 | $(GSLDESFYDWFERQLGKK\text{-}Lig')_2$-21 | 4.7 * 10-7 | ++++ |
| S351 | 1969 and 1970 | F1-F1 | N-N | 1-1 | 21-$(Lig'\text{-}GSLDESFYDWFERQLGKK)_2$ | 1.4 * 10-6 | +++ |
| S352 | 1971 and 1972 | F1-F1 | N-N | 1-1 | 21-$(Lig'\text{-}GGGFHENFYDWFVRQVSKK)_2$ | 6.6 * 10-7 | +++ |
| S353 | 1973 and 1974 | F1-F1 | C-C | 1-1 | $(GSLDESFYDWFERQLGKK\text{-}Lig')_2$-14 | 1.1 * 10-8 | ++++++ |
| S354 | 1975 and 1976 | F1-F1 | N-N | 1-1 | 14-$(Lig'\text{-}GSLDESFYDWFERQLGKK)_2$ | 3.9 * 10-8 | ++++ |
| S359 | 1977 and 1978 | F1-F1 | N-N | 1-1 | 9-$(Lig'\text{-}DWLQRNANFYDWFVAEL)_2$ | 7.0 * 10-7 | + |
| S360 | 1979 and 1980 | F1-F1 | N-N | 1-1 | 23-$(Lig'\text{-}DWLQRNANFYDWFVAEL)_2$ | 9.9 * 10-7 | |
| S361 | 1981 and 1982 | F1-F1 | C-C | 1-1 | $(GSLDESFYDWFERQLGKK\text{-}Lig')_2$-24 | 2.2 * 10-6 | +++ |
| S362 | 1983 and 1984 | F1-F1 | N-N | 1-1 | 24-$(Lig'\text{-}GSLDESFYDWFERQLGKK)_2$ | 1.1 * 10-7 | ++++ |
| S363 | 1985 and 1986 | F1-F1 | N-N | 1-1 | 24-$(Lig'\text{-}GGGFHENFYDWFVRQVSKK)_2$ | 2.2 * 10-7 | +++ |
| S365 | 1987 | F1 | - | 1 | RMYFSTGAPQNFYDWFVQEWD | 1.0 * 10-5 | 0 |
| S366 | 1988 | F1 | - | 1 | PLRESRNFYDWFVQQLE | 3.7 * 10-7 | 0 |
| S368 | 1989 | F2 | - | 1 | RGTRSDPFYHKLSELLQGH | >2 * 10-5 | 0 |
| S371 | 1558 | F1 | - | 1 | GSLDESFYDWFERQLGKK | 6.3 * 10-7 | + |
| S372 | 1990 | F1 | - | 1 | SGSLDESFYDWFERQLGKK | 2.0 * 10-7 | ++ |
| S373 | 1991 | F1 | - | 1 | GSLDESFYDWFERQLGKKK(S) | 1.2 * 10-7 | +++ |
| S374 | 1992 and 1993 | F1-F1 | N-N | 1-1 | 17-$(Ald\text{-}GSLDESFYDWFERQLGKK)_2$ | 1.8 * 10-7 | ++++ |
| S375 | 1994 | F1-F1 | C-N | 1-1 | (GSLDESFYDWFERQLGKKK-Ald)-14-(Ald-GSLDESFYDWFERQLGKK) | 2.0 * 10-7 | ++++ |
| S376 | 1995 and 1996 | F1-F1 | N-N | 1-1 | 19-$(Ald\text{-}GSLDESFYDWFERQLGKK)_2$ | 1.6 * 10-7 | ++++ |
| S378 | 1997 and 1998 | F1-F1 | C-C | 1-1 | $(GSLDESFYDWFERQLGKKK\text{-}Ald)_2$-17 | 6.5 * 10-8 | +++++ |
| S379 | 1999 and 2000 | F1-F1 | C-C | 1-1 | $(GSLDESFYDWFERQLGKKK\text{-}Ald)_2$-19 | 5.6 * 10-8 | +++++ |

TABLE 7-continued

| Pep-tide | SEQ ID NO: | Formula | Linkage | SiteIR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S380 | 2001 and 2002 | F1-F1 | C-C | 1-1 | (EEDWLQRNANFYDWFVAEL-Lig')$_2$-9 | 5.1 * 10-7 | ++ |
| S381 | 2003 and 2004 | F1-F1 | C-C | 1-1 | (EEDWLQRNANFYDWFVAEL-Lig')$_2$-23 | 1.2 * 10-7 | ++++ |
| S386 | 1559 | F1 | - | 1 | GSLDESFYDWFERQLG | 3.2 * 10-7 | + |
| S387 | 2005 | F1 | - | 1 | SLDESFYDWFERQLG | 6.3 * 10-7 | + |
| S388 | 2006 | F1 | - | 1 | LDESFYDWFERQLG | 3.4 * 10-7 | + |
| S389 | 2007 | F1 | - | 1 | DESFYDWFERQLG | 1.1 * 10-6 | + |
| S390 | 1794 | F1 | - | 1 | ESFYDWFERQLG | 6.2 * 10-7 | + |
| S391 | 2008 | F1 | - | 1 | SFYDWFERQLG | 1.5 * 10-6 | + |
| S392 | 2009 | F1 | - | 1 | FYDWFERQLG | 3.8 * 10-6 | 0 |
| S394 | 1788 | F1 | - | 1 | GSLDESFYDWFERQ | 9.1 * 10-8 | + |
| S395 | 1787 | F1 | - | 1 | GSLDESFYDWFERQL | 8.1 * 10-8 | ++ |
| S396 | 1789 | F1 | - | 1 | GSLDESFYDWFER | >2 * 10-5 | 0 |
| S397 | 2010 | F1 | - | 1 | GSLDESFYDWFE | >2 * 10-5 | 0 |
| S398 | 2011 | F1 | - | 1 | GSLDESFYDWF | >2 * 10-5 | 0 |
| S399 | 1790 | F1 | - | 1 | ESFYDWFERQL | 9.5 * 10-8 | ++ |
| S400 | 1791 | F1 | - | 1 | ESFYDWFERQ | 6.3 * 10-7 | 0 |
| S401 | 2012 | F1 | - | 1 | ESFYDWFER | >2 * 10-5 | 0 |
| S402 | 2013 | F1 | - | 1 | ESFYDWFE | >2 * 10-5 | 0 |
| S403 | 2014 | F1 | - | 1 | ESFYDWF | >2 * 10-5 | 0 |
| S414 | 2015 and 2016 | F1-F1 | C-C | 1-1 | (ESFYDWFERQLGK-Lig')$_2$-14 | 3.8 * 10-7 | ++++ |
| S415 | 2017 and 2018 | F1-F1 | C-C | 1-1 | (ESFYDWFERQLGK-Lig')$_2$-23 | 1.0 * 10-7 | ++++ |
| S416 | 2019 and 2020 | F1-F1 | N-N | 1-1 | 14-(Lig'-ESFYDWFERQLG)$_2$ | 9.3 * 10-7 | +++ |
| S417 | 2021 and 2022 | F1-F1 | N-N | 1-1 | 23-(Lig'-ESFYDWFERQLG)$_2$ | 9.2 * 10-7 | +++ |
| S418 | 2023 and 2024 | F1-F1 | C-C | 1-1 | (ESFYDWFERQLGK-Ald)$_2$-17 | 1.2 * 10-7 | ++++ |
| S419 | 2025 and 2026 | F6-F6 | N-N | 2-2 | 14-(Lig'-EWLDQEWAWVQCEVYGRGCPSEE)$_2$ | | 0 |
| S420 | 2027 and 2028 | F1-F1 | N-N | 1-1 | 17-(Ald-ESFYDWFERQLG)$_2$ | | ++ |

TABLE 7-continued

| Peptide | SEQ ID NO: | Formula | Linkage | Site | IR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|---|---|
| S423 | 2029 and 2030 | F1-F8 | C-C | 1-3 | | ESFYDWFERQLG<br>K<br>ACAWPTYWNCG | 6.2 * 10-8 | 0 |
| S425 | 2031 | F1-F6 | C-N | 1-2 | | GSLDESFYDWFERQLGKK-Lig'-14-Lig'-<br>EWLDQEWAWVQCEVYGRSCPSEE | 2.4 * 10-9 | - |
| S429 | 2032 | F6-F1 | C-N | 2-1 | | EWLDQEWAWVQCEVYGRGCPSEE-Lig'-14-Lig'-<br>GSLDESFYDWFERQLGKK | 6.0 * 10-10 | |
| S432 | 2033 and 2034 | F1-F6 | C-C | 1-2 | | ESFYDWFERQLGGGG<br>K<br>CEVYGRGCPS | 1.8 * 10-7 | + |
| S433 | 2035 and 2036 | F1-F6 | C-C | 1-2 | | ESFYDWFERQLGGGG<br>K<br>WLDQEWAWVQ | 1.1 * 10-7 | + |
| S436 | 2037 and 2038 | F1-F6 | C-C | 1-2 | | ESFYDWFERQLGGGG<br>K<br>WLDQEWAWVQCEVYGRGCPS | 5.2 * 10-10 | +++ |
| S437 | 2039 and 2040 | F1-F6 | C-C | 1-2 | | ESFYDWFERQLGGGG<br>K<br>LDQEWAWVQCEVYGRGCPS | 6.9 * 10-10 | +++ |
| S438 | 2041 and 2042 | F1-F6 | C-C | 1-2 | | ESFYDWFERQLGGGG<br>K<br>DQEWAWVQCEVYGRGCPS | 3.0 * 10-8 | ++ |
| S439 | 2043 and 2044 | F1-F6 | C-C | 1-2 | | ESFYDWFERQLGGGG<br>K<br>QEWAWVQCEVYGRGCPS | 4.6 * 10-8 | |
| S440 | 2045 and 2046 | F1-F6 | C-C | 1-2 | | ESFYDWFERQLGGGG<br>K<br>EWAWVQCEVYGRGCPS | 9.9 * 10-8 | |
| S441 | 2047 and 2048 | F1-F6 | C-C | 1-2 | | ESFYDWFERQLGGGG<br>K<br>WAWVQCEVYGRGCPS | 1.2 * 10-7 | |
| S442 | 2049 and 2050 | F1-F6 | C-C | 1-2 | | ESFYDWFERQLGGGG<br>K<br>AWVQCEVYGRGCPS | 1.6 * 10-7 | |
| S443 | 2051 and 2052 | F1-F6 | C-C | 1-2 | | ESFYDWFERQLGGGG<br>K<br>WVQCEVYGRGCPS | 1.7 * 10-7 | |
| S444 | 2053 and 2054 | F1-F6 | C-C | 1-2 | | ESFYDWFERQLGGGG<br>K<br>VQCEVYGRGCPS | 1.9 * 10-7 | |
| S445 | 2055 and 2056 | F1-F6 | C-C | 1-2 | | ESFYDWFERQLGGGG<br>K<br>QCEVYGRGCPS | 2.3 * 10-7 | |
| S453 | 2057 | F1-F6 | C-N | 1-2 | | GSLDESFYDWFERQLGKKK-Ald-17-Ald-<br>KEWLDQEWAWVQCEVYGRGCPSEE | 5.7 * 10-10 | |
| S454 | 2058 and 2059 | F1-F6 | C-C | 1-2 | | GSLDESFYDWFERQLGKKK-Ald<br>17<br>EWLDQEWAWVQCEVYGRGCPSEEK-Ald | 3.8 * 10-10 | +++ |
| S455 | 2060 | F6-F1 | C-N | 2-1 | | EWLDQEWAWVQCEVYGRGCPSEEK-Ald-18-Ald-<br>GSLDESFYDWFERQLGKK | 1.1 * 10-9 | ++++ |

TABLE 7-continued

| Peptide | SEQ ID NO: | Formula | Linkage | SiteIR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S456 | 2061 and 2062 | F1-F6 | N-N | 1-2 | Ald-GSLDESFYDWFERQLGKK 17 Ald-KEWLDQEWAWVQCEVYGRGCPSEE | 2.4 * 10-9 | +++ |
| S457 | 2063 | F6-F1 | C-N | 2-1 | WLDQEWAWVQCEVYGRGCPSGGSGGSGSLDESFYDWFERQLG | 1.6 * 10-9 | ++++ |
| S458 | 2064 | F6-F1 | C-N | 2-1 | WLDQEWAWVQCEVYGRGCPSGGSGGSGSLDESFYDWFERQLG | 3.2 * 10-9 | ++++ |
| S459 | 2065 | F1-F6 | C-N | 1-2 | GSLDESFYDWFERQLGGGSGGSWLDQEWAWVQCEVYGRGCPS | 7.6 * 10-11 | - |
| S467 | 2066 | F6-F1 | C-N | 2-1 | EWLDQEWAWVQCEVYGRGCPSEEK-Ald-16-Ald-GSLDESFYDWFERQLGKK | 6.8 * 10-10 | ++++ |
| S468 | 2067 | F6-F1 | C-N | 2-1 | EWLDQEWAWVQCEVYGRGCPSEEK-Ald-19-Ald-GSLDESFYDWFERQLGKK | 4.0 * 10-10 | ++++ |
| S471 | 2068 | F6-F1 | C-N | 2-1 | LDQEWAWVQCEVYGRGCPSESFYDWFERQLG | 6.7 * 10-10 | ++++ |
| S481 | 2069 | F6-F1 | C-N | 2-1 | HHHHHHKLDQEWAWVQCEVYGRGCPSESFYDWFERQLG | 1.3 * 10-9 | |
| S482 | 2070 | F6-F1 | C-N | 2-1 | LDQEWAWVQCEVYGRGCPSESFYDWFERQLG | | |
| S483 | 2071 | F6-F1 | C-N | 2-1 | LDEWAWVQCVEYGRGCPSESFYDWFERQLG | 5.2 * 10-8 | 0 |
| S484 | 2072 | F6-F1 | C-N | 2-1 | LDQEWAVQCEVYGRGCPSESFYDWFERQLG | 8.7 * 10-8 | 0 |
| S485 | 2073 | F6-F1 | C-N | 2-1 | LDQEWAWVCEVYGRGCPSESFYDWFERQLG | 1.6 * 10-7 | 0 |
| S486 | 2074 | F6-F1 | C-N | 2-1 | LDQEWAWVQCVYGRGCPSESFYDWFERQLG | 5.7 * 10-8 | 0 |
| S487 | 2075 | F6-F1 | C-N | 2-1 | LDQEWAWVQCEVYGRCPSESFYDWFERQLG | | |
| S488 | 2076 | F6-F1 | C-N | 2-1 | LDQEWAWVQCEVYGRGCSESFYDWFERQLG | | |
| S489 | 2077 | F6-F1 | C-N | 2-1 | LDQEWAWVQCEVYGRGCPESFYDWFERQLG | | |
| S490 | 2078 | F6-F1 | C-N | 2-1 | LDQEWAWVQCEVYGRGCESFYDWFERQLG | | |
| S491 | 2079 | F6-F1 | C-N | 2-1 | LDQEWAWVQCEVYGRGCPSEFYDWFERQLG | | |
| S492 | 2080 | F6-F1 | C-N | 2-1 | LDQEWAWVQCEVYGRGCPSESFYDWFRQLG | | |
| S493 | 2081 | F6-F1 | C-N | 2-1 | EWLDQEWAWVQCEVYGRGCPSEE-POX-Lys(biotin) | | |
| S494 | 2082 | F6-F1 | C-N | 2-1 | ADQEWAWVQCEVYGRGCPSESFYDWFERQLG | 1.7 * 10-8 | + |
| S495 | 2083 | F6-F1 | C-N | 2-1 | LAQEWAWVQCEVYGRGCPSESFYDWFERQL | 2.6 * 10-9 | |
| S496 | 2084 | F6-F1 | C-N | 2-1 | LDAEWAWVQCEVYGRGCPSESFYDWFERQL | | |
| S497 | 2085 | F6-F1 | C-N | 2-1 | LDQAWAWVQCEVYGRGCPSESFYDWFERQL | 2.5 * 10-9 | +++ |
| S498 | 2086 | F6-F1 | C-N | 2-1 | LDQEAAWVQCEVYGRGCPSESFYDWFERQL | 5.6 * 10-8 | + |
| S499 | 2087 | F6-F1 | C-N | 2-1 | LDQEWAAVQCEVYGRGCPSESFYDWFERQL | 6.2 * 10-10 | ++++ |
| S500 | 2088 | F6-F1 | C-N | 2-1 | LDQEWAWAQCEVYGRGCPSESFYDWFERQL | | |
| S501 | 2089 | F6-F1 | C-N | 2-1 | LDQEWAWVACEVYGRGCPSESFYDWFERQL | | |
| S502 | 2090 | F6-F1 | C-N | 2-1 | LDQEWAWVQCAVYGRGCPSESFYDWFERQL | 3.0 * 10-9 | +++ |
| S503 | 2091 | F6-F1 | C-N | 2-1 | LDQEWAWVQCEAYGRGCPSESFYDWFERQL | 2.1 * 10-9 | |
| S504 | 2092 | F6-F1 | C-N | 2-1 | LDQEWAWVQCEVAGRGCPSESFYDWFERQL | 1.3 * 10-8 | |
| S505 | 2093 | F6-F1 | C-N | 2-1 | LDQEWAWVQCEVYARGCPSESFYDWFERQL | | |
| S506 | 2094 | F6-F1 | C-N | 2-1 | LDQEWAWVQCEVYGAGCPSESFYDWFERQL | | |
| S507 | 2095 | F6-F1 | C-N | 2-1 | LDQEWAWVQCEVYGRACPSESFYDWFERQL | | |

TABLE 7-continued

| Pep-tide | SEQ ID NO: | Formula | Linkage | SiteIR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S508 | 2096 | F6-F1 | C-N | 2-1 | LDQEWAWVQCEVYGRGCASESFYDWFERQL | | |
| S509 | 2097 | F6-F1 | C-N | 2-1 | LDQEWAWVQSEVYGRGSPSESFYDWFERQL | 5.7 * 10-9 | |
| S510 | 2098 | F6-F1 | C-N | 2-1 | SLEEEWAQVECEVYGRGCPSGGSGGSGLLDESFYHWFDRQLR | 6.2 * 10-11 | +++++ |
| S511 | 2099 | F6-F1 | C-N | 2-1 | WLDQEWAWVQCEVYGRGCPSGGSGGSGRVDWLQRNANFYDWF VAELG | 3.8 * 10.9 | ++ |
| S512 | 2100 | F6-F1 | C-N | 2-1 | WLDQEWAWVQCEVYGRGCPSGGSGGSSQAGSAFYAWFDQVLRTV | 2.8 * 10-8 | ++ |
| S513 | 2101 | F6-F1 | C-N | 2-1 | WLDQEWAWVQCEVYGRGCPSGGSGGSQSDAFYSGLWALIGLSDG | | |
| S515 | 2102 | F6 | - | 2 | LDQEWAWVQCEVYGRGCPSPOX-Lys(Biotin) | | |
| S516 | 2103 | F4-F1 | C-N | 2-1 | H-Acy-CLEEwGASL-Tic-QCSGSESFYDWFERQL | | |
| S517 | 2104 | F6-F1 | C-N | 2-1 | SIEEEWAQIKCDVWGRGCPSESFYDWFERQL | 6.0 * 10-12 | ++++++ |
| S518 | 2105 | F6-F1 | C-N | 2-1 | RLEEEWAWVQCEVYGRGCPSGSLDESFYDWFERQLG | 1.6 * 10-10 | +++++ |
| S519 | 2106 | F6-F1 | C-N | 2-1 | SLEEEWAQVECEVYGRGCPSGSLDESFYDWFERQLG | 2.0 * 10-11 | +++++++ |
| S520 | 2107 | F6-F1 | C-N | 2-1 | SIEEEWAQIKCDVWGRGCPPGLLDESFYHWFDRQLR | 2.0 * 10-11 | ++++++ |
| S521 | 2108 | F4-F1 | C-N | 2-1 | HLCVLEELFWGASLFGYCSGGSLDESFYDWFERQL | 2.7 * 10-8 | + |
| S522 | 2109 | F4-F1 | C-N | 2-1 | HLCVLEELFWGASLFGYCSGGRVDWLQRNANFYDWFVAELG | | |
| S523 | 2110 | F6-F1O | C-N | 2-1 | WLDQEWAWVQCEVYGRGCPSDSDWAGYEWFEEQLD | 4.3 * 10-9 | ++ |
| S524 | 2111 | F6-F1 | C-N | 2-1 | HHHHHHKSLEEEWAQVECEVYGRGCPSGSLDESFYDWFERQLG | 1.1 * 10-11 | ++++++ |
| S527 | 2228 2229 | F4-F1 | C-N | 2-1 | H-Acy-CAQEwGSEL-Tic-QCSGSESFYDWFERQL | 2.4 * 10-9 | |
| S530 | 2230 | F6-F1 | C-N | 2-1 | SLEEEWAQVECEVYGRGCPSESFYDWFERQL | 8.0 * 10-12 | +++++++ |
| S531 | 2231 | F6-F1 | C-N | 2-1 | SLEEEWAQVECEVYGRGCPSFYDWFERQL | 7.5 * 10-11 | +++ |
| S532 | 2232 | F6-F1 | C-N | 2-1 | SLEEEWAWVECEVYGRGCPSGSLDESFYDWFERQL | 3.7 * 10-11 | +++++ |
| S533 | 2233 | F6-F1 | C-N | 2-1 | LDQEWAQVQCEVYGRGCPSESFYDWFERQL | 6.7 * 10-11 | ++++ |
| S534 | 2234 | F6-F1 | C-N | 2-1 | SLEEEWAWVQCEVYGRGCPSESFYDWFERQL | 1.0 * 10-11 | ++++++ |
| S535 | 2235 | F6-F1 | C-N | 2-1 | QLDEEWAGVQCEVYGRGCSLDESFYDWFERQLG | | |
| S536 | 2236 | F6-F1 | C-N | 2-1 | LEEEWAQVECEVYGRGCPSESFYDWFERQL | 8.3 * 10-11 | ++++ |
| S537 | 2237 | F6-F1 | C-N | 2-1 | SLEHEWAQVECEVYGRGCPSGSLDESFYDWFERQLG | 4.4 * 10-11 | ++++ |
| S538 | 2238 | F6-F1 | C-N | 2-1 | SLEQEWAQVECEVYGRGCPSGSLDESFYDWFERQLG | 3.8 * 10-11 | ++++ |
| S539 | 2239 | F6-F1 | C-N | 2-1 | SLELEWAQVECEVYGRGCPSGSLDESFYDWFERQLG | 9.8 * 10-11 | ++++ |
| S540 | 2240 | F6-F1 | C-N | 2-1 | SLEEEWAQVKCEVYGRGCPSGSLDESFYDWFERQLG | 1.3 * 10-11 | +++++ |
| S541 | 2241 | F6-F1 | C-N | 2-1 | SLEEEWAQVECEVWGRGCPSGSLDESFYDWFERQLG | 7.8 * 10-12 | +++++++ |
| S542 | 2242 | F6-F1 | C-N | 2-1 | SLEEEWAQVECEVYGRGCPSGSLDESFYHWFERQLG | 2.7 * 10-11 | ++++++ |
| S543 | 2243 | F1-F6 | C-N | 1-2 | GSLDESFYDWFERQLGGGSGGSWLDEEWAQVQCEVYGRGCPS | 1.9 * 10-11 | --- |
| S544 | 2244 | F1-F6 | C-N | 1-2 | ESFYDWFERQLGWLDQEWAWVQCEVYGRGCPS | | |
| S545 | 2245 | F1-F6 | C-N | 1-2 | ESFYDWFERQLGWLDEEWAQVQCEVYGRGCPS | | |
| S546 | 2246 2247 | F6-F1 | C-N | 2-1 | SLEEEWAQVECEV-Bpa-GRGCPSGSLDESFYDWFERQ-Bpa-GK (Biotin) | 2.6 * 10-8 | |

TABLE 7-continued

| Pep-tide | SEQ ID NO: | Formula | Linkage | SiteIR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S547 | 2248 | F6 | | 2 | SLEEEWAQVECEVYGRGCPS | 4.9 * 10-8 | - |
| S548 | 2249 | F6 | | 2 | SLEEEWAQVECEVWGRGCPS | 4.1 * 10-9 | - |
| S549 | 2250 | F6-F1 | C-N | 2-1 | SLEEEWAQVECEVYGRGCSGSLDESFYDWFERQLG | 1.3 * 10-11 | ++++++ |
| S550 | 2251 | F1 | | 1 | Ac-GSLDESFYDWFERQLG-POX-K | 4.0 * 10-8 | |
| S551 | 2252 | F6-F1 | C-N | 2-1 | SLEEEWAQVEAEVYGRGAPSGSLDESFYDWFERQLG | 7.2 * 10-11 | |
| S552 | 2253 | F6-F1 | C-N | 2-1 | SLEEEWAQVECEVYGRGCPSGSLDESFYDWFERQLGKHHHHHH | | |
| S553 | 2254 | F6-F1 | C-N | 2-1 | SLEEEWAQVECEVYGRGCPPGLLDESFYHWFDRQLR | 7.3 * 10-12 | |
| S554 | 2255 | F6-F1 | C-N | 2-1 | SLEEEWAQIECEVYGRGCPSESFYDWFERQLG | 6.4 * 10-12 | +++++++ |
| S555 | 2256 | F6-F1 | C-N | 2-1 | SLEEEWAQVECEVYGRGCPSESFYDWFVRQLG | 5.7 * 10-11 | ++++ |
| S556 | 2257 | F6-F1 | C-N | 2-1 | SIEEEWAQIKCDVWGRGCSESFYDWFERQL | 3.2 * 10-11 | ++++ |
| S557 | 2258 | F6-F1 | C-N | 2-1 | SLEEEWAQIECEVYGRGCPSESFYDWFERQL | 2.0 * 10-11 | |
| S558 | 2259 | F6-F1 | C-N | 2-1 | SLEEEWAQIECEVWGRGCPSESFYDWFERQL | 1.9 * 10-11 | +++++++ |
| S559 | 2260 | F6-F1 | C-N | 2-1 | SLEEEWAQIECEVWGRGCSESFYDWFERQL | 2.1 * 10-11 | +++++++ |
| S560 | 2261 | F6-F1 | C-N | 2-1 | SLEEEWAQIECEVWGRGCPSGSLDESFYDWFERQL | 1.4 * 10-11 | +++++++ |
| S561 | 2262 | F6-F1 | C-N | 2-1 | SLEEEWAQIECEVWGRGCSGSLDESFYDWFERQL | 1.8 * 10-11 | +++++++ |
| S562 | 2263 | F6-F1 | C-N | 2-1 | SIEEEWAQIKCDVWGRCSESFYDWFERQL | 1.8 * 10-11 | ++++ |
| S563 | 2264 2265 | F6-F1 | C-N | 2-1 | SLEEEWAQIQCEVWG RncSESFYDWFERQL | 1.4 * 10-11 | +++++ |
| S564 | 2266 | F6-F1 | C-N | 2-1 | SLEEEWAQIQCEVWGRCSESFYDWFERQL | 1.3 * 10-11 | ++++++ |
| S565 | 2267 2268 | F6-F1 | C-N | 2-1 | SIEEEWAQIQCEVWG RpcSESFYDWFERQL | | |
| S566 | 2269 | F6-F1 | C-N | 2-1 | SIEEEWAQVECEVWGRGCPSESFYDWFERQLG | | |
| S567 | 2270 | F6-F1 | C-N | 2-1 | SI EEEWAQIECDVWGRGPSESFYDWFERQLG | | |
| S568 | 2271 | F6-F1 | C-N | 2-1 | AcSIEEEWAQIKCDVWGRGPSESFYDWFERQLG | 4.3 * 10-12 | +++++++ |
| S569 | 2272 | F6-F1 | C-N | 2-1 | SLEEEWAQIEEVWGRGPSESFYDWFERQLG | 1.5 * 10-10 | +++ |
| S570 | 2273 | F6-F1 | C-N | 2-1 | SLEEEWAQIEEVWGRPSESFYDWFERQLG | 7.3 * 10-10 | +++ |
| S571 | 2274 | F6-F1 | C-N | 2-1 | SLEEEWAQIEEVWGRGSESFYDWFERQLG | 1.6 * 10-9 | |
| S572 | 2275 | F6-F1 | C-N | 2-1 | SLEEEWAQIEEVWGRSESFYDWFERQLG | 4.8 * 10-9 | |
| S573 | 2276 | F6-F1 | C-N | 2-1 | SLEEEWAQIESEVWGRSESFYDWFERQLG | 3.6 * 10-11 | +++ |
| S574 | 2277 | F6-F1 | C-N | 2-1 | SLEEEWAQIEAEVWGRGAPSESFYDWFERQLG | 9.2 * 10-12 | ++++ |
| S575 | 2278 | F6-F1 | C-N | 2-1 | SLEEEWAQIEAEVWGRAPSESFYDWFERQLG | | |
| S576 | 2279 | F6-F1 | C-N | 2-1 | SLEEEWAQIEAEVWGRGASESFYDWFERQLG | | |
| S577 | 2280 | F6-F1 | C-N | 2-1 | SLEEEWAQIEAEVWGRSESFYDWFERQLG | | |
| S578 | 2281 | F6-F1 | C-N | 2-1 | SLEEEWAQIECEVYGRGCSESFYDWFERQLG | | |
| S579 | 2282 | F6-F1 | C-N | 2-1 | SLEEEWAQIECEVYGRGCSESFYDWFERQLG | | |
| S580 | 2283 2284 | F6-F1 | C-N | 2-1 | SLEEEWAQVECEVYGRGC-βturn-ESFYDWFERQLG | 1.2 * 10-11 | ++++ |

TABLE 7-continued

| Pep-tide | SEQ ID NO: | Formula | Linkage | SiteIR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S581 | 2285 2286 | F6-F1 | C-N | 2-1 | SLEEEWAQIESEVWGR-βturn-ESFYDWFERQLG | 1.2 * 10-11 | +++ |
| S582 | 2287 | F6-F1 | C-N | 2-1 | SLEEEWAQIECEVWGRGCPKGFYGWFRRRG | 2.5 * 10-9 | |
| S583 | 2288 | F6-F1 | C-N | 2-1 | ELEEEWAQIECEVWGRGCPKGFYGWFRRRGK | | |
| S584 | 2289 | F6-F1 | C-N | 2-1 | SLEEEWAQIECEVWGRGCPKGFYGWFRRRRG | 9.3 * 10-9 | |
| S585 | 2290 | F6-F1 | C-N | 2-1 | SLEREWAQIECEVWGRGCSESFYDWFERQL | | |
| S586 | 2291 | F6-F1 | C-N | 2-1 | SLEEEWAQIECEVWGRGCPSESFYDWFERQL | | |
| S587 | 2292 | F6-F1 | C-N | 2-1 | ELEEEWAQIECEVWGRGCPKGFYGWFRRRRGK | | |
| S588 | 2293 2294 | F6-F1 | C-N | 2-1 | LEEEWAQVECEV-IodoTyr-GRGCSGSLDESFYDWFERQLG | 1.8 * 10-10 | ++++ |
| S589 | 2295 2296 | F6-F1 | C-N | 2-1 | LEEEWAQVECEVYGRGCSGSLDESFY-IodoTyr-DWFERQLG | | |
| S590 | 2297 2298 | F6-F1 | C-N | 2-1 | LEEEWAQIECEV-IodoTyr-GRGCSGSLDESFYDWFERQLG | 5.8 * 10-11 | +++++ |
| S591 | 2399 2300 | F6-F1 | C-N | 2-1 | LEEEWAQIECEVWGRGCSGSLDESF-IodoTyr-DWFERQLG | 1.3 * 10-10 | ++++ |
| S592 | 2301 | F6 | | 2 | SLEEEWAQIECEVWGRGCPSY | 1.7 * 10-9 | |
| S593 | 2302 | F6 | | 2 | SIEEEWAQIKCDVWGRGCPSY | 2.2 * 10-9 | |
| S594 | 2303 | F6-F1 | C-N | 2-1 | SLEEEWAQIECEVWGRCWHHSFYDWFERQL | 7.1 * 10-11 | +++++ |
| S595 | 2304 | F6-F1 | C-N | 2-1 | LEEEWAQIQREVWHSPASESFYDWFERQL | 6.2 * 10-10 | ++++ |
| S596 | 2305 | F6-F1 | C-N | 2-1 | SLEEEWAQIQHELYGPAESESFYDWFERQL | 4.5 * 10-11 | ++++ |
| S597 | 2306 | F6-F1 | C-N | 2-1 | Ac-SLEEEWAQIECEVYGRGCPSESFYDWFERQL | 8.5 * 10-12 | +++++++ |
| S600 | 2307 | F6-F1 | C-N | 2-1 | Ac-SIEEEWAQIKCDVWGRGSESFYDWFERQL | 7.6 * 10-12 | |
| S601 | 2308 | F6-F1 | C-N | 2-1 | SLEEEWAQIQEDLYGANHNESFYDWFERQL | 1.8 * 10-10 | |
| S602 | 2309 | F6-F1 | C-N | 2-1 | SLEEEWAQIQAEVYGNPNSESFYDWFERQL | 3.1 * 10-11 | |
| S603 | 2310 | F6-F1 | C-N | 2-1 | Ac-SLEEEWAQIQEDLYGANHNESFYDWFERQL | 1.5 * 10-11 | |
| S604 | 2311 | F6-F1 | C-N | 2-1 | SLEEEWAQIQCEVWGRGCWRRHFYDWFERQL | | |
| S605 | 2312 | F6-F1 | C-N | 2-1 | SLEEEWAQIQHELWPVEKGESFYDWFERQL | 9.4 * 10-11 | ++++ |
| S606 | 2313 | F6-F1 | C-N | 2-1 | SLEEEWAQIQCEVWGRGCPSESFYDWFERQL | 4.0 * 10-12 | ++++++++ |
| S607 | 2314 | F6-F1 | C-N | 2-1 | SLEEEWAQIQCKLYGRNCKESFYDWFERQL | | |
| S608 | 2315 | F6-F1 | C-N | 2-1 | SLEEEWAQIQCKVWGKCKESFYDWFERQL | | |
| S609 | 2316 | F6-F1 | C-N | 2-1 | SLEEEWAQIQCKLYGRNCKSESFYDWFERQL | | |
| S610 | 2317 | F6-F1 | C-N | 2-1 | SLEEEWAQIQCKLWGKNCKESFYDWFERQL | | |
| S611 | 2318 | F6-F1 | C-N | 2-1 | SLEEEWAQIECEVWGRGCPSESFYDWFERQLPK | | |
| S612 | 2319 | F6-F1 | C-N | 2-1 | HQLEEEWQAIQCELWGRGCPSESFYDWFERQL | | |
| S613 | 2320 | F6-F1 | C-N | 2-1 | HLEEEWSEIQCELWGRGCPSESFYDWFERQL | | |
| S614 | 2321 | F6-F1 | C-N | 2-1 | SLEFEWAQIECEVYGRGCPSEDFYDWFEAQLHA | | |
| S615 | 2322 | F6-F1 | C-N | 2-1 | Ac-SLEFEWAQIECEVYGRGCPSEDFYDWFEAQLHA | | |

TABLE 7-continued

| Pep-tide | SEQ ID NO: | Formula | Linkage | SiteIR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S616 | 2323 | F6-F1 | C-N | 2-1 | HQLEEEWQAIQCELWGRGCPSEDFYDWFEAQLHA | | |
| S617 | 2324 | F6-F1 | C-N | 2-1 | HLEEEWSEIQCELWGRGCPSEDFYDWFEAQLHA | | |
| S618 | 2325 | F6-F1 | C-N | 2-1 | HELEEEWKRIECELWGRGCPSEDFYDWFEAQLHA | | |
| S619 | 2326 | F6-F1 | C-N | 2-1 | Ac-HQLEEEWQAIQCELWGRGCPSEDFYDWFEAQLHA | | |
| S620 | 2327 | F6-F1 | C-N | 2-1 | Ac-HLEEEWSEIQCELWGRGCPSEDFYDWFEAQLHA | | |
| S621 | 2328 | F6-F1 | C-N | 2-1 | Ac-HELEEEWKRIECELWGRGCPSEDFYDWFEAQLHA | | |
| S622 | 2329 | F6-F1 | C-N | 2-1 | SLEEEWAQIECEVWGRGCPSESFYDWFERQLG | | |
| S623 | 2330 | F6-F1 | C-N | 2-1 | Ac-SLEEEWAQIECEVWGRGCPSESFYDWFERQLG | | |
| S624 | 2331 2332 | F6-F1 | C-N | 2-1 | SLEEEWAQVECEV-(3-iodo-Tyr)-GRGCPSGSLDESFYDWFE RQLG-NH2 | | |
| S625 | 2333 | E8 | | 1 | KVRGFQGGTVWPGYEWLRNAAKK | | |
| S626 | 2334 | F6-E8 | C-N | 2-1 | SLEEEWAQIECEVYGRGCPSVRGFQGGTVWPGYEWLRNAA | | |
| S627 | 2335 | F6-F1 | C-N | 2-1 | Ac-SLEEEWAQIQHELWPVEKGESFYDWFERQL | | |
| S628 | 2336 | F6-F1 | C-N | 2-1 | Ac-HGLEEEWAQIQHELWPVEKGESFYDWFEAQLHA | | |
| S629 | 2337 | F6-F1 | C-N | 2-1 | HLEEEWRQIQCELWGRGCPSESFYDWFERQL | | |
| S630 | 2338 | F6-F1 | C-N | 2-1 | Ac-HLEEEWRQIQCELWGRGCPSESFYDWFEAQLHA | | |
| S631 | 2339 | F6-F1 | C-N | 2-1 | HPLEEEWSQIQCELWGRGCPSESFYDWFERQL | | |
| S632 | 2340 | F6-F1 | C-N | 2-1 | Ac-HPLEEEWSQIQCELWGRGCPSESFYDWFEAQLHA | | |
| S633 | 2341 | F6-F1 | C-N | 2-1 | HGLEEEWAQIQCEVWGRGCPSESFYDWFEAQLHA | | |
| S634 | 2342 | F6-F1 | C-N | 2-1 | Ac-SLEEEWAQIQCEVWGRGCPSESFYDWFEAQLHA | | |
| S635 | 2343 | F6-F1 | C-N | 2-1 | Ac-SLEEEWAQIECEVYGRGCPSEDFYDWFEEQLHN | | |
| S636 | 2344 | F6-F1 | C-N | 2-1 | Ac-SLEEEWAQIQCEVWGRGCPSESFYDWFERQL | | |
| S637 | 2345 | F6-F2 | C-N | 2-1 | Ac-SLEEEWAQIECEVYGRGCPSDGFYNAIELLS | | |
| S638 | 2346 | F6-F1 | C-N | 2-1 | Ac-HGLEEEWAQIQCEVWGRGCQRPEPFYDWFEAQLHA | | |
| S639 | 2347 | F6-F1 | C-N | 2-1 | Ac-HGLEEEWAQIQCEVWGRGCPSESFYDWFEAQLHA | | |
| S640 | 2348 | F6 | | 2 | SLEEEWAQIQHELWPVEAGESY | | |
| S641 | 2349 | F6-F1 | C-N | 2-1 | Ac-SLEEEWAQIQAEVWGRGAPSESFYDWFEAQLHA | | |
| S642 | 2350 | F6-F1 | C-N | 2-1 | Ac-SLEEEWAQIQCEVWGRGCQRPEPFYDWFERQL | | |
| S643 | 2351 | F6-F1 | C-N | 2-1 | Ac-SLEEEWAQIQCELWGRGCPSESFYDWFERQL | | |
| S644 | 2352 | F6-F1 | C-N | 2-1 | SLEEEWAQHEEDVYHPPAESFYDWFERQL | | |
| S645 | 2353 | F6-F1 | C-N | 2-1 | Ac-HGLEEEWAQHEEDVYHPPAESFYDWFEAQLHA | | |
| S646 | 2354 | F6-F1 | C-N | 2-1 | Ac-SLEEEWAQIQCEVWGRGCHNHLPFYDWFERQL | | |

TABLE 7-continued

| Peptide | SEQ ID NO: | Formula | Linkage | SiteIR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|---|
| S647 | 2355 | F6-F1 | C-N | 2-1 | Ac-SLEEEWAQIQCEVWGRGCPSEPFYDWFAHDNGD | | |
| S648 | 2356 | F6-F1 | C-N | 2-1 | Ac-SLEEEWAQIQCEVWGRGCPSEAFYDWFAEQLDD | | |

7, 9, 12, 13, 14, 17, 19, 20, 21, 22, 23, and 24 represent specific chemical linkers (see Table 3); For FFC: 0 is no effect, + is agonist, - is antagonist. Peptides listed on 3 lines consist of two different peptides, linked N-N or C-C, either by chemical linkage or by being synthesized on the two branches of an amino acid with two amino groups such as, e.g., lysine. Acy = 1-amino-1-cyclohexanecarboxylic acid; Cha = cyclohexylalanine; Aib = 2-aminoisobutyric acid; Hyp = Hydroxyproline; Amino acids which are not capitalized are D-amino acids; Lig = Diaminopropionic acid with a 2-aminohydroxyacetyl group (CO-CH2-O-NH2) on the side chain amino group; Lig' = lysine with a 2-aminohydroxyacetyl group (CO-CH2-O-NH2) on the side chain amino group; Ald = an aldehyde group obtained by periodate oxidation of a serine, either N-terminal or attached to the side chain amino group of lysine.

[0342] Results further indicated that S175-S175 dimer peptides (Site 1-Site 1) were less agonistic than S175 monomer peptides (++vs. +++). S175-S175 dimer peptides having a C—N linkage were less agonistic or equally agonistic as compared to S175-S175 dimer peptides having C—C or N—N linkages. F8-F8 dimer peptides, like the parent monomer, showed no agonist activity.

[0343] Table 7 further indicates that, relative to peptide S519, a potent insulin mimetic, the alterations that are most influential in increasing receptor affinity and potency are: acetylation of the N-terminal amino group; replacing V at position 9 with I; replacing E at position 10 with Q; replacing Y at position 14 with W; and deleting the sequence GSLD at positions 21 to 24.

## Example 5

### Substrate Phosphorylation Assay (HIR Kinase)

[0344] WGA (wheat germ agglutinin)-purified recombinant human insulin receptor was mixed with either insulin or peptide in varying concentrations in substrate phosphorylation buffer (50 mM HEPES (pH 8.0), 3 mM $MnCl_2$, 10 mM $MgCl_2$, 0.05% Triton X-100, 0.1% BSA, 12.5 μM ATP). A synthetic biotinylated substrate peptide (Biotin-KSRGDYMTMQIG) was added to a final concentration of 2 μg/ml. Following a 1 hr incubation at RT, the reactions were stopped by the addition of 50 mM EDTA. The reactions were transferred to Streptavidin coated 96-well microtiter plates (NUNC, Cat. No. 236001) and incubated for 1 hr at RT. The plates were washed 3 times with TBS (10 mM Tris (pH 8.0), 150 mM NaCl).

[0345] Subsequently, a 2000-fold dilution of horseradish peroxidase (HRPO) conjugated phosphotyrosine antibody (Transduction Laboratories, Cat. No. E120H) in TBS was added. The plates were incubated for 30 min and washed 3 times with TBS. TMB (3,3',5,5'-tetramethylbenzidine; Kem-En-Tec, Copenhagen, Denmark) was added. One substrate from Kem-En-Tec was added. After 10-15 min, the reaction was stopped by the addition of 1% acetic acid. The absorbance, representing the extent of substrate phosphorylation, was measured in a spectrophotometer at a wavelength of 450 nM.

[0346] The results indicated that the potency of the Site 1-Site 2 dimer, peptide 539, was 0.1 to 1% of that of insulin in all assays tested (Table 8), and the dose-response curves (FIGS. **17**A-**17**B) had a shape similar to that of insulin dose-response curves, suggesting an insulin-like action mechanism. In addition, Site 1-Site 2 dimer peptides 537 and 538 were also active as specific insulin receptor antagonists (Table 8; FIGS. **16**A-**16**C). Notably, Site 2-Site 1 dimer peptide 539 was more active in the kinase assay than Site 1-Site 1 homodimer peptides 521 and 535 (FIGS. **19**A-**19**B), despite lower FFC potency (FIGS. **14**A-**14**C; FIGS. **17**A-**17**B). Similar results are shown in FIGS. **20**A-B and FIGS. **21**A-B. This data suggested that homodimer and heterodimer peptides used different mechanisms of action.

TABLE 8

| Pep. | Mon./ Link. | Sequence | SEQ ID NO: | Form | Site IR | HIR Kd (nM) | HIGF-1R kd (nM) | FFC Pot. (nM) | Kinase Pot. (nM) |
|---|---|---|---|---|---|---|---|---|---|
| HI | | | | na | na | | | | |
| HIGF-1R | | | | na | na | | | | |
| 521 | RP9-6aa-RP9 | MADYKDDDDKGSLDESFYDWF ERQLGKKGGSGGSGSLDESFY DWFERQLGKKAAA(ETAG)PG | 2112 | 1-1 | 1-1 | 25 | - | A 3 | 1400 |
| 535 | RP9-12aa- | MADYKDDDDKGSLDESFYDWF ERQLGKKGGSGGSGGSGGSGS | 2113 | 1-1 | 1-1 | 15 | - | A 2 | 1000 |

TABLE 8-continued

| Pep. | Mon./ Link. | Sequence | SEQ ID NO: | Form | Site IR | HIR Kd (nM) | HIGF-1R kd (nM) | FFC | Pot. (nM) | Kinase Pot. (nM) |
|---|---|---|---|---|---|---|---|---|---|---|
| | RP9 | LDESFYDWFERQLGKKAAA (ETAG)PG | | | | | | | | |
| 537 | RP9-6aa-D8 | MADYKDDDDKGSLDESFYDWF ERQLGKKGGSGGSWLDQEWAW VQCEVYGRGCPSAAA(ETAG) PG | 2114 | 1-6 | 1-2 | 0.092 | 980 | N | 10 | Inactive |
| 538 | RP9-12aa-D8 | MADYKDDDDKGSLDESFYDWF ERQLGKKGGSGGSGGSGGSWL DQEWAWVQCEVYGRGCPSAAA (ETAG)PG | 2115 | 1-6 | 1-2 | 0.080 | 710 | N | 10 | Inactive |
| 539 | D8-6aa-RP9 | MADYKDDDDKWLDQEWAWVQC EVYGRGCPSGGSGGSGSLDES FYDWFERQLGKKAAA(ETAG) PG | 2116 | 6-1 | 2-1 | 0.530 | 1500 | A | 10 | 110 |

A = agonist; N = antagonist; na = not applicable; Form. = formula; Mon. = constituent monomers; Link. = linker; Pot. = potency; HI and HIGF-1R are controls; All with tags at both ends; All dimers are linked C-N; Linker sequences are underlined.

## Example 6

### IR Autophosphorylation Assays

[0347] IR activation was assayed by detecting autophosphorylation of an insulin receptor construct transfected into 32D cells (Wang et al., 1993, *Science* 261:1591-1594; clone 969). The IR transfected 32D cells were seeded at $5\times10^6$ cells/well in 96-well tissue culture plates and incubated overnight at 37° C. Samples were diluted 1:10 in the stimulation medium (PRIM1640 with 25 nM HEPES pH 7.2) plus or minus insulin. The culture media was decanted from the cell culture plates, and the diluted samples were added to the cells. The plates were incubated at 37° C. for 30 min. The stimulation medium was decanted from the plates, and cell lysis buffer (50 mM HEPES pH 7.2, 150 mM NaCl, 0.5% Triton X-100, 1 mM AEBSF, 10 KIU/ml aprotinin, 50 μM leupeptin, and 2 mM sodium orthovanadate) was added. The cells were lysed for 30 min.

[0348] In the ELISA portion of the assay, the cell lysates were added to the BSA-blocked anti-IR unit mAb (Upstate Biotechnology, Lake Placid, N.Y.) coated ELISA plates. After a 2 hr incubation, the plates were washed 6 times with PBST and biotinylated anti-phosphotyrosine antibody (Upstate Biotechnology) is added. After another 2 h incubation, the plates were again washed 6 times. Streptavidin-Eu was then added, and the plates were incubated for 1 h. After washing the plates again, EG&G Wallac enhancement solution (100 mM acetone-potassium hydrogen pthalate, pH 3.2; 15 mM 2-naphtyltrifluoroacetate; 50 mM tri(n-octyl)-phosphine oxide; 0.1% Triton X-100) was added into each well, and the plates were placed onto a shaker for 20 min at RT. Fluorescence of samples in each well was measured at 615 nm using a VICTOR1420 Multilabel Counter (EG&G Wallac).

[0349] Alternatively, IR autophosphorylation was determined using a holoenzyme phosphorylation assay. In accordance with this assay, 1 μl of purified insulin receptor (isolated from a Wheat Germ Agglutinin Expression System) was incubated with 25 nM insulin, or 10 or 50 μM peptide in 50 μl autophosphorylation buffer (50 mM HEPES pH. 8.0, 150 mM NaCl, 0.025% Triton-X-100, 5 mM $MnCl_2$, 50 μM sodium orthovanadate) containing 10 μM ATP for 45 min at 22° C. The reaction was stopped by adding 50 μl of gel loading buffer containing β-mercaptoethanol (Bio-Rad Laboratories, Inc., Hercules, Calif.). The samples were run on 4-12% SDS-polyacrylamide gels. Western Blot analysis was performed by transferring the proteins onto nitrocellulose membrane. The membrane was blocked in PBS containing 3% milk overnight. The membrane was incubated with anti-phosphotyrosine 4G10 HRP labeled antibody (Upstate Biotechnology) for 2 h. Protein bands were visualized using SuperSignal West Dura Extended Duration Substrate Chemiluminescence Detection System (Pierce Chemical Co.).

## Example 7

### Fluorescence-Based HIR Binding Assays

[0350] A. Time-Resolved Fluorescence Resonance Energy Transfer Assays

[0351] Time-resolved fluorescence resonance energy transfer assays (TR-FRET) were used for peptide competition studies. In one set of assays, monomer and dimer peptides were tested for the ability to compete with biotinylated RP-9 monomer peptide (b-RP9) for binding to HIR-immunoglobulin heavy chain chimera (sIR-Fc; Bass et al., 1996). The assays were performed using a 384-well white microplate (NUNC) with a final volume of 30 μl. Final incubation conditions were in 22 nM b-RP9, 1 nM SA-APC (streptavidin-allophycocyanin), 1 nM $Eu^{3+}$-sIR-Fc (LANCE™ labeled, PE Wallac, Inc.), 0.05 M Tris-HCl (pH

8 at 25° C.), 0.138 M NaCl, 0.0027 M KCl, and 0.1% BSA (Cohn Fraction V). After 16-24 hr of incubation at RT, the fluorescence signal at 665 nm and 620 nm was read on a Victor$^2$ 1420 plate reader (PE Wallac, Inc.). Primary data were background corrected, normalized to buffer controls, and then expressed as percent of specific binding.

[0352] Results are shown in FIGS. **22**A-**22**B. FIG. **21**A shows b-RP9 competition data. For these figures, the Z'-factor was greater than 0.5 ($Z'=1-(3\sigma_+ + 3\sigma_-)/|\mu_+ - \mu_-|$; Zhang et al., 1999, *J. Biomol. Screen.* 4:67-73), and the signal-to-background (S/B) ratio was ~4-5. In FIG. **22**A, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis of the data according to the following formula: y=min+(max−min)/(1+10^((logIC$_{50}$−x)*Hillslope)). This was used to determine $IC_{50}$ values.

[0353] In another set of assays, monomer and dimer peptides were tested for the ability to compete with biotinylated-S175 (b-S175) or b-RP9 for binding to sIR-Fc. The TR-FRET assays were performed in a 384-well white microplate with a final volume of 30 μl. Final incubation conditions were in 33 nM b-S175 or 22 nM b-RP9, 1 nM SA-APC, 1 nM $Eu^{3+}$-sIR-Fc, 0.05 M Tris-HCl (pH 8 at 25° C.), 0.138 M NaCl, 0.0027 M KCl, and 0.1% BSA. After 16-24 hr of incubation at RT, the fluorescence signal at 665 nm and 620 nm was read on a Victor$^2$ 1420 plate reader. Primary data were background corrected, normalized to buffer controls, and then expressed as % Specific Binding.

[0354] Results are shown in FIGS. **23**A-**23**B. For these figures, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis of the data, which was used to determine $IC_{50}$ values. FIG. **23**A shows b-S175 competition data; FIG. **23**B shows b-RP9 competition data.

[0355] B. Fluorescence Polarization Assays

[0356] Fluorescence polarization assays (FP) were used for peptide competition studies. In one set of assays monomer and dimer peptides were tested for the ability to compete with fluorescein-RP-9 (FITC-RP9) for binding to soluble HIR ectodomain (sIR; Kristensen et al., 1998, *J. Biol. Chem.* 273:17780-17786). The assays were performed in a 384-well black microplate (NUNC) with a final volume of 30 μl. Final incubation conditions were 1 nM FITC-RP9, 10 nM sIR, 0.05 M Tris-HCl (pH 8 at 25° C.), 0.138 M NaCl, 0.0027 M KCl, 0.05% BGG (bovine gamma globulin), 0.005% Tween-20. After 16-24 hr of incubation at RT, the fluorescence signal at 520 nm was read on an Analyst™ AD plate reader (LJL BioSystems, Inc.). Primary data were background corrected using 10 nM sIR without FITC-RP9 addition, normalized to buffer controls, and then expressed as percent of specific binding. The Z'-factor was greater than 0.5 and the assay dynamic range was ~125 mP. In FIGS. **24**-**27**, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis of the data, which was used to determine $IC_{50}$ values. The Z'-factor was greater than 0.5 and the assay dynamic range was ~125 mP. Results are shown in FIGS. **24**A-**24**B.

[0357] In another set of assays, monomer and dimer peptides were tested for the ability to compete with FITC-RP9 for binding to soluble human insulin mini-receptor (mIR; Kristensen et al., 1999, *J. Biol. Chem.* 274:37351-37356). The FP assays were performed in a 384-well black microplate with a final volume of 30 μl. Final incubation conditions were 2 nM FITC-RP9, 20 nM mIR, 0.05 M Tris-HCl (pH 8 at 25° C.), 0.138 M NaCl, 0.0027 M KCl, 0.001% BGG, 0.005% Tween-20. After 16-24 hr of incubation at RT, the fluorescence signal at 520 nm was read on an Analyst™ AD plate reader. Primary data were background corrected using 20 nM mIR without FITC-RP9 addition, normalized to buffer controls and then expressed as percent of specific binding. Results are shown in FIGS. **25**A-**25**B.

[0358] Monomers and dimer peptides were also tested for the ability to compete with fluorescein-insulin (FITC-Insulin) for binding to sIR. The FP assays were performed in a 384-well black microplate with a final volume of 30 μl. Final incubation conditions were in 2 nM FITC-Insulin, 20 nM sIR, 0.05 M Tris-HCl (pH 8 at 25° C.), 0.138 M NaCl, 0.0027 M KCl, 0.05% BGG, 0.005% Tween-20. After 16-24 hr of incubation at RT, the fluorescence signal at 520 nm was read on an Analyst™ AD plate reader. Primary data were background corrected using 20 nM sIR without FITC-Insulin addition, normalized to buffer controls and then expressed as percent of specific binding. Results are shown in FIGS. **26**A-**26**B.

[0359] In other assays, peptide monomers and dimer peptides were tested for the ability to compete with FITC-Insulin for binding to mIR. The FP assays were performed in a 384-well black microplate with a final volume of 30 μl. Final incubation conditions were 2 nM FITC-Insulin, 20 nM mIR, 0.05 M Tris-HCl (pH 8 at 25° C.), 0.138 M NaCl, 0.0027 M KCl, 0.05% BGG (bovine gamma globulin), 0.005% Tween-20. After 16-24 hr of incubation at RT, the fluorescence signal at 520 nm was read on an Analyst™ AD plate reader. Primary data were background corrected using 20 nM mIR without FITC-RP9 addition, normalized to buffer controls and then expressed as % Specific Binding. Results are shown in FIGS. **27**A-**27**B.

## SUMMARY

[0360] Table 9, below, summarizes the binding data calculated from competition assays using the IR constructs, sIR-Fc, sIR, and mIR, in TR-FRET and FP formats. The data in Table 9 indicate that most dimer peptides (e.g., S291 and S375 or S337), showed greater agonist activity than the corresponding monomer peptides (e.g., H2C or RP9, respectively) in the FFC assay. It was previously demonstrated that an inequality between monomer peptides and insulin was exhibited in competition assays where the assay reporter was a monomer peptide (i.e., RP9 or S175). This inequality was also demonstrated by dimer peptides as seen in Table 9. Table 9 further shows that Group 6 monomer peptides such as E8 (D120) were able to compete with FITC-RP9 or b-RP9 peptides for binding to sIR-Fc, but did not compete peptide ligands, such as FITC-RP9 for binding to mIR. Experiments using different IR constructs thereby allowed differentiation of Site I peptides based on sequence motifs (i.e., Group 6 (Formula 10) vs. Group 1 (Formula 1; A6)).

TABLE 9

| Monomer or Dimer | SEQ ID NO: | Link-age | TARGET□ Label□ Sequence | sIR-Fc b-S175 FRET IC50 (nM) | sIR-Fc b-S175 FRET Hill | sIR-Fc b-RP9 FRET IC50 (nM) | sIR-Fc b-RP9 FRET Hill | sIR-Fc FITC-RP9 FP IC50 (nM) | sIR-Fc FITC-RP9 FP Hill | sIR FITC-RP9 FP IC50 (nM) | sIR FITC-RP9 FP Hill | mIR FITC-RP9 FP IC50 (nM) | mIR FITC-RP9 FP Hill | HIR $^{125}$I-insulin RRA IC50 (nM) | HIR $^{125}$I-insulin RRA FFC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| H2C | 2117 | | **FHENFYDWFVQRVSKK** | 410 | -0.82 | 626 | -1.03 | 50 | -0.27 | 37 | -0.49 | 770 | -0.89 | 700 | + |
| S291 | 1916 and 1917 | N-N | **(Lig-GGG-H2C)$_2$**-9 | 81 | -0.96 | 250 | -0.69 | | | 12 | -0.35 | 668 | -0.38 | 1200 | ++++ |
| RP9 | 1558 | | **GSLDESFYDWFERQLGKK** | 6 | -0.45 | 42 | -0.69 | 10 | -0.41 | 0.03 | -0.29 | 49 | -0.53 | 44 | +/0 |
| S375 | 1994 | C-N | **(RP9-Lig)-14-(RP9-Lig)** | 7 | -0.80 | 86 | -0.67 | | | 0.2 | -0.22 | 91 | -0.80 | 200 | ++++ |
| S337 | 1960 and 1961 | C-C | **(RP9-Lig)$_2$**-23 | 0.2 | -0.36 | 14 | -0.57 | 1 | -0.37 | 0.2 | -0.28 | 111 | -0.70 | 11 | +++++ |
| S391 | 2008 | | **truncated-(-GSLDE)RP9(-KK)** | 59 | -0.59 | 610 | -0.56 | | | 119 | -0.49 | 284 | -0.77 | 1500 | NN |
| S390 | 1794 | | **truncated(-GSLD)RP9(-KK)** | 27 | -0.49 | 127 | -0.49 | | | 19 | -0.64 | 94 | -0.94 | 620 | + |
| S414 | 2015 and 2016 | C-C | **(truncated(-GSLD)RP9(-KK))$_2$**-14 | 92 | -0.62 | 164 | -0.73 | | | 0.2 | -0.25 | 151 | -0.69 | NN | NN |
| S175 | 1560 | | **GRVDWLQRNANFYDWFVAELG** | 22 | -0.58 | 64 | -0.74 | 10 | -0.56 | 1 | -0.36 | 167 | -1.72 | 230 | +++ |
| S380 | 2001 and 2002 | C-C | **(EE-short-S175-Lig)$_2$**-9 10 | -0.55 | 23 | -0.64 | | | 0.5 | -0.29 | 27 | -0.49 | 510 | ++ | |
| E8 (D120) | 2118 | | **GGTVWPGYEWLRNA** | 755 | -0.74 | | | 207 | -0.49 | | >100000 | | | 2200 | - |
| Insulin | | | | 59 | -0.37 | 63 | -0.46 | >100000 | -0.25 | 1250 | — | 172 | -0.78 | 0.04 | +++++ |

FRET = Time-Resolved Fluorescence Resonance Energy Transfer Assay; FP = Fluorescence Polarization Assay; RRA = Radio-Receptor Assay; FFC = Free Fat Cell Assay; N-N = N-terminal linkage; C-C = C-terminal linkage;; All are site 1 (formula 1) monomers or site 1-site 1 (formula 1-formula 1) dimers;

[0361] Based on the functional studies outlined above, the following peptide dimers were designed.

| SEQ ID NO: | Monom./ Linkers | Sequence |
|---|---|---|
| 2119 | F8-6aa-RP9 | HLCVLEELFWGASLFGYCSG**GGSGGS**GSLDESFYDWFERQL |
| 2120 | F8-12aa-RP9 | HLCVLEELFWGASLFGYCSG**GGSGGSGGSGGS**GSLDESFYDWFERQL |
| 2121 | D8-6aa-S175 | WLDQEWAWVQCEVYGRGCPS**GGSGGS**GRVDWLQRNANFYDWFVAELG |
| 2122 | D8-12aa-S175 | WLDQEWAWVQCEVYGRGCPS**GGSGGSGGSGGS**GRVDWLQRNANFYDWFVAELG |
| 2123 | F8-6aa-S175 | HLCVLEELFWGASLFGYCSG**GGSGGS**GRVDWLQRNANFYDWFVAELG |
| 2124 | F8-12aa-S175 | HLCVLEELFWGASLFGYCSG**GGSGGSGGSGGS**GRVDWLQRNANFYDWFVAELG |
| 2125 | D8-6aa-RP15 | HLCVLEELFWGASLFGYCSG**GGSGGS**SQAGSAFYAWFDQVLRTV |
| 2126 | D8-6aa-RP6 | HLCVLEELFWGASLFGYCSG**GGSGGS**TFYSCLASLLTGTPQPNRGPWERCR |
| 2127 | D8-6aa-RP17 | HLCVLEELFWGASLFGYCSG**GGSGGS**QSDAFYSGLWALIGLSDG |
| 2128 | D8-6aa-Grp 6 | HLCVLEELFWGASLFGYCSG**GGSGGS**DSDWAGYEWFEEQLD |

[0362] Linker sequences are underlined and in bold; Monomer sequences are shown below; All dimers are linked C—N.

| SEQ ID NO: | Monomer | Formula | Site | Sequence |
|---|---|---|---|---|
| 1576 | F8 | 4 | 2 | HLCVLEELFWGASLFGYCSG |
| 1558 | RP9 | 1 | 1 | GSLDESFYDWFERQL |
| 2129 | D8 | 6 | 2 | WLDQEWAWVQCEVYGRGCPS |
| 1560 | S175 | 1 | 1 | GRVDWLQRNANFYDWFVAELG |
| 2130 | RP15 | 1 | 1 | SQAGSAFYAWFDQVLRTV |
| 1635 | Rp6 | 2 | 1 | TFYSCLASLLTGTPQPNRGPWERCR |
| 2131 | RP17 | 1 | 1 | QSDAFYSGLWALIGLSDG |
| 1595 | Group 6 | 10 | 1 | DSDWAGYEWFEEQLD |

## Example 8

### Peptide Fusions to the Maltose Binding Protein

[0363] A. Cloning

[0364] The transfer of interesting peptide sequences from phage display to maltose binding protein (MBP) fusions is desirable for several reasons. First, to obtain a more sensitive affinity estimate, the polyvalency of phage display peptides should be converted to a monovalent system. For this purpose, the peptide sequences are fused to MBP that generally exists as a monomer with no cysteine residues. Second, competition experiments can be carried out with the same or different peptides, one phage displayed and the other fused to MBP. Lastly, purified peptides can be obtained by cleavage of the fusion protein at a site engineered in the DNA sequence.

[0365] FIG. **28** shows a schematic drawing of the MBP-peptide construct. In the construct, the N-terminus of the peptide sequence is fused to the C-terminus of the MBP. Two peptide-flanking epitope tags are included, a shortened-FLAG® at the N-terminus and E-Tag at the C-terminus. The corresponding gene fusion was generated by ligating a vector fragment encoding the MBP in frame with a PCR product encoding the peptide of interest. The vector fragment was obtained by digesting the plasmid pMAL-c2 (New England Biolabs) with EcoRI and HindIII and then treating the fragment with shrimp alkaline phosphatase (SAP; Amersham). The digested DNA fragment was resolved on a 1% agarose gel, excised, and purified by QIAEXII (QIAGEN). The 20-amino acid peptide sequences of interest were initially encoded in the phage display vector pCANTAB5E (Pharmacia). To obtain these sequences, primers were synthesized which anneal to sequences encoding the shortened FLAG® or E-Tag epitopes and also contain the required restriction enzyme sites EcoRI and HindIII. PCR products were obtained from individual phage clones and digested with restriction enzymes to yield the insert fragment. The vector and insert were ligated overnight at 15° C. The ligation product was purified using QIAquick spin columns (QIAGEN) and electroporations were performed at 1500 v in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 10 ng of DNA and 40 μl of *E. coli* strain ER2508 (RR1 lon:miniTn10($Tet^r$) (malB) (argF-lac)U169 $Pro^+$ zjc::Tn5($Kan^r$) fhuA2) electrocompetent cells (New England Biolabs). Immediately after the pulse, 1 ml of pre-warmed (40° C.) 2xYT medium containing 2% glucose (2xYT-G) was added and the transformants were grown at 37° C. for 1 h. Cell transformants were plated onto 2xYT-AG plates and grown overnight at 37° C. Sequencing confirmed the clones contained the correct constructs.

[0366] C. Small-Scale Expression of Soluble MBP-Peptide Fusion Proteins

[0367] *E. coli* ER2508 (New England Biolabs) carrying the plasmids encoding MBP-peptide fusion proteins were grown in 2xYT-AG at 37° C. overnight (250 rpm). The following day the cultures were used to inoculate media (2×YT containing-G) to achieve an $OD_{600}$ of 0.1. When the cultures reached an $OD_{600}$ of 0.6, expression was induced by the addition of IPTG to a final concentration of 0.3 mM and then cells were grown for 3 h. The cells were pelleted by centrifugation and samples from total cells were analyzed by SDS-PAGE electrophoresis. The production of the correct molecular weight fusion proteins was confirmed by Western blot analysis using the monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia).

[0368] Large-Scale Expression of Soluble MBP-Peptide Fusion Proteins

[0369] *E. coli* ER2508 carrying plasmids encoding the MBP-peptide fusion proteins were grown in 2xYT-AG media for 8 h (250 rpm, 37° C.). The cultures were subcultured in 2xYT-AG to achieve an $OD_{600}$ of 0.1 and grown at 30° C. overnight. This culture was used to inoculate a fermentor with medium of following composition (g/l): glucose (3.00); $(NH_4)_2SO_4$ 5.00; $MgSO_4*7H_2O$ (0.25); $KH_2PO_4$ (3.00); citric acid (3.00); peptone (10.00); and yeast extract (5.00); pH 6.8.

[0370] The culture was grown at 700 rpm, 37° C. until the glucose from the medium was consumed ($OD_{600}$=~6.0-7.0). Expression of the fusion protein was induced by the addition of 0.3 mM IPTG and the culture was grown for 2 h in fed-batch mode fermentation with feeding by 50% glucose at a constant rate of 2 g/l/h. The cells were removed from the medium by centrifugation. Samples of the cell pellet were analyzed by SDS-PAGE followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product.

[0371] Purification

[0372] The cell pellets were disrupted mechanically by sonication or chemically by treatment with the mild detergent Triton X-100. After removal of cell debris by centrifugation, the soluble proteins were prepared for chromatographic purification by dilution or dialysis into the appropriate starting buffer. The MBP fusions were initially purified either by amylose affinity chromatography or by anion exchange chromatography. Final purification was performed using anti-E-Tag antibody affinity columns (Pharmacia). The affinity resin was equilibrated in TBS (0.025 M Tris-buffered saline, pH 7.4) and the bound protein was eluted with Elution buffer (100 mM glycine, pH 3.0). The purified proteins were analyzed for purity and integrity by SDS-PAGE and Western blot analysis according to standard protocols.

[0373] For MBP fusions, IR agonist activity was observed for the Site 1-Site 1 dimer peptides shown in Table 10, below. Additional binding data for the MBP fusions are shown in Table 11, also below.

TABLE 10

| Fus | Monomer/ Linker | Sequence | SEQ ID NO: | Form. | Act. | Site IR | Fus. Conc. | MW (kDa) | $K_d$ (HIR) |
|---|---|---|---|---|---|---|---|---|---|
| 426 | D8 | MBP . . . NNNNLGIEGRISEFIEGR AQPAMA WLDQEWAWVQCEVYGRGCPSAAA(ETAG)AA | 2132 | 6 | N | 2 | 0.76 | 52.2 | $1.4 \times 10^{-6}$ |
| 429 | D8-6AA-D8 | MBP . . . NNNNLGIEGRISEFIEGRAQPAMAWLDQE WAWVQCEVYGRGCPSGGSGGSKWLDQEWAWVQCEVYGRG CPSAAA(ETAG)AA | 2133 | 6-6 | N-N | 2-2 | 3.2 | 55.3 | $1.3 \times 10^{-6}$ |
| 430 | H2C-4aa-RB6 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDKFHE NFYDWFVRQVSGSGSLDALDRLMRYFEERPSLETAG | 2134 | 1-6 | A- | 1-1 | 0.17 | 54.5 | $2.1 \times 10^{-6}$ |
| 431 | H2C-6aa-F8 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDKFHEN FYDWFVRQVSGGSGGSHLCVLEELFWGASLFGYCSGAAA (ETAG)AA | 2135 | 1-4 | A-N | 1-2 | 3.3 | 54.8 | $4.7 \times 10^{-8}$ |
| 432 | H2C-12aa-F8 | MBP-NNNNLGIEGRISEFIEGRDYKDDDKFHENFYDWFV RQVSGGSGGSGGSGGS HLCVLEELFWGASLFGYCSGAA A(ETAG)AA | 2136 | 1-4 | A-N | 1-1 | 2.9 | 55.5 | $3.5 \times 10^{-8}$ |
| 433 | H2C-9aa-F8 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDKFHEN FYDWFVRQVSGGSGGSGGSHLCVLEELFWGASLFGYCSG AAA(ETAG)AA | 2137 | 1-4 | A-N | 1.2 | 2.8 | 55.2 | $2.1 \times 10^{-8}$ |
| 434 | G3-12aa-G3 | MBP . . . NNNNLGIEGRISEFIEVRAQPAMARGGGT FYEWFESALRKHGAGGGSGGSGGSGGSRGGGTFYEWFES ALRKHGAGAAA(ETAG)AA | 2138 | 1-1 | N-N | 1-1 | 0.01 | 56 | $3.2 \times 10^{-6}$ |
| 436 | H2C-9aa-H2C | MBP . . . NNNNLGIEGRISEFIEGRAQPAMAFHENF YDWFVRQVSGGSGGSGGSFHENFYDWFVRQVSAAA (ETAG)AA | 2139 | 1-1 | A | 1-1 | 1.1 | 54.2 | $4.1 \times 10^{-7}$ |
| 437 | H2C | MBP . . . NNNNLGIEGRISEFIEGRAQPAMA FHENFYDWFVRQVSAAA(ETAG)AA | 2140 | 1 | N-N | 1 | 0.3 | 51.5 | $8.3 \times 10^{-6}$ |
| 427 | G3-6aa-G3 | MBP . . . NNNNLGIEGRISEFIEGRAQPAMARGGGT FYEWFESTLRKHGAGGGSGGSRGGGTFYEWFESALRKHG AGAAA(ETAG)AA | 2141 | 1-1 | N-N | 1-1 | 0.02 | 55.36 | $3.3 \times 10^{-6}$ |
| 435 | H2C-3aa-H2C-3aa-H2C | MBP . . . NNNNLGIEGRISEFIEGRAQPAMAFHENF YDWFVRQVSGGSFHENFYDWFVRQVSGGSFHENFYDWFV RQVSAAA(ETAG)AA | 2142 | 1-1-1 | A-A-A | 1-1-1 | 2.1 | 55.5 | $2.0 \times 10^{-6}$ |
| 439 | H2C-6aa-H2C | MBP . . . NNNNLGIEGRISEFIEGRAQPAMAFHENF YDWFVRQVSGGSGGSFHEN FYDWFVRQVS(ETAG)AA | 2143 | 1-1 | A-A | 1-1 | 1.4 | 53.9 | $5.5 \times 10^{-7}$ |

TABLE 10-continued

| Fus | Monomer/ Linker | Sequence | SEQ ID NO: | Form. | Act. | Site IR | Fus. Conc. | MW (kDa) | $K_d$ (HIR) |
|---|---|---|---|---|---|---|---|---|---|
| 449 | H2C-12aa-H2C | MBP . . . NNNNLGIEGRISEFIEGRAQPAMAFHENF YDWFVRQVSGGSGGSGGSGGSAQPAMAFHENFYDWFVRQ VSAAA(ETAG)AA | 2144 | 1-1 | | 1-1 | 1.5 | 51.8 | $6.2 \times 10^{-7}$ |
| 452 | G3 | MBP . . . NNNNLGIEGRISEFIEGRAQPAMARGGGT FYEWFESALRKHGAGAAA(ETAG)AA | 2145 | 1 | | 1 | 0.15 | 48.8 | $7.8 \times 10^{-7}$ |
| 463 | H2C-3aa-H2C | MBP . . . NNNNLGIEGRISEFIEGRAQPAMAFHENF YDWFVRQVSGGSFHENFYDWFVRQVSAAA(ETAG)AA | 2146 | 1-1 | A-A | 1-1 | 1.8 | 50.1 | $9.6 \times 10^{-7}$ |
| 464 | LF-H2C | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDK FHENFYDWFVRQVSAA(ETAG)AA | 2147 | 1 | | 1 | 0.045 | 48.4 | $3.9 \times 10^{-8}$ |
| 446 | LF-F8 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDKHLC VLEELFWGASLFGYCSGAAA(ETAG)AA | 2148 | 1 | | 2 | 1.9 | 49.1 | $7.7 \times 10^{-7}$ |
| 459 | SF-RB6 | MBP . . . NNNNLGIEGRISEFGSADYKDLDALDRLM RYFEERPSLAAA(ETAG)AA | 2149 | 3 | | 1 | 0.069 | 48.1 | $7.7 \times 10^{-8}$ |
| MBP*IacZ | | ** | | na | | | 5.1 | 50 | $>1 \times 10^{-5}$ |

*MBP (negative control for the fusions) is fused to a small fragment of beta-galactosidase (IacZ); **MBP-IacZ fusion protein was derived from the plasmid pMal-c2 as purchased form NEB. Fus. = fusion; Act. = activity; Conc. = concentration; N = Antagonist; A = Agonist; LF = Long FLAG ® epitope (DYKDDDDK; SEQ ID NO:1777); SF = Short FLAG ® epitope (DYKD; SEQ ID NO:1545); na = not applicable; Form. = formula; All dimers are linked C-N; Linker sequences are underlined.

[0374]

TABLE 11

| Fusion | Monomer/ Linker | Sequence | SEQ ID NO: | Form | Site IR | High conc. tested ($\mu$M) | Kd (HIR) $\mu$M |
|---|---|---|---|---|---|---|---|
| 431- | H2C-6aa-F8 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDKFHENFYDWFVRQVSGGSG GSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA | 2150 | 1-6 | 1-2 | 0.2 | 0.033 |
| 431+ | H2C-6aa-F8 | DYKDDDKFHENFYDWFVRQVSGGSGGSHLCVLEELFWGASLFGYCS GAAA(ETAG)AA | 2151 | 1-6 | 1-2 | 0.2 | 0.0074 |
| 432- | H2C-12aa-F8 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDKFHENFYDWFVRQVSGGSG GSGGSGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA | 2152 | 1-6 | 1-2 | 0.2 | 0.02 |
| 432+ | H2C-12aa-F8 | DYKDDDKFHENFYDWFVRQVSGGSGGSGGSGGSHLCVLEELFWGAS LFGYCSGAAA(ETAG)AA | 2153 | 1-6 | 1-2 | 0.2 | 0.0038 |
| 433- | H2C-9aa-F8 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDKFHENFYDWFVRQVSGGSG GSGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA | 2154 | 1-6 | 1-2 | 0.2 | 0.03 |
| 433+ | H2C-9aa-F8 | DYKDDDK FHENFYDWFVRQVSGGSGGSGGS HLCVLEELFWGASLFGYCSGAAA(ETAG)AA | 2155 | 1-6 | 1-2 | 0.2 | 0.004 |

The concentrations of these fusions vary depending on the expression quality. There are 2 sets of each fusion: uncleaved (-) and cleaved with factor Xa (+). The fusion proteins are in Tris buffer (20 mM Tris, 200 mM NaCI, 1 mM EDTA, 50 mM maltose, pH 7.5) and the cleaved fusions (+) are in the same Tris buffer (500 $\mu$l) + 12 $\mu$g Factor Xa. (Source of Factor Xa: New England Biolabs). Conc. = concentration; Form. = formula; All dimers are linked C-N; Linker sequences are underlined.

[0375] The A7 (20A4), D8, and D10 peptide sequence are shown in FIGS. **8** and **9**A-**9**B. The 447 peptide sequence is: LCQRLGVGWPGWLSGWCA (SEQ ID NO:2156).

TABLE 13

BIAcore Results - Synthetic peptides compete for binding to IR

| Incubation Mix | % Binding | Result (RUs) | Sequence Type |
|---|---|---|---|
| IR | 100 | 128 | Positive control |
| IR + 20D1 | 41 | 51.8 | Formula 1 Motif |
| IR + D8 | 33 | 41.6 | Formula 6 Motif |
| IR + 20C11 | 38 | 49 | Formula 2 Motif (bkg high) |
| IR + H2 | 27 | 34.6 | IGF (phosphorylated band) |
| IR + 2A9 | 100 | 128 | IGF(bkg high) |
| IR + 20A4 | 33 | 41.8 | Formula 6 Motif |
| IR + p53wt | 97 | 124.5 | P53 wild type |

The concentration of each peptide was about 40 μM and the concentration of IR was 450 nM.
The 20D1, 20A4, and D8 peptide sequences are shown in FIGS. 8 and 9A-9B.
The remaining peptide sequences are as follows: 447 = LCQRLGVGW-PGWLSGWCA (SEQ ID NO: 2156); 2A9 = LCQSWGVRIGWLTGLCP (SEQ ID NO: 2157); 20C11 = DRAFYNGLRDLVGAVYGAWD (SEQ ID NO: 1659); H2 = VTFTSAVFHENFYDWFVRQVS (SEQ ID NO: 1784).

[0376] Regarding preparation of a Site 1 agonist comprising two D117 (H2C) peptides, a linker of only 3 amino acids (12 Å) provided a ligand of greater affinity for Site 1 of IR than a corresponding ligand prepared with a 9 amino acid (36 Å) linking region (FIG. **29**).

[0377] Stimulation of Autophosphorylation of IR by MBP-Fusion Peptides

[0378] MBP fusion peptides were prepared as described above, and then assayed for autophosphorylation of a insulin receptor construct transfected into 32D cells (Wang et al., 1993; clone 969) (see Example, above). The results of these experiments shown in FIG. **30** indicate that the H2C monomer and H2C-H2C homodimer peptides stimulate autophosphorylation of IR in vivo. H2C dimer peptides (Site 1-Site 1) with a 6 amino acid linker (H2C-6aa-H2C) were most active in the autophosphorylation assay. Other active dimer peptides are also shown in FIG. **30**, particularly H2C-9aa-H2C, H2C-12aa-H2C, H2C-3aa-H2C, and F8.

[0379] Insulin Receptor Binding Affinity and Fat Cell Potency of MBP-Fusion Peptides

[0380] Results of assays to determine binding affinity for insulin receptor and fat cell potency of the MBP-fusion peptides are shown in Table 14, below.

TABLE 14

| Peptide | SEQ ID NO: | Formula | Site IR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|
| RB426 | 2158 | F6 | 2 | MBP . . . NNNNLGIEGRISEFIEGRAQPAMAWLDQEWAWVQCEVYGRGCPSAAA (ETAG)AA | 1.4 * 10-8 | |
| RB429 | 2159 | F6-F6 | 2-2 | MBP . . . NNNNLGIEGRISEFIEGRAQPAMAWLDQEWAWVQCEVYGRGCPSGGSGG SKWLDQEWAWVQCEVYGRGCPSAAA(ETAG)AA | 1.3 * 10-8 | |
| RB505M | 2160 | F4 | 2 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDK HLCVLEELFWGASLFGYCSGAA A(ETAG)AA | | |
| RB517M | 2161 | F4-F4 | 2-2 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDK HLCVLEELFWGASLFGYCSGGG SGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA | | |
| RB515 | 2162 | F4-F4 | 2-2 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDK HLCVLEELFWGASLFGYCSGGG SGGSGGSGGSH LCVLEELFWGASLFGYCSGAAA(ETAG)AA | | |
| RB510 | 2163 | F4-F4-F4 | 2-2-2 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDK HLCVLEELFWGASLFGYCSGGG SGGSHLCVLEELFWGASLFGYCSGGGSGGS HLCVLEELFWGASLFGYCSGAAA (ETAG)AA | | |
| RB437 | 2164 | F1 | 1 | MBP . . . NNNNLGIEGRISEFIEGRAQPAMA FHENFYDWFVRQVSAAA(ETAG) AA | 8.3 * 10-8 | |
| RB463 | 2165 | F1-F1 | 1-1 | MBP . . . NNNNLGIEGRISEFIEGRAQPAMAFHENFYDWFVRQVSGGSFHENFYDW FVRQVSAAA(ETAG)AA | 9.6 * 10-7 | |
| RB439 | 2166 | F1-F1 | 1-1 | MBP . . . NNNNLGIEGRISEFIEGRAQPAMA FHENFYDWFVRQVSGGSGGSFHEN FYDWFVRQVS-ETAG | 5.5 * 10-7 | |
| RB436 | 2167 | F1-F1 | 1-1 | MBP . . . NNNNLGIEGRISEFIEGRAQPAMAFHENFYDWFVRQVSGGSGGSGGSFH ENFYDWFVRQVSAAA(ETAG)AA | 4.1 * 10-7 | |
| RB449 | 2168 | F1-F1 | 1-1 | MBP . . . NNNNLGIEGRISEFIEGR AQPAMAFHENFYDWFVRQVSGGSGGSGGSG GSAQPAMAFHENFYDWFVRQVSAAA(ETAG)AA | 6.2 * 10-7 | |
| RB435 | 2169 | F1-F1-F1 | 1-1-1 | MBP . . . NNNNLGIEGRISEFIEGRAQPAMAFHENFYDWFVRQVSGGSFHENFYDW FVRQVSGGSFHENFYDWFVRQVSAAA(ETAG)AA | 2.0 * 10-8 | |
| RB502 | 2170 | F1 | 1 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDKVRVDWLQRNANFYDWFVAELVAA A(ETAG)AA | | |

TABLE 14-continued

| Peptide | SEQ ID NO: | Formula | Site IR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|
| RB508M | 2171 | F1-F1 | 1-1 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDK**V**RVDWLQRNANFYDWFVAELGGG SGGSGRVDWLQRNANFYDWFVAELGAAA(ETAG)AA | | |
| RB509M | 2172 | F1-F1 | 1-1 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDK**V**RVDWLQRNANFY**A**WFVAELGGG SGGSGGSGGSGRVDWLQRNANFYDWFVAELGAAA(ETAG)AA | | |
| RB452 | 2173 | F1 | 1 | MBP . . . NNNNLGIEGRISEFIEGRAQPAMARGGGTFYEWFES**A**LRKHGAGAAA (ETAG)AA | 7.8 * 10-7 | |
| RB427 | 2174 | F1-F1 | 1-1 | MBP . . . NNNNLGIEGRISEFIEGRAQPAMARGGGTFYEWFESTLRKHGAGGGSGG SRGGGTFYEWFESALRKHGAGAAA(ETAG)AA | 3.3 * 10-8 | |
| RB434 | 2175 | F1-F1 | 1-1 | MBP . . . NNNNLGIEGRISEFIEVRAQPAMARGGGTFYEWFESALRKHGAGGGSGG SGGSGGSRGGGTFYEWFESALRKHGAGAAA(ETAG)AA | 3.2 * 10-8 | |
| RB513 | 2176 | F1 | 1 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDKGSLDESFYDWFERQLGKKAA (ETAG)AA | | |
| RB516 | 2177 | F1-F1 | 1-1 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDKGSLDESFYDWFERQLGKKGGSGG SGSLDESFYDWFERQLGKKAAA(ETAG)AA | | |
| RB512 | 2178 | F1-F1 | 1-1 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDKGSLDESFYDWFERQLGKKGGSGG SGGSGGSGSLDESFYDWFERQLGKKAAA(ETAG)AA | | |
| RB464 | 2179 | F1 | 1 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDK FHENFYDWFVRQVSAA(ETAG) AA | 3.8 * 10-18 | |
| RB446 | 2180 | F4 | 2 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDKHLCVLEELFWGASLFGYCSGAAA (ETAG)AA | 7.7 * 10-7 | |
| RB459 | 2181 | F3 | 1 | MBP . . . NNNNLGIEGRISEFGSADYKDLDALDRLMRYFEERPSLAAA(ETAG) AA | 7.7 * 10-8 | |
| RB430 | 2182 | F1-F3 | 1-1 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDDKFHENFYDWFVRQVSGGSGGS LD ALDRLMRYFEERPSLETAG | 2.1 * 10-8 | |
| RB430 | 2183 | F1-F3 | 1-1 | cleaved DYKDDDKFHENFYDWFVRQVSGSGSLDALDRLMRYFEERPSLAAA(ETAG) AA | ~4 * 10-9 | |
| RB431 | 2184 | F1-F4 | 1-2 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDKFHENFYDWFVRQVSGGSGGSH LC VLEELFWGASLFGYCSGAAA(ETAG)AA | 4.7 * 10-8 | |
| RB431 | 2185 | F1-F4 | 1-2 | cleaved DYKDDDKFHENFYDWFVRQVSGGSGGSHLCVLEELFWGASLFGYCSGAAA (ETAG)AA | ~8 * 10-9 | |
| RB432 | 2186 | F1-F4 | 1-2 | MBP-NNNNLGIEGRISEFIEGRDYKDDDKFHENFYDWFVRQVSGGSGGSGGSGGSH LC VLEELFWGASLFGYCSGAAA(ETAG)AA | 3.5 * 10-8 | |
| RB432 | 2187 | F1-F4 | 1-2 | cleaved DYKDDDKFHENFYDWFVRQVSGGSGGSGGSGGSHLCVLEELFWGASLFGYC SGAAA(ETAG)AA | ~6 * 10-9 | |
| RB433 | 2188 | F1-F4 | 1-2 | MBP . . . NNNNLGIEGRISEFIEGRDYKDDDK FHENFYDWFVRQVSGGSGGSGGS H LCVLEELFWGASLFGYCSGAAA(ETAG)AA | 2.1 * 10-8 | |
| RB508 | 2189 | F1-F1 | 1-1 | DYKDDDDKVRVDWLQRNANFYDWFVAELGGGSGGSGRVDWLQRNANFYDWFVAELGAAA GAPVPYPDPLEPRSA | 1.5 * 10-7 | ++ |
| RB509 | 2190 | F1-F1 | 1-1 | DYKDDDDKVRVDWLQRNANFY**A**WFVAELGGGSGGSGGSGGSGRVDWLQRNANFYDWF VAELGAAAGAPVPYPDPLEPRAA | 5.5 * 10-8 | ++ |
| RB505 | 2191 | F4 | 2 | DYKDDDDKHLCVLEELFWGASLFGYCSGAAA(ETAG)AA | 4.8 * 10-7 | - |

TABLE 14-continued

| Peptide | SEQ ID NO: | Formula | Site IR | Sequence | HIR Kd (mol/l) | FFC |
|---|---|---|---|---|---|---|
| RB517 | 2192 | F4-F4 | 2-2 | DYKDDDDKHLCVLEELFWGASLFGYCSGGGSGGGSHLCVLEELFWGASLFGYCSGAAA (ETAG)AA | 6.0 * 10-8 | - |
| RB521 | 2193 | F1-F1 | 1-1 | MADYKDDDDKGSLDESFYDWFERQLGKKGGSGGGSGSLDESFYDWFERQLGKKAAA (ETAG)PG | 4.4 * 10-8 | +++++ |
| RB535 | 2194 | F1-F1 | 1-1 | MADYKDDDDKGSLDESFYDWFERQLGKKGGSGGGSGGSGGSGSLDESFYDWFERQLGKKA AA(ETAG)PG | ~1.0 * 10-7 | +++++ |
| RB540 | 2195 | F6 | 2 | MADYKDDDDKWLDQEWAWVQCEVYGRGCPSAAA(ETAG)PG | ~1.0 * 10-7 | |
| RB539 | 2196 | F6-F1 | 2-1 | MADYKDDDDKWLDQEWAWVQCEVYGRGCPSGGSGGSGSLDESFYDWFERQLGKKAAA (ETAG)PG | 7 * 10-10 | ++++ |
| RB537 | 2197 | F1-F6 | 1-2 | MADYKDDDDKGSLDESFYDWFERQLGKKGGSGGGSWLDQEWAWVQCEVYGRGCPSAAA (ETAG)PG | 5.9 * 10-11 | - |
| RB538 | 2198 | F1-F6 | 1-2 | MADYKDDDDKGSLDESFYDWFERQLGKKGGSGGGSGGSGGSWLDQEWAWVQCEVYGRGCP SAAA(ETAG)PG | 1.7 * 10-11 | - |
| RB626 | 2199 | F6-F1 | 2-1 | MADYKDEIEAEWGRVRCLVYGRCVGGGGSGGSGGSGGSGSLDESFYDWFERQLGKKAAA (ETAG)PG | 3.0 * 10-10 | +++ |
| RB625 | 2200 | F6-F1 | 2-1 | MADYKDDDDKWLDQEWAWVQCEVYGRGCPSQPPPPDITTHRADPQGSLDESFYDWFERQ LGKKAAA(ETAG)PG | 3.8 * 10-10 | +++++ |
| RB622 | 2201 | F6-F1 | 2-1 | MADYKDDDDKWLDQEWAWVQCEVYGRGCPSTPKPPTPPPLSADGSLDESFYDWFERQLG KKAAA(ETAG)PG | 1.0 * 10-9 | ++++ |
| RB596 | 2202 | F1 | 1 | MQNDDGSLDESFYDWFERQLGHHHHHHPG | 9.4 * 10-8 | |
| RB569 | 2203 | F1 | 1 | MGSLDESFYDWFERQLGEEEGGDHHHHHHPG | 2.1 * 10-7 | |
| RB570 | 2204 | F1 | 1 | MQNDDGSLDESFYDWFERQLGEEEGGDHHHHHHPG | 2.5 * 10-8 | |

ETAG = GAPVPYPDPLEPR(SEQ ID NO: 2205); MBP . . . NNNNL = fusion junction to MBP at c-terminus of MBP; All dimers are linked C-N.

## Example 9

### In Vivo Assays for Insulin Agonists

[0381] To test the in vivo activity of dimer peptide S519, an intravenous blood glucose test was carried out on Wistar rats. Male Mol:Wistar rats, weighing about 300 g, were divided into two groups. A 10 μl sample of blood was taken from the tail vein for determination of blood glucose concentration. The rats were anaesthetized with Hypnorm/Dormicum at t=−30 min and blood glucose was measured again at t=−20 min and at t=0 min. After the t=0 sample was taken, the rats were injected into the tail vein with vehicle or test substance in an isotonic aqueous buffer at a concentration corresponding to a 1 ml/kg volume of injection. Blood glucose was measured at times 10, 20, 30, 40, 60, 80, 120, and 180 min. The Hypnorm/Dormicum administration was repeated at 20 minute intervals. Results shown in FIG. **33** demonstrate that the S519 (at 20 nmol/kg) peptide lowered blood glucose levels similar to levels observed for human insulin (at 2.5 nmol/kg) (n=8). The S519 peptide and human insulin showed comparable in vivo effects, both in magnitude and onset of response (FIG. **33**).

## Example 10

### IGF-1 Surrogates

[0382] Three major groups of peptide IGF-1 surrogates were obtained from IGF-1R panning experiments: Site 1 A6 (FyxWF) (SEQ ID NO: 1596); Site 1 B6 (FyxxLxxL) (SEQ ID NO: 1732), and Site 2 (C—C looped). See Beasley et al. International Application PCT/US00/08528, filed Mar. 29, 2000, and Beasley et al., U.S. application Ser. No. 09/538,038, filed Mar. 29, 2000. Active surrogates included 20E2 and RP6 (B6-like; Formula 2), S175 (A6-like; Formula 1), G33 (A6-like; Formula 1), RP9 (A6-like; Formula 1), D815 (Site 2), and D8B12 (Site 2) peptides. The IGF-1 surrogates were analyzed by various assays, described as follows.

[0383] D. Phage Competition

[0384] Phage competition studies were performed with Site 1 (RP9) and Site 2 (D815) monomer peptides. Plates were coated with IGF-1R (100 ng/well in carbonate buffer, pH 9.6) overnight at 4° C. Wells were blocked with 4% non-fat milk in PBS for 60 min at room temperature. One

hundred microliters of rescued phage were added to each well. Peptides in varying concentrations were added and the mixtures were incubated for 2 hr at room temperature. Plates were washed three times with PBS and 100 μl of anti-M13 antibody conjugated to horseradish peroxidase was added to each well. The labeled antibody was incubated at room temperature for 60 min. After washing, 100 μl of ABTS was added per well and the plates read in a microtiter reader at 450 nM.

[0385] Phage included RP9 (A6-like; Formula 1); RP6 (B6-like; Formula 2); D8B12 (Site 2); and D815 (Site 2). Peptides included RP9 and D815.

| Peptide | Formula | Site IGF-1R | Sequence | SEQ ID NO: |
|---|---|---|---|---|
| D8B12 | 6 | 2 | WLEQERAWIWCEIQGSGCRA | 1884 |
| D815 | 6 | 2 | WLDQERAWLWCEISGRGCLS | 2206 |
| RP6 | 2 | 1 | TFYSCLASLLTGTPQPNRGP WERCR | 1635 |
| RP9 | 1 | 1 | GSLDESFYDWFERQLG | 1559 |

[0386] Results shown in FIGS. **34**A-**34**E demonstrate that that RP9 and D815 peptides competed both Site 1 and Site 2 phage. These results illustrate the allosteric nature of the interaction with IGF-1R.

[0387] Phage competition studies were also performed with Site 2-Site 1 dimer peptides containing 6- or 12-amino acid linkers. Plates were coated with IGF-1R (100 ng/well in carbonate buffer, pH 9.6) overnight at 4° C. Wells were blocked with 4% non-fat milk in PBS for 60 min at room temperature. One hundred microliters of rescued phage were added to each well. Peptides in varying concentrations were added and the mixture incubated for 2 hr at room temperature. Plates were washed three times with PBS and 100 μl of anti-M13 antibody conjugated to horseradish peroxidase was added to each well. The labeled antibody was incubated for 60 min at room temperature. After washing, 100 μl of ABTS was added per well and the plates read in a microtiter reader at 450 nM. Phage included RP9, RP6, D8B12, and D815. Peptides included D815-6L-RP9 and D815-12L-RP9. Linker sequences are underlined and shown below.

| Peptide | Formula | Site IGF-1R | Sequence | SEQ ID NO: |
|---|---|---|---|---|
| D815-6L-RP9 | 6-1 | 2-1 | LDQERAWLWCEISGRGCLSGGSGG SGSLDESFYDWFERQLGKK | 2207 |
| D815-12L-RP9 | 6-1 | 2-1 | WLDQERAWLWCEISGRGCLSGGSG GSGGSGGSGSLDESFYDWFERQLG KK | 2208 |

[0388] D8B12, D815, RP6, and RP9 amino acid sequences are shown in the previous section. Results shown in FIGS. **35**A-**35**E demonstrate that dimers competed both Site 1 and Site 2 phage. This indicates that both dimer units were active at IGF-1R.

[0389] IGF-1 Proliferation Assays

[0390] FDCP-2 cells expressing the IL-3 and human IGF-1R receptors were grown in RPMIk-1640 medium supplemented with 15% fetal bovine serum (FBS) and 5% WEHI conditioned medium (containing IL-3) in accordance with routine methods. Prior to an experiment, the cells were pelleted and washed two times in PBS. Following this, cells were resuspended in RPMI-1640 medium with 2% FBS and added to a 96-well plate at a concentration of $2\times10^4$ cells/well in 75 μl. This was designated as the cell plate.

[0391] Peptides were suspended in PPMI-15% FBS (test medium). For the agonist assay, medium was added to rows 2-12 of a 96 well plate. The peptide was added to row 1 in 200 μl of test medium at a final concentration of 60 μM. The peptide was serially diluted (1:1) across rows 2-11. No peptide was added to row 12 (control; cells without IGF-1). For the antagonist assay, test medium containing 10 ng/ml IGF-1 ($ED_{50}$ test medium) was added to all wells of a 96 well plate. To row 1 was added 100 μl of peptide in $ED_{50}$ test medium at a concentration of 120 μM. The peptide was serially:diluted (1:1) across rows 2-11. No peptide was added to row 12 (control; cells with IGF-1).

[0392] For both agonist and antagonist assays, 75 μl from the working plates was transferred to the appropriate rows in comparable cell plates. The starting peptide concentration for both agonist and antagonist assays was 30 μM. Each peptide was done in duplicate. Plates were incubated at 37° C. for 45-48 hr. Ten microliters of WST-1 (Cell Proliferation Reagent, Roche cat #1 644 807) were added to each well and the plates were read in an ELISA reader (440/700 dual wavelength) each hour for 4 hr. Graphs were prepared from the raw data using Sigma Plot. Peptides included:

| Peptide | Formula | Site IGF-1R | Sequence | SEQ ID NO: |
|---|---|---|---|---|
| 20E2 | 2 | 1 | DYKDFYDAIDQLVRGSARAGGTRD | 2209 |
| D815 | 6 | 2 | WLDQERAWLWCEISGRGCLS | 2206 |
| G33 | 1 | 1 | GIISQSCPESFYDWFAGQVSDPWW CW | 1600 |
| RP6 | 2 | 1 | TFYSCLASLLTGTPQPNRGPWERC | 1635 |
| RP9 | 1 | 1 | RGSLDESFYDWFERQLG | 1559 |
| S175 | 1 | 1 | GRVDWLQRNANFYDWFVAELG | 1560 |

[0393] Results of the IGF-1 proliferation assays are shown in FIGS. **36-42**. FIG. **36** demonstrates that that peptides G33 (Site 1; $ED_{50}$~10 μM) and D815 (Site 2; $ED_{50}$~2 μM) showed agonist activity at IGF-1R, whereas peptides RP9 and RP6 showed no agonist activity. FIG. **37** demonstrates that that peptides RP6 (Site 1; $ED_{50}$~1 μM) and RP9 (Site 1; $ED_{50}$~7 μM) showed antagonist activity at IGF-1R, whereas peptides G33 and D815 showed no antagonist activity. FIG.

**38** demonstrates that peptides S175 and 20E2 exhibited weak agonist activity at IGF-1R ($ED_{50}$>10 μM). FIG. **39** shows that D815-RP9 dimers with 6- or 12-amino acid linkers acted as agonists at IGF-1R. FIG. **40** shows that dimer peptide D815-6-G33 was inactive as an agonist at IGF-1R. FIG. **41** shows that monomer peptide RP6 acted as an antagonist at IGF-1R. The IGF-1 standard curve determined for FDCP-2 cells is shown in FIG. **42**.

[**0394**] The IGF-1R data for the Site 1 and Site 2 peptides is summarized in Table 15, below.

TABLE 15

| Mon./ Dimer | Form. | Site IGF-1R | Link. | Sequence | SEQ ID NO: | nM Ki app Kd | nM $ED_{50}$ Growth | Max Action | nM $IC_{50}$ Antag. | Ki/ ED50 | Class |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IGF-1 | | | NA | | | 0.69 | 0.30 | 100 | 2 | 2.3 | A |
| rG33 | 1 | 1 | NA | GIISQSCPESFYDWFAGQVSDPWWCW | 1600 | 1450 | 500 | >50 | — | 2.9 | A |
| rD815 | 6 | 2 | NA | WLDQERAWLWCEISGRGCLS | 2206 4080 | 500 | >50% | — | 8.2 | A | |
| RP9 | 1 | 1 | NA | GSLDESFYDWFERQLG | 1559 | 417 | — | <10% | 900 | 0.5 | N |
| D815-G33 | 6-1 | 2-1 | 6 aa | WLDQERAWLWCEISGRGCLSGGSG GSGIISQSCPESFYDWFAGQVSDPW WCW | 2210 | 624 | — | <10% | nd | | nd |
| D815-RP9 | 6-1 | 2-1 | 6 aa | WLDQERAWLWCEISGRGCLSGGSG GSGSLDESFYDWFERQLGKK | 2211 | 36 | 50 | >50% | >500 | 0.8 | A |
| D815-RP9 | 6-1 | 2-1 | 12 aa | WLDQERAWLWCEISGRGCLSGGSG GSGGSGGSGSLDESFYDWFERQLG KK | 2212 | 3 | 10,000 | 100 | — | 0.0003 | A |

A = agonists; N = antagonist; nd = not determined; NA = not applicable; Form.= formula; Mon. = monomer; Antag. = antagonism; Link. = linker; Linker sequences are underlined.

## Example 11

### Panning Peptide Libraries

[**0395**] E. Panning IGF-1 Surrogate Secondary Libraries

[**0396**] Soluble IGF-1R ("sIGF-1R") was obtained from R&D Systems. The soluble protein (>95% pure) included the heterotetrameric (alpha 2-beta 2) extracellular domain of IGF-1R isolated from a mouse myeloma cell line. sIGF-1R (500 ng/well) was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, NUNC) and incubated overnight at 4° C. Wells were then blocked with MPBS (PBS buffer pH 7.5 containing 2% Carnation® non-fat dry milk) at room temperature (RT) for 1 h. Eight wells were used for each round of panning for the G33 and RP6 secondary libraries. The phage were incubated with MPBS for 30 min at RT, then 100 μl was added to each well.

[**0397**] For the first round, the input phage titer was $4\times10^{13}$ cfu/ml. For rounds 2 and 3, the input phage titer was approximately $10^{11}$ cfu/ml. Phage were allowed to bind for 2 to 3 h at RT. The wells were then quickly washed 13 times with 200 μl/well of MPBS. Bound phage were eluted by incubation with 100 μl/well of 20 mM glycine-HCl, pH 2.2 for 30 s. The resulting solution was then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, then plated onto two 24 cm×24 cm plates containing 2xYT-AG. The plates were incubated at 30° C. overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at −80° C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above. A minimum of 72 clones was picked at random from the second, third, and fourth rounds of panning and screened for binding activity. DNA sequencing of the clones determined the amino acid sequences summarized in FIG. **43**A-**43**B.

[**0398**] Panning Peptide Dimer Libraries

[**0399**] Microtiter plates were coated and blocked by standard methods, as follows. Plates were coated with sIGF-1R (see Example, above) or soluble IR (Bass construct; Bass et al., 1996, *J. Biol. Chem.* 271:19367-19375) in 0.2 M $NaHCO_3$, pH 9.4. One hundred microliters of solution containing either 50 ng IR or IGF-1R (rounds 1 and 2), 25 ng IR or IGF-1R (round 3), or 12.5 ng IR or IGF-1R (round 4) was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nalge NUNC) and incubated overnight at 4° C. Wells were then blocked with a solution of 2% non-fat milk in PBS (MPBS) at RT for at least 1 h.

[**0400**] Eight wells coated with IR or IGF-1R were used for each round of panning. One hundred microliters of phage were added to each well. For the first round, the input phage titer was $3\times10^{13}$ cfu/ml. For subsequent rounds, the input phage titer was approximately $10^{12}$ cfu/ml. Phage were incubated for 2-3 h at RT. The wells were then quickly washed 13 times with 300 μl/well of PBS. Bound phage were eluted by incubation with 150 μl/well of 50 mM glycine-HCl, pH 2.0 for 15 min. The resulting solution was pooled and then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, in 2xYT medium for 1 hr at 37° C. prior to the addition of helper phage, ampicillin, and glucose (2% final concentration).

[0401] After incubation for 1 hr at 37° C., the cells were spun down and resuspended in 2xYT-AK medium. The cells were then returned to the shaker and incubated overnight at 37° C. Phage amplified overnight were then precipitated and subjected to the next round of panning. A total of 96 clones were picked at random from rounds 3 and 4 and screened for binding activity. Several clones from each pan were further tested for binding to IR or IGF-1R in phage ELISA by competition with soluble peptides as described in Beasley et al. International Application PCT/US00/08528, filed Mar. 29, 2000, and Beasley et al., U.S. application Ser. No. 09/538,038, filed Mar. 29, 2000. Competition was performed by addition of 5 μl of RP9 peptide, recombinant D8 peptide, or both per well, followed by addition of 100 μl of phage per well. Representative peptides are shown in FIGS. **44**A-**44**B and in Table 16, below.

TABLE 16

| Pep. | SEQ ID NO: | Form. | Site IR | Sequence | Description |
|---|---|---|---|---|---|
| RP27 | 2213 | 6-1 | 2-1 | GLDQEQAWVECEVYGRGCPYGSLDESFYDW FERQLG | No linker |
| RP28 | 2214 | 6-1 | 2-1 | RLEEEWAWVQCEVYGRGCPS**GGSGGS**GSLD ESFYDWFERQLG | EEE Stretch in D8 |
| RP29 | 2215 | 6-1 | 2-1 | SLDREWACVKCEVYGRGCPC**GGSGGS**GSLD ESFYDWFERQLG | Repeat isolate |
| RP30 | 2216 | 6-1 | 2-1 | SLEEEWAQVECEVYGRGCPS**GGSGGS**GSLD ESFYDWFERQLG | D8 by Design |
| RP31 | 2217 | 6-1 | 2-1 | SLEEEWAQVECEVYGRGCPS**GGSGGS**GLLD ESFYHWFDRQLR | D8 & RP9 by design |
| RP32 | 2218 | 6-1 | 2-1 | SIEEEWAQIKCDVWGRGCPP**GGSGGS**GLLD ESFYHWFDRQLR | D8 & RP9 by design |
| RP33 | 2219 | 6-1 | 2-1 | QLDLEWAWVQCEVYGRGC**GGS**GSLDESFYD WFERQLG | 3 amino acid linker |
| RP34 | 2220 | 6-1 | 2-1 | QLDEEWAGVQCEVYGRGCSLDESFYDWFER QLG | No linker |
| RP35 | 2221 | 6-1 | 2-1 | RLEEEWRWVQCEVYGRGCAA**GGSGGS**GSLD ESFYDWFERQLG | EEE Stretch in D8 |
| RP36 | 2222 | 6-10 | 2-1 | SLDQEWAWVQCEVYGRGCPS**GGSGGS**DSDW AGYEWFEEQLD | D8 (W1->S)- Group 6 by design |

Pep. = peptide; Form. = formula; Linker sequences are shown in bold and underlined; All dimers are linked C-N

[0402] Determination of Amino Acid Preferences

[0403] For both monomer and dimer peptides, amino acid preferences for each peptide were determined as follows. The expected frequency of each of the 20 amino acids at that position was calculated based on codon usage and % doping for that library. This was then compared to the actual frequency of occurrence of each amino acid at every position after four rounds of biopanning. Any amino acid that occurred at a frequency >2-fold was considered preferred. Most preferred amino acid(s) were those that have the greatest fold enrichment after panning. Preferred amino acid sequences for RP9, D8, and Formula 10 (Group 6) peptides are shown below.

TABLE 17

| Peptide | Sequence | SEQ ID NO: |
|---|---|---|
| RP9 | GSLDESFYDWFERQLG | 1559 |
| Regular | G**LA**DEDFY**E**WFERQL**R** **L** | 2223 |
| w/Peptide | G**Q**LDE**D**FY**E**WF**D**RQL**S** **A** | 2224 |

TABLE 17-continued

| Peptide | Sequence | SEQ ID NO: |
|---|---|---|
| w/Insulin | G**FM**DESFYEWFERQL**R** **W** **A** | 2225 |

[0404] Table 17 shows preferred amino acid sequences for RP9 peptides. Residues in bold indicate strong preference; underlined residues indicate positions where more than one

amino acid preference is seen. The first column indicates the conditions used for the panning procedure. "RP9" indicates sequence of the parent RP9; "Regular" indicates regular pan as described in methods for panning of random libraries; "w/peptide" indicates panning in the presence of 2 nM RP9 peptide; "w/insulin" indicates panning in the presence of 2 nM insulin.

TABLE 18

| Peptide | Sequence | SEQ ID NO: |
|---|---|---|
| D8 Parent: | WLDQEWAWVQCEVYGRGCPS | 2129 |
| Dimer Consensus | sLEE**EW**aQIE**CEVY/WGRGC**ps | 2226 |
| Monomer Consensus | sL**EEEW**aQIq**CEIY/WGRGC**ry **W** | 1548 |

[0405] Table 18 shows preferred amino acid sequences for D8 peptides. Upper case residues in bold indicate strong preference (>90% frequency); upper case letters, non-bold, indicate some preference (5-15% higher frequency than expected); lower case letters indicate less preference (2-5% higher frequency than expected); similar preferences seen in D8 in both monomer and dimer libraries. The underlined Y/W indicates that both residues are equally preferred at that position. In the original D8 sequence that position is occupied by Y.

TABLE 19

| Peptide | Sequence | Type | SEQ ID NO: |
|---|---|---|---|
| Group 6 | W(A/E)GYEW(F/L) | preferred core | 1549 |
| Group 6 | DSDWAGYEWFEEQLD | preferred sequence | 1595 |

[0406] Table 19 shows preferred amino acid sequences for Group 6 peptides. Underlined residues indicate preferred N-terminal and C-terminal extensions.

Example 12

Fluorescence-Based HIGF-1R Binding Assays

[0407] F. Heterogeneous Time-Resolved Fluorometric Assays

[0408] The effect of recombinant peptide surrogate G33 (rG33) on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R) was determined using heterogeneous time-resolved fluorometric assays (TRF; DELFIA®, PE Wallac, Inc.). The rhIGF-1R protein included the extracellular domain of the receptor pre-propeptide, up to amino acid residue 932 (A. Ullrich et al., 1986, *EMBO J.* 5:2503-2512). Duplicate data points were collected at each concentration of competitor and the lines were designed to represent the best fit to a four-parameter non-linear regression analysis (y=min+(max−min)/(1+10^((logIC$_{50}$−x)*Hillslope))) of the data, which was used to determine IC$_{50}$ values.

[0409] The assay was performed using a 96-well clear microplate (NUNC MaxiSorp) with a final volume of 100 µl. Microtiter plates were coated with 0.1 µg rhIGF-1R in 100 µl of $NaHCO_3$, pH 8.5 buffer, and incubated overnight at room temperature (RT). The plates were washed 3-times with 0.05 M Tris-HCl (pH 8 at 25° C.), 0.138 M NaCl, 0.0027 M KCl (TBS). This was followed by addition of 200 µl blocking buffer (TBS containing 0.05% Bovine Serum Albumin (BSA, Cohn Fraction V)), and incubated for 1 hr at RT. The plates were washed 6-times with a 1× solution of Wallac's DELFIA wash concentrate. Competitor was added in a volume of 50 µl and serially diluted across the microtiter plate in TBS containing 0.05% BSA. Non-specific binding (background) was determined in the presence of 60 µM hIGF-1.

[0410] Fifty microliters of b-rhIGF-1, 10 nM, diluted in TBS containing 0.05% BSA was added. The plates were incubated for 2 hr at RT. After incubation, plates were washed 6-times with a 1× solution of Wallac's DELFIA wash concentrate. Then the plates were treated with 100 µL of Wallac's DELFIA Assay Buffer containing a 1:1000 dilution of europium-labeled streptavidin and incubated for 2 hours at RT. This was followed by washing 6-times with a 1× solution of Wallac's DELFIA wash concentrate. One hundred microliters of Wallac's DELFIA enhancer was added, and the plates were shaken for 30 min at RT. After shaking, the fluorescence signal at 620 nm was read on a Victor$^2$ 1420 plate reader (PE Wallac, Inc.). Primary data were background corrected, normalized to buffer controls, and then expressed as % Specific Binding. The Z'-factor was greater than 0.5 ($Z'=1-(3\tau_+ + 3\tau_-)/|\mu_+ - \mu_-|$; Zhang et al., 1999, *J. Biomol. Screen.* 4:67-73) and the signal-to-background (S/B) ratio was ~20. The results of these experiments are shown in FIG. **45**. The IC$_{50}$ value calculated for rG33 is shown in Table 20, below.

[0411] The effect of recombinant peptide surrogates D815 (rD815), RP9, D815-6aa-G33, D815-6aa-RP9, and D815-12aa-RP9 on the binding of b-rhIGF-1 to rhIGF-1R was determined using the fluorometric assay described above. IGF-1 was used as a control. Duplicate data points were collected at each concentration of competitor and the lines represent the best fit to a four-parameter non-linear regression analysis, which was used to determine IC$_{50}$ values. Results for rD815 are show in FIG. **46**; results for RP9 are shown in FIG. **47**; results for D815-6-G33 are shown in FIG. **48**; results for D815-6-RP9 are shown in FIG. **49**; and results for D815-12-RP9 are shown in FIG. **50**; the results for IGF-1 are shown in FIG. **51**. The IC$_{50}$ values for the rD815, RP9, D815-6aa-G33, D815-6aa-RP9, and D815-12aa-RP9 peptides, and IGF-1 are shown in Table 20, below. Linker sequences are underlined.

TABLE 20

| Competitor | Sequence | SEQ ID NO: | IC$_{50}$ (M) |
|---|---|---|---|
| rG33 | GIISQSCPESFYDWFAGQV SDPWWCW | 1600 | $1.45 \times 10^{-6}$ M |
| rD815 | WLDQERAWLWCEISGRGCL | 2206 | $4.08 \times 10^{-6}$ M |
| RP9 | SGSLDESFYDWFERQLG | 1559 | $4.17 \times 10^{-7}$ M |

TABLE 20-continued

| Competitor | Sequence | SEQ ID NO: | $IC_{50}$ (M) |
|---|---|---|---|
| D815-6aa-G33 | WLDQERAWLWCEISGRGCL SGGSGGSGIISQSCPESFY DWFAGQVSDPWWCW | 2210 | $6.24 \times 10^{-7}$ M |
| D815-6aa-RP9 | WLDQERAWLWCEISGRGCL SGGSGGSGSLDESFYDWFE RQLGKK | 2211 | $3.57 \times 10^{-8}$ M |
| D815-12aa-RP9 | WLDQERAWLWCEISGRGCL SGGSGGSGGSGGSGSLDES FYDWFERQLGKK | 2212 | $3.22 \times 10^{-9}$ M |
| IGF-1 | | | $6.85 \times 10^{-10}$ M |

[0412] The order of potency of all peptides or dimers compared to IGF-1 was determined as: IGF-1>D815-12aa-RP9>>D815-6aa-RP9>RP9≅D815-6aa-G33>rG33>rD815. These results suggest that the coupling of D815 with RP9 using an extended linker (12 versus 6 amino acids) produced a potent competitor that approximates the affinity of IGF-1 for its own receptor.

[0413] G. Time-Resolved Fluorescence Resonance Energy Transfer Assays

[0414] The effect of Site 1 peptide surrogates, Site 2 peptide surrogates, and rhIGF-1 on the dissociation of biotinylated-20E2 (b-20E2, Site 1) from recombinant human IGF-1R was determined using time-resolved fluorescence resonance energy transfer assays (TR-FRET). Best fit non-linear regression analysis of the data, was used to determine dissociation rate constants. Each data point represents a single observation.

[0415] The assay was performed using a 96-well white microplate (NUNC) with a final volume of 100 μl. Final incubation conditions were 16.5 nM b-20E2, 2.2 nM SA-APC (streptavidin-allophycocyanin), 2.2 nM $Eu^{3+}$-rhIGF-1R (LANCE™ labeled, PE Wallac, Inc.), 0.05 M Tris-HCl (pH 8 at 25° C.), 0.138 M NaCl, 0.0027 M KCl, and 0.1% BSA (Cohn Fraction V). Reactions were allowed to reach equilibrium for 6 hr at RT. Following this, various peptide surrogates or IGF-1 were added at a final concentration of 100 μM or 30 μM, respectively. The addition of peptides or IGF-1 initiated the measurement of dissociation (Time Zero, sec). The fluorescence signal at 665 nm was read on a Victor$^2$ 1420 plate reader (PE Wallac, Inc.) at 30 sec intervals.

[0416] Results of these experiments are shown in FIG. **52**. The buffer controls did not vary over the time interval of study, which demonstrated that the equilibrium was not disturbed by the addition of diluent at Time zero. The addition of excess (>1000-fold 20E2 $K_d$ for IGF-1R) Site 1 peptides such as H2C, 20E2, or RP6 did not differ depending on specific the peptide used, and the dissociation rates of b-20E2 were similar for these peptides. D8B12 (Site 2 peptide) and IGF-1 (binds both Site 1 and Site 2) did demonstrate significant differences in the rate of dissociation of b-20E2. This would suggest that these agents act as non-competitive or allosteric regulators of Site 1 binding.

[0417] The effect of various peptide surrogates or peptide dimers on the binding of biotinylated-20E2 (B-20E2) to recombinant human IGF-1R was determined using TR-FRET assays, described above. For these experiments, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis (y=min+(max−min)/(1+10^((log$IC_{50}$−x)*Hillslope))) of the data, which was used to determine $IC_{50}$ values.

[0418] The assays were performed using a 384-well white microplate (NUNC) with a final volume of 30 μl. Final incubation conditions were 15 nM b-20E2, 2 nM SA-APC, 2 nM $Eu^{3+}$-rhIGF-1R (LANCE™ labeled, PE Wallac, Inc.), 0.05 M Tris-HCl (pH 8 at 25° C.), 0.138 M NaCl, 0.0027 M KCl, and 0.1% BSA (Cohn Fraction V). After 16-24 hr of incubation at RT, the fluorescence signal at 665 nm and 620 nm was read on a Victor$^2$ 1420 plate reader (PE Wallac, Inc.). Primary data were background corrected, normalized to buffer controls, and then expressed as % Specific Binding. The Z'-factor was greater than 0.5 ($Z'=1-(3\sigma_+ + 3\sigma_-)/|\mu_+ - \mu_-|$; Zhang et al, 1999, *J. Biomol. Screen.* 4:67-73) and the signal-to-background (S/B) ratio was ~4. Results of these experiments are shown in FIG. **53**. Table 21, below, shows the $IC_{50}$ values calculated for these experiments. Notably, the Cl peptide showed IGF-1R affinities of ~1 nM (FIG. **53**) and ~10 nM (Table 21) in these assays.

TABLE 21

| Competitor | Sequence | SEQ ID NO: | For-mula | Site IGF-1R | $IC_{50}$ (M) |
|---|---|---|---|---|---|
| C1 | CWARPCGDAANFYDWFVQQ AS | 1550 | 1 | 1 | 8.80E-10 |
| IGF-1 | | | | | 2.93E-09 |
| RP9 | GSLDESFYDWFERQLG | 1559 | 1 | 1 | 3.93E-08 |
| 20E2 | DYKDFYDAIDQLVRGSARA GGTRD | 2209 | 2 | 1 | 1.04E-07 |
| E8 | GGTVWPGYEWLRNA | 2118 | 10 | 2 | 2.53E-07 |
| H2C | FHENFYDWFVQRVSKK | 2117 | 1 | 1 | 4.60E-07 |

TABLE 21-continued

| Competitor | Sequence | SEQ ID NO: | For-mula | Site IGF-1R | $IC_{50}$ (M) |
|---|---|---|---|---|---|
| S173 | LDALDRLMRYFEERPSL | 1830 | 3 | 1 | 6.29E-06 |
| D8B12 | WLEQERAWIWCEIQGSGCRA | 1884 | 6 | 2 | 1.13E-05 |
| A6 | SAKNFYDWFVKK | 1551 | 1 | 1 | 3.10E-05 |

[0419] H. Fluorescence Polarization Assays

[0420] The effect of various peptide monomers and dimers on the binding of fluorescein-RP-9 (FITC-RP9) to soluble human insulin receptor-immunoglobulin heavy chain chimera (sIR-Fc; Bass et al., 1996, *J. Biol. Chem.* 271:19367-19375) was determined using fluorescence polarization assays (FP). For these experiments, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis of the data, which was used to determine $IC_{50}$ values.

[0421] The assays were performed in a 384-well black microplate (NUNC) with a final volume of 30 μl. Final incubation conditions were 1 nM FITC-RP9, 10 nM sIR, 0.05 M Tris-HCl (pH 8 at 25° C.), 0.138 M NaCl, 0.0027 M KCl, 0.05% BGG (bovine gamma globulin), 0.005% Tween-20. After 16-24 hr of incubation at RT, the fluorescence signal at 520 nm was read on an Analyst™ AD plate reader (LJL BioSystems, Inc.). Primary data were background corrected using 10 nM sIR without FITC-RP9 addition, normalized to buffer controls and then expressed as % Specific Binding. The Z'-factor was greater than 0.5 ($Z'=1-(3\sigma_+ + 3\sigma_-)/|\mu_+ - \mu_-|$; Zhang et al, 1999, *J. Biomol. Screen.* 4:67-73) and the assay dynamic range was ~125 mP. In parallel with these experiments, TR-FRET assays were performed using rhIGF-1R and b-20E2, as described above. Results of the FP and TR-FRET experiments are shown in Table 22, below.

TABLE 22

| Peptide | FP sIR-Fc | TR-FRET rhIGF 1R | Binding Ratio IGF-1R/IR | Formula | Site IGF-1R | SEQ ID NO: | Sequence |
|---|---|---|---|---|---|---|---|
| RP4 | 17 | 8100 | 476 | 2 | 1 | 1552 | PPWGARFYDAIEQ LVFDNL |
| S175 | 10 | 1650 | 165 | 1 | 1 | 1560 | GRVDWLQRNANFY DWFVAELG |
| RP15 | 28 | 706 | 25 | 1 | 1 | 2130 | SQAGSAFYAWFDQ VLRTV |
| H2C (D117) | 66 | 600 | 9 | 1 | 1 | 2117 | FHENFYDWFVQRV SKK |
| 20E2 (D118) | 51 | 100 | 1.9 | 2 | 1 | 2209 | DYKDFYDAIDQLV RGSARAGGTRD |
| RP9 | 24 | 33 | 1.4 | 1 | 1 | 1559 | GSLDESFYDWFER QLG |
| G33 | 139 | 178 | 1.3 | 1 | 1 | 1600 | GIISQSQPESFYD WFAGQVSDPWWCW |
| E8 (D120) | 206 | 175 | 0.85 | 10 | 2 | 2118 | GGTVWPGYEWLRN A |
| C1 (D112) | 52 | 10 | 0.19 | 1 | 1 | 1550 | CWARPCGDAANFY DWFVQQAS |
| RP16 | 6400 | 961 | 0.15 | | | 1553 | VMDARDDPFYHKL SELVT |

FP sIR-Fc column shows $IC_{50}$ (nM) values obtained (vs. FITC-RP9); TR-FRET rhIGF-1R column shows $IC_{50}$ (nM) values obtained (vs. b-20E2); for Binding Ratio: higher values indicated higher affinity for IR than IGF-1R.

[0422] These results demonstrated that S175, RP4, and RP15 showed high affinities for IR and showed high binding ratios for IGF-1R over IR. H2C, 20E2, RP9, and Cl were slightly less potent than S175, RP4, and RP15 at IR, and these peptides had lower binding ratios for IGF-1R over IR. G33 and E8 were less potent than S175, RP4, and RP15 at IR, and showed comparable binding to IGF-1R and IR. RP16 had poor potency at IR and IGF-1R, but had higher affinity for IGF-1R than IR.

Example 13

Insulin Receptor Surrogates with Enhanced Specificity

[0423] Peptide S597 was tested for its bioactivity relative to insulin. SGBS cells (a human adipocyte cell line) were incubated with various concentrations of human insulin or peptide S597 and cellular uptake of $^{14}$C-glucose was measured essentially as described in Example 4. The results (as illustrated in FIG. **54**) indicate that the potency of S597 in stimulating glucose uptake is at least as good as that of human insulin.

[0424] The glucose-lowering effect of peptide S597 and peptide S557 in rats was compared with that of insulin as follows: Eighteen male Wistar rats, 200-225 g, fasted for 18 h, were anesthetized using Hypnorm-Dormicum (1.25 mg/ml Dormicum, 2.5 mg/ml fluanisone, 0.079 mg/ml fentanyl citrate) 2 ml/kg as a priming dose 30 min prior to test substance dosing and additional 1 ml/kg every 20 minutes (at time points −10 min, 10 min and 30 min relative to test substance dosing).

[0425] The rats were allocated into three groups. The animals were dosed with an intravenous injection (tail vein), 2 ml/kg, of either human insulin 1.25 nmol/kg (n=6) or S557 peptide 5 nmol/kg (n=6) or S597 peptide 5 nmol/kg (n=6). Blood samples for the determination of whole blood glucose concentration were collected in heparinized 10 μl glass tubes by puncture of the capillary vessels in the tail tip at times −20 min and 0 min (before dosing), and at times 10, 20, 30, 40, 60, 80, 120, and 180 min after dosing. Blood glucose concentrations were measured after dilution in analysis buffer by the immobilized glucose oxidase method using an EBIO Plus autoanalyzer (Eppendorf, Germany).

[0426] The results (as illustrated in FIG. **55**) indicate that the blood glucose lowering effect of S597 in rats is about 4 times lower than that of human insulin. The improved effect of S597 relative to S557 shows the effect of N-terminal acetylation.

[0427] The glucose-lowering effect of different concentrations of peptide S597 was also tested by intravenous administration to fasted Goettingen minipigs weighing about 15 kg. The results (as illustrated in FIG. **56**) indicate that the glucose-lowering effect at 3 nmol/kg S597 is comparable to that of 0.3 nmol/kg human insulin.

Example 14

Co-Administration of Therapeutic Peptides

[0428] The rate of disappearance of two co-administered peptides was tested as follows:

[0429] Mixtures containing 600 nmol/ml peptide S557 and 1800 nmol/ml $B^{29}$-$N^{\epsilon}$-(N-lithocolyl-γ-glutamyl)-des(B30) human insulin included $^{125}$I-labeled peptides were injected into the neck of a pig. Radioactivity at the injection site was monitored over time using an external gamma counter.

[0430] The results (as illustrated in FIG. **57**) indicate that the disappearance of either peptide was not influenced by the presence of the second peptide.

[0431] Incorporated herein by reference in its entirety is the Sequence Listing for the application, comprising SEQ ID NO:1 to SEQ ID NO:2227. The Sequence Listing is disclosed on three CD-ROMs, designated "CRF", "Copy 1", and "Copy 2". The Sequence Listing is a computer-readable ASCII file named "18784051 US1.app.txt", created on Aug. 8, 2002, in IBM-PC machine format, on a MS-Windows®98 operating system. The 18784051 US1.app.txt file is 927,737 bytes in size.

[0432] As various changes can be made in the above compositions and methods without departing from the scope and spirit of the invention, it is intended that all subject matter contained in the above description, shown in the accompanying drawings, or defined in the appended claims be interpreted as illustrative, and not in a limiting sense.

[0433] The contents of all patents, patent applications, published articles, books, reference manuals, texts and abstracts cited herein are hereby incorporated by reference in their entirety to more fully describe the state of the art to which the present invention pertains.

SEQUENCE LISTING

The patent application contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US20070265189A1). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

What is claimed is:

**1**. A method of modulating insulin receptor activity in mammalian cells comprising administering to the cells an effective amount of an amino acid sequence that comprises a first subsequence that binds to Site 1 of insulin receptor and The method according to claim **28**, wherein the Site 1 sequence consists essentially of comprises a Formula 1 sequence $X_1X_2X_3X_4X_5$ and a second subsequence that binds

to Site 2 of insulin receptor and the Site 2 sequence consists essentially of and comprises a Formula 6 sequence $X_{62}$ $X_{63}$ $X_{64}$ $X_{65}$ $X_{66}$ $X_{67}$ $X_{68}$ $X_{69}$ $X_{70}$ $X_{71}$ $X_{72}$ $X_{73}$ $X_{74}$ $X_{75}$ $X_{76}$ $X_{77}$ $X_{78}$ $X_{79}$ $X_{80}$ $X_{81}$,

wherein $X_1$, $X_2$, $X_4$, and $X_5$ are aromatic amino acids; and $X_3$ is any polar amino acid;, and wherein $X_{62}$, $X_{65}$, $X_{66}$ $X_{68}$, $X_{69}$, $X_{71}$, $X_{73}$, $X_{76}$, $X_{77}$, $X_{78}$, $X_{80}$ and $X_{81}$ are any amino acids acid; $X_{63}$, $X_{70}$, and $X_{74}$ are hydrophobic amino acids; $X_{64}$ is a polar amino acid; $X_{67}$ and $X_{75}$ are aromatic amino acids; and $X_{72}$ and $X_{79}$ are cysteines; and the Formula 1 and Formula 2 sequences are linked C-terminus to N-terminus and oriented Site 2 to Site 1.

**2**. The method according to claim 1, wherein $X_1$, $X_2$, and $X_5$ are selected from the group consisting of phenylalanine and tyrosine, $X_3$ is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and $X_4$ is selected from group consisting of tryptophan, tyrosine and phenylalanine.

**3**. The method according to claim 2, wherein $X_{63}$ is selected from the group consisting of leucine, isoleucine, methionine and valine; $X_{70}$ and $X_{74}$ are selected from group consisting of valine, isoleucine, leucine and methionine; $X_{64}$ is selected from group consisting of aspartic acid and glutamic acid; $X_{67}$ is tryptophan; and $X_{75}$ is selected from group consisting of tyrosine and tryptophan.

**4**. The method of claim 3, wherein the amino acid sequence increases insulin receptor activity.

**5**. The method according to claim 1, wherein the Formula 1 sequence is SEQ ID NO:1554.

**6**. The method according to claim 1, wherein the Formula 6 sequence is SEQ ID NO:2129.

**7**. The method according to claim 1, wherein the Formula 1 sequence is selected from the group consisting of SEQ ID NOS:1-712; SEQ ID NOS:1221-1243; and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776.

**8**. The method according to claim 1, wherein the Formula 6 sequence is selected from the group consisting of SEQ ID NOS:926-1061; SEQ ID NOS:1244-1253; and SEQ ID NOS:1596, 1718-1719, 1556, 1560, and 1720-1776.

**9**. The method according to claim 1, wherein the Formula 1 sequence is selected from the group consisting of S105-S116 (SEQ ID NOS:1791-1805, 1556, and 1806-1807), S131 (SEQ ID NO:1820), S137 (SEQ ID NO:1821), S158 (SEQ ID NO:1780), S165-S168 (SEQ ID NOS:1554, and 1824-1826), S171 (SEQ ID NO:1831), S175-S176 (SEQ ID NOS:1560 and 1836), S179-S184 (SEQ ID NOS:1839-1844), S214-216 (SEQ ID NOS:1845-1847), S219-223 (SEQ ID NOS:1852-1856), S227 (SEQ ID NO:1858), S234-245 (SEQ ID NOS:1869-1880), S248-S251(SEQ ID NOS:1883-1886), S264-S265 (SEQ ID NOS:1990-1991), S268 (SEQ ID NO:1903), S278 (SEQ ID NO:1905), S287 (SEQ ID NO:1911), S294-S295 (SEQ ID NOS:1922-1923), S315 (SEQ ID NO:1937), S319-322 (SEQ ID NOS:1940-1943), S326 (SEQ ID NO:1600), S342 (SEQ ID NO:1962), S365-S366 (SEQ ID NOS:1987-1988), S371-S373 (SEQ ID NOS:1558, 1900-1901), S386-S403 (SEQ ID NOS:1559, 2005-2007, 1794, 2008-2009, 1788, 1787, 1789, 2010-2011, 1791, and 2012-2014), RB437 (SEQ ID NO:2164), RB502 (SEQ ID NO:2170), RB452 (SEQ ID NO:2173), RB513 (SEQ ID NO:2176), RB464 (SEQ ID NO:2179), RB596 (SEQ ID NO:2202), RB569 (SEQ ID NO:2203), and RB570 (SEQ ID NO:2204).

**10**. The method according to claim 1, wherein the Formula 6 sequence is selected from the group consisting of S256 (SEQ ID NO:1893), S263 (SEQ ID NO:1899), S266 (SEQ ID NO:1902), S284-285 (SEQ ID NOS:1909-1910), S515 (SEQ ID NO:2102), and RB426 (SEQ ID NO:2158).

**11**. The method according to claim 1, wherein the Formula 1 sequence is selected from the group consisting of SEQ ID NO:1556; SEQ ID NO:1557; SEQ ID NO:1558; SEQ ID NO:1559; SEQ ID NO:1561; SEQ ID NO:1562; SEQ ID NO:1563; SEQ ID NO:1564; SEQ ID NO:1565; SEQ ID NO:1566; SEQ ID NO:1567; SEQ ID NO:1568; SEQ ID NO:2130; and SEQ ID NO:1560.

**12**. The method according to claim 1, wherein the Formula 6 sequence is a D8 sequence selected from the group consisting of: SEQ ID NO:2227; SEQ ID NO:1579; SEQ ID NO:1580; SEQ ID NO:1581; SEQ ID NO:1582; SEQ ID NO:1583; and SEQ ID NO:1584.

**13**. The method according to claim 1, wherein the amino acid sequence is sequence 539 (SEQ ID NO:2116).

**14**. The method according to claim 1, wherein the amino acid sequence is selected from the group consisting of RP27 (SEQ ID NO:2213), RP28 (SEQ ID NO:2214), RP29 (SEQ ID NO:2215), RP30 (SEQ ID NO:2216), RP31 (SEQ ID NO:2217), RP32 (SEQ ID NO:2218), RP33 (SEQ ID NO:2219), RP34 (SEQ ID NO:2220), RP35 (SEQ ID NO:2221), and RP36 (SEQ ID NO:2222).

**15**. The method according to claim 1, wherein the amino acid sequence is selected from the group consisting of D8-6aa-S175 (SEQ ID NO:2121), D8-12aa-S175 (SEQ ID NO:2122), D8-6aa-RP6 (SEQ ID NO:2126), and D8-6aa-RP17 (SEQ ID NO:2127).

**16**. The method according to claim 1, wherein the amino acid sequence is selected from the group consisting of S429 (SEQ ID NO:2032), S455 (SEQ ID NO:2060), S457-S458 (SEQ ID NOS:2063-2064), S467-S468 (SEQ ID NOS:2066-2067), S471 (SEQ ID NO:2068), S481-S513 (SEQ ID NOS:2069-2101), S517-S520 (SEQ ID NOS:2104-2107), S524 (SEQ ID NO:2111), RB539 (SEQ ID NO:2196), RB625—RB626 (SEQ ID NOS:2200 and 2199), and RB622 (SEQ ID NO:2201).

**17**. The method according to claim 1, wherein the amino acid sequence is selected from the group consisting of: S527-S546 (SEQ ID NOS:2228-2247); S549 (SEQ ID NO:2250), S551-S591 (SEQ ID NOS:2252-2300); S594-S624 (SEQ ID NOS:2303-2332); S626-S639 (SEQ ID NOS:2334-2347); and S641-S648 (SEQ ID NOS:2349-2356).

**18**. The method according to claim 16, wherein the amino acid sequence is selected from the group consisting of S557 (SEQ ID NO:2258) and S597 (SEQ ID NO:2306).

**19**. The method according to claim 4, wherein the mammalian cell is in a mammal.

**20**. The method according to claim 19, wherein the mammal is a human suffering from diabetes and the method comprises delivering a therapeutically effective amount of the amino acid to the human as a diabetes treatment.

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