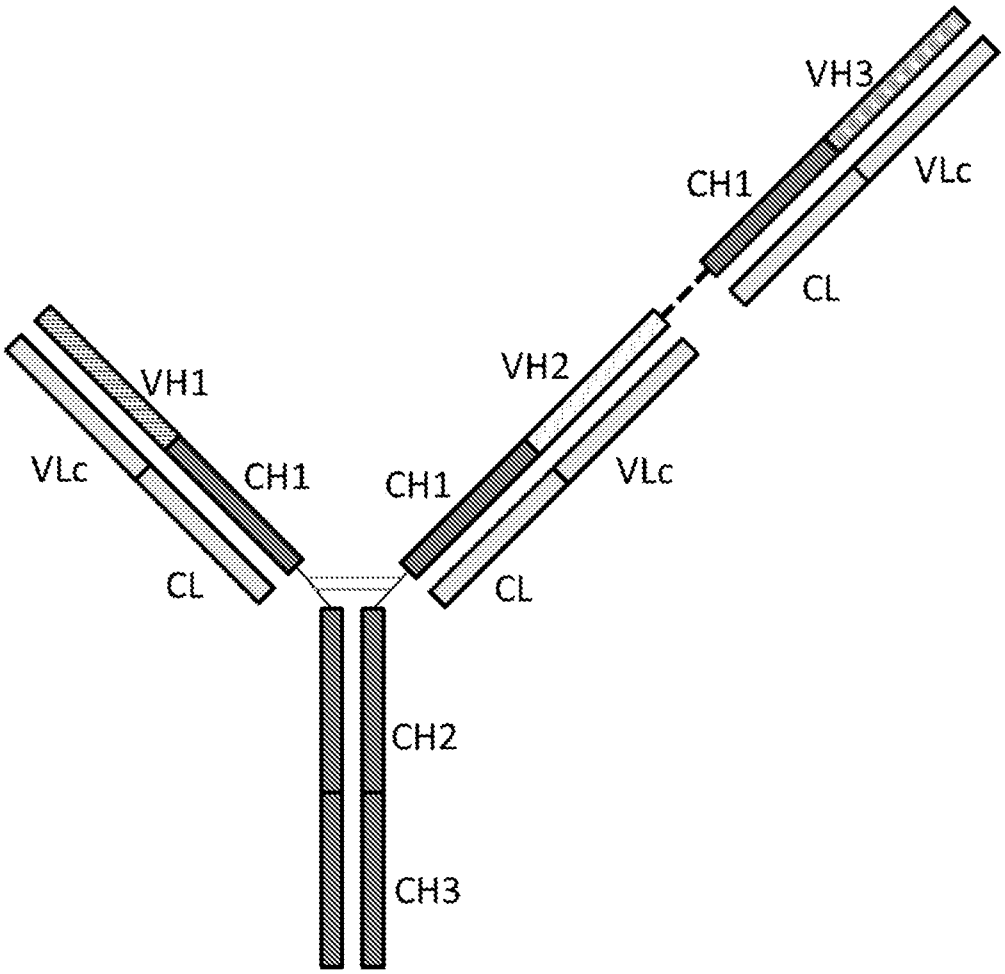



Fig. 1a

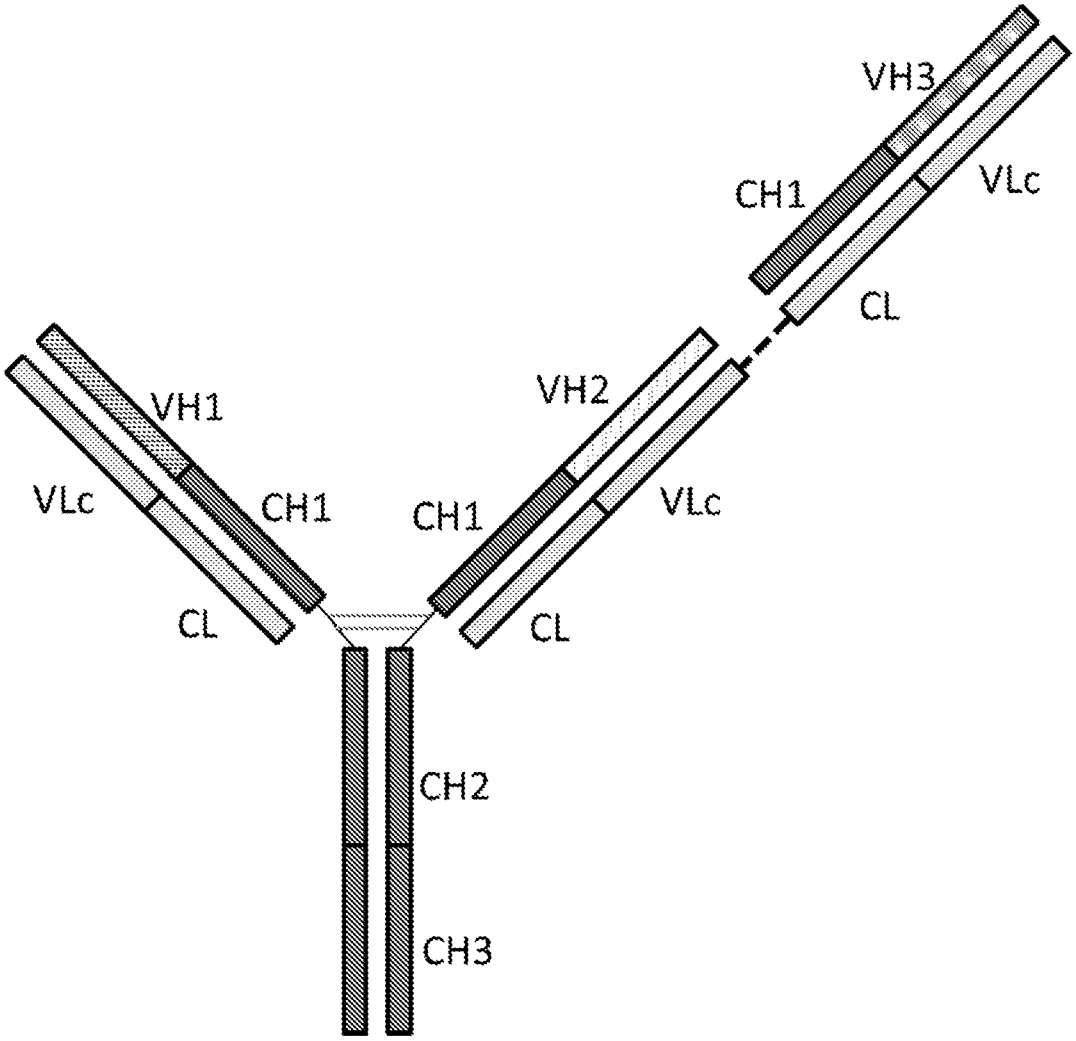



Fig. 1b

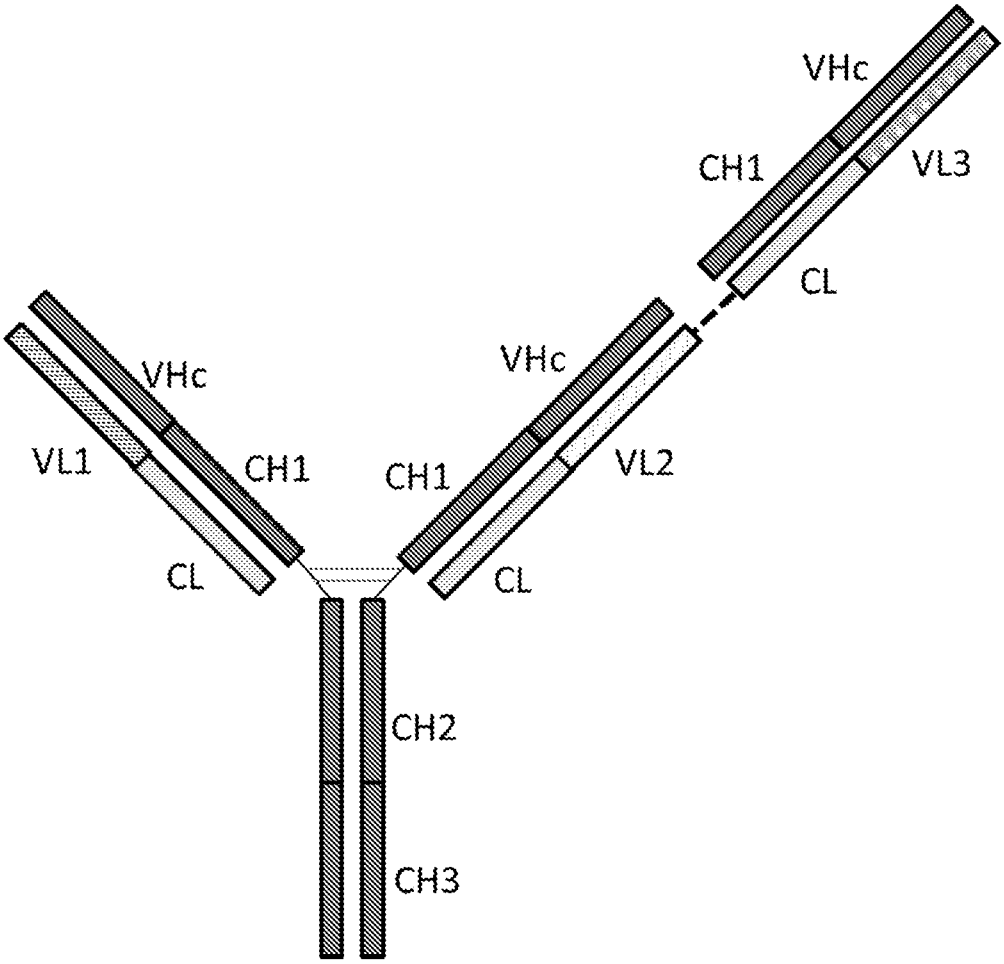



Fig. 1c

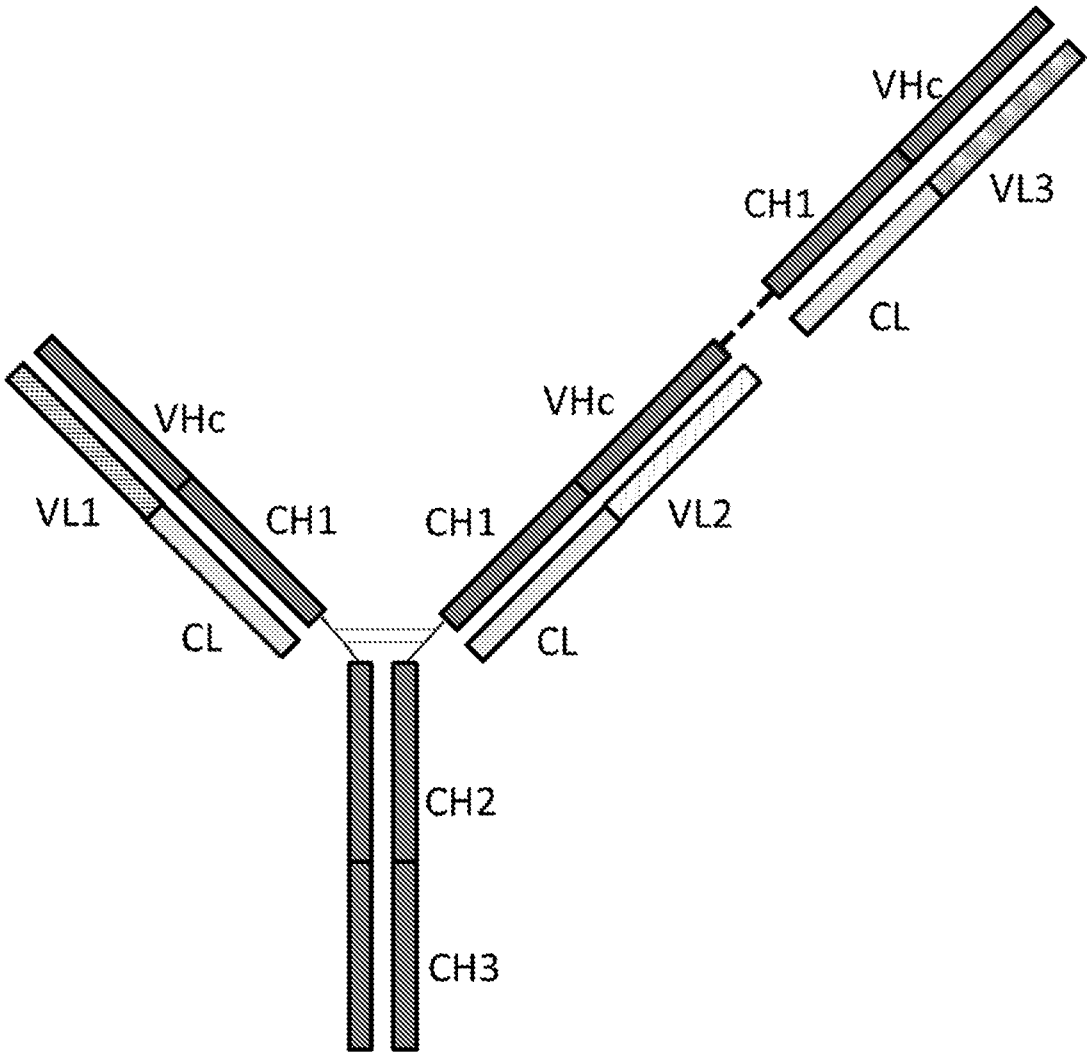



Fig. 1d

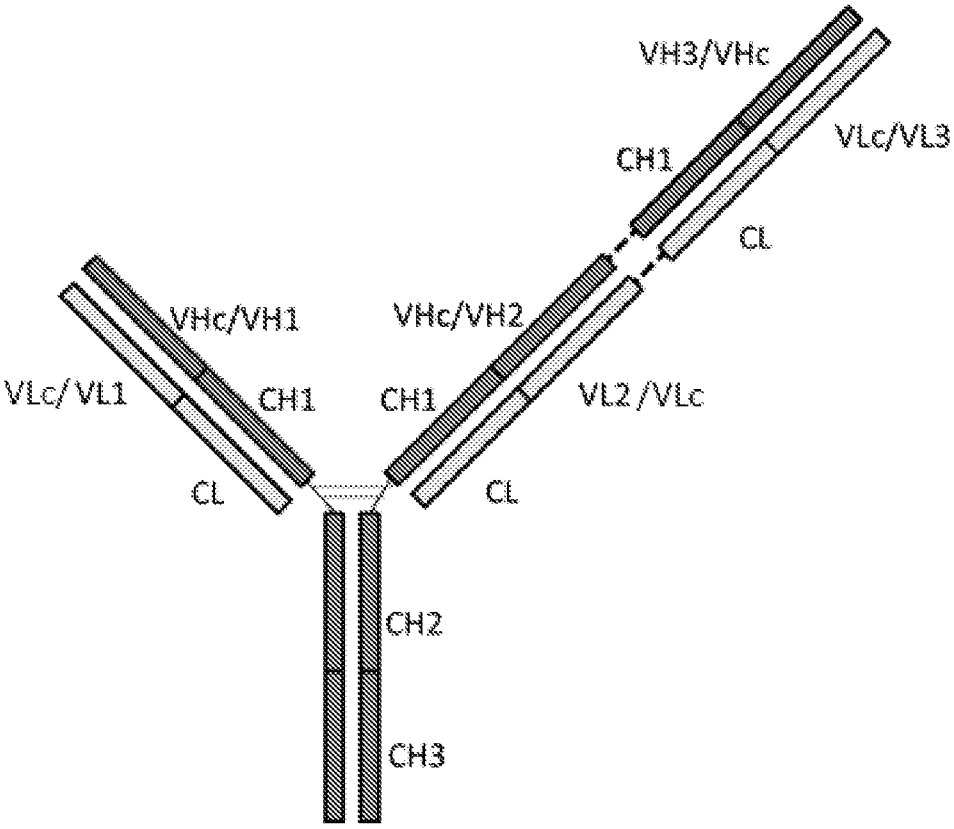

VH3/VHc
VLc/VL3
CH1
CL
VHc/VH1
VHc/VH2
VLc/VL1
CH1
CH1
VL2/VLc
CL
CL
CH2
CH3

Fig. 1e

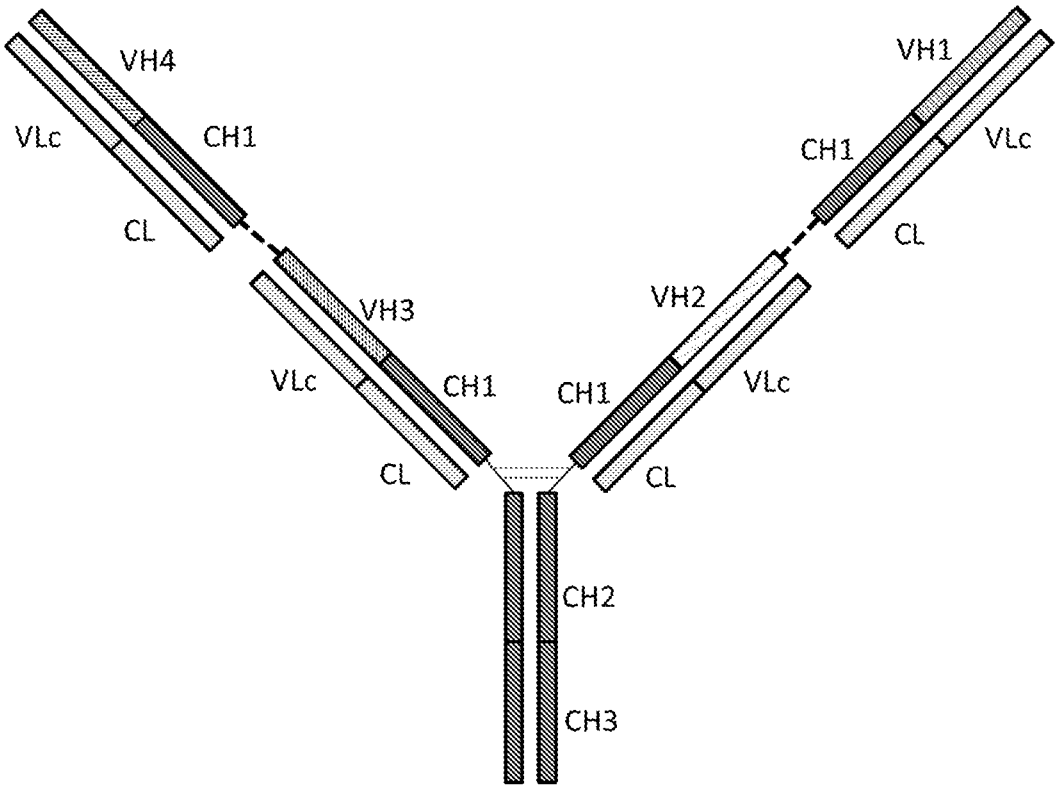

VH4
VLc
CH1
CL
VH3
VLc
CH1
CL
VH2
CH1
VLc
CL
VH1
CH1
VLc
CL
CH2
CH3

Fig. 1f

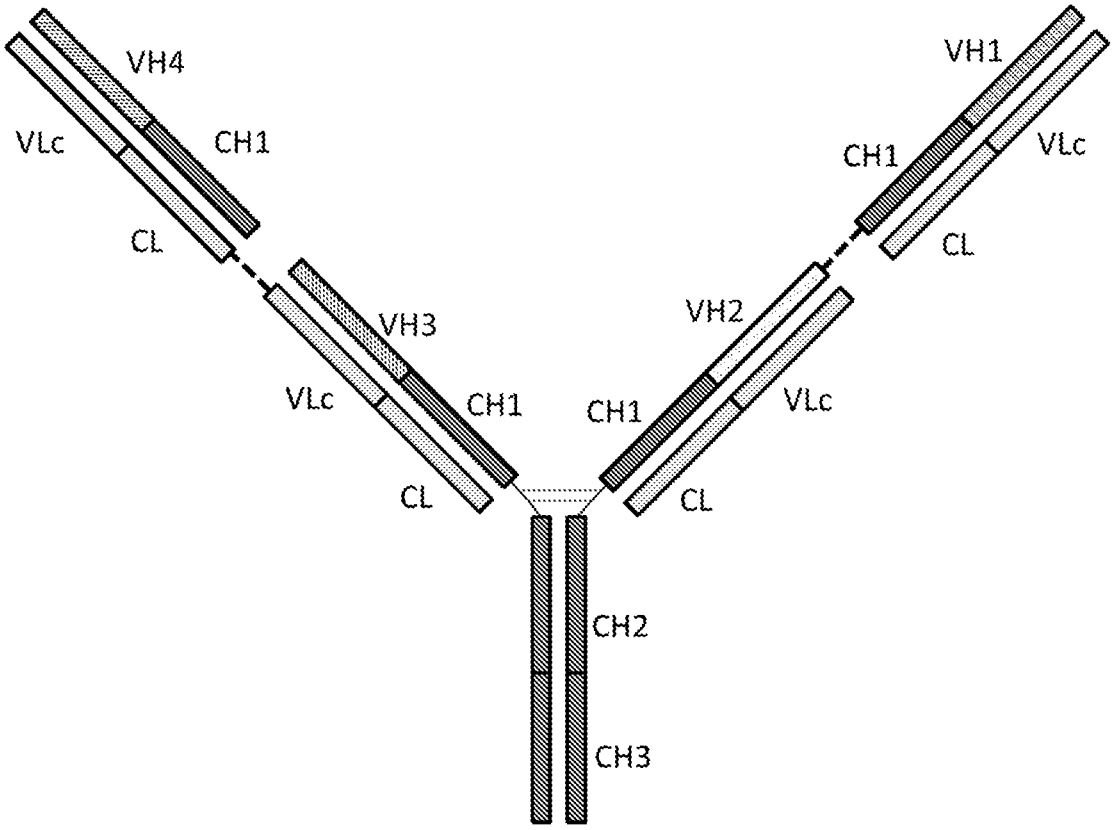

VH4
VLc
CH1
CL
VH3
VLc
CH1
CL
VH2
CH1
VLc
CL
VH1
CH1
VLc
CL
CH2
CH3

Fig. 1g

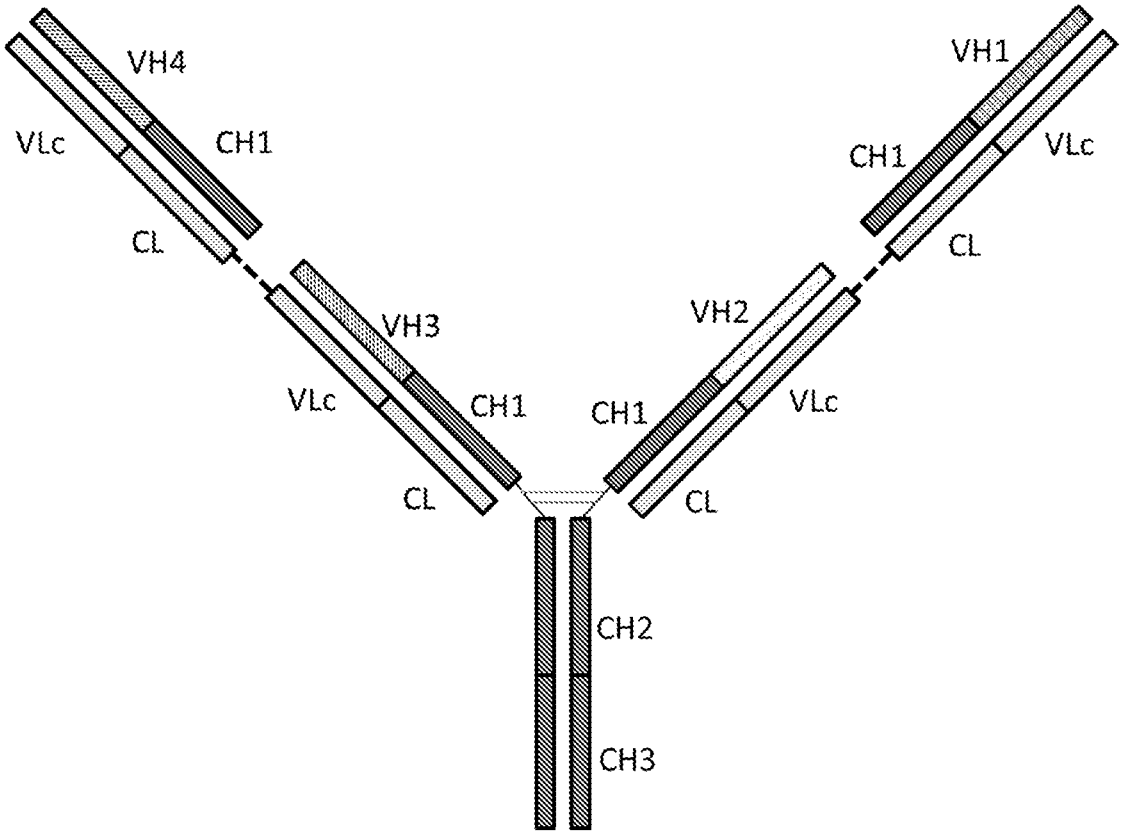

VH4
VLc
CH1
CL
VH3
VLc
CH1
CL
VH2
CH1
VLc
CL
VH1
CH1
VLc
CL
CH2
CH3

Fig. 1h

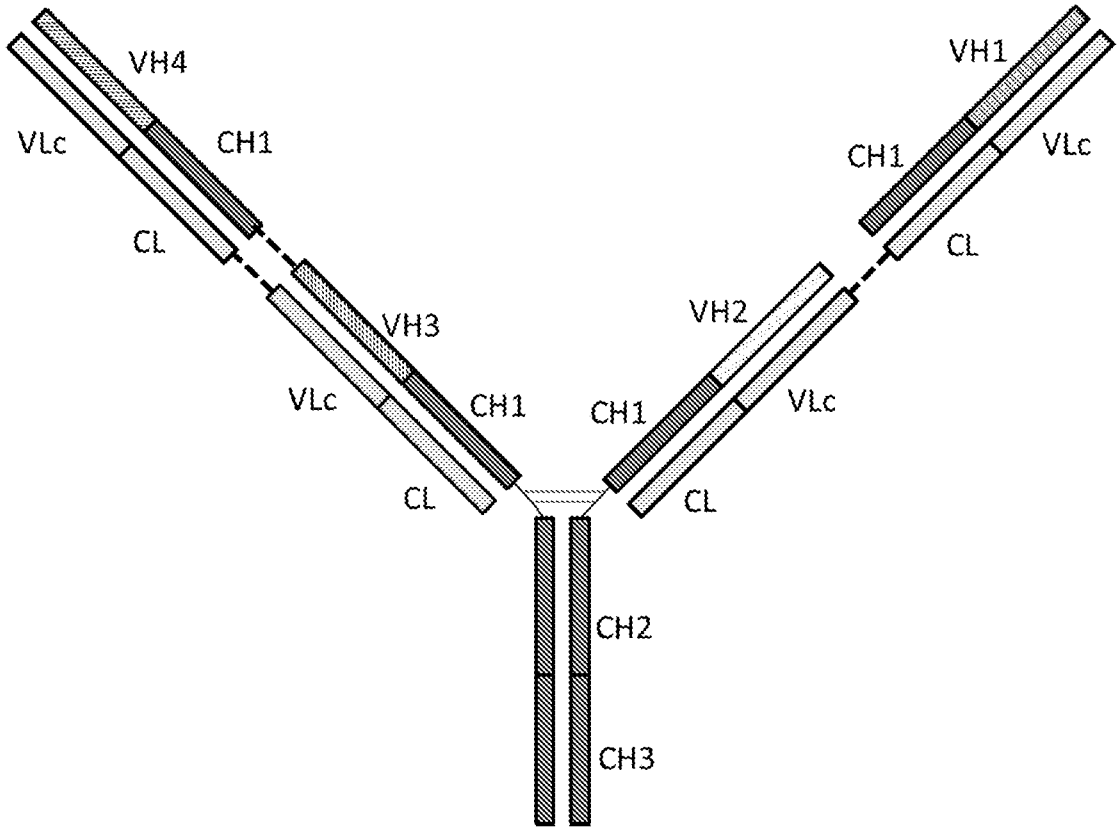

VH4
VLc
CH1
CL
VH3
VLc
CH1
CL
VH2
CH1
VLc
CL
VH1
CH1
VLc
CL
CH2
CH3

Fig. 1i

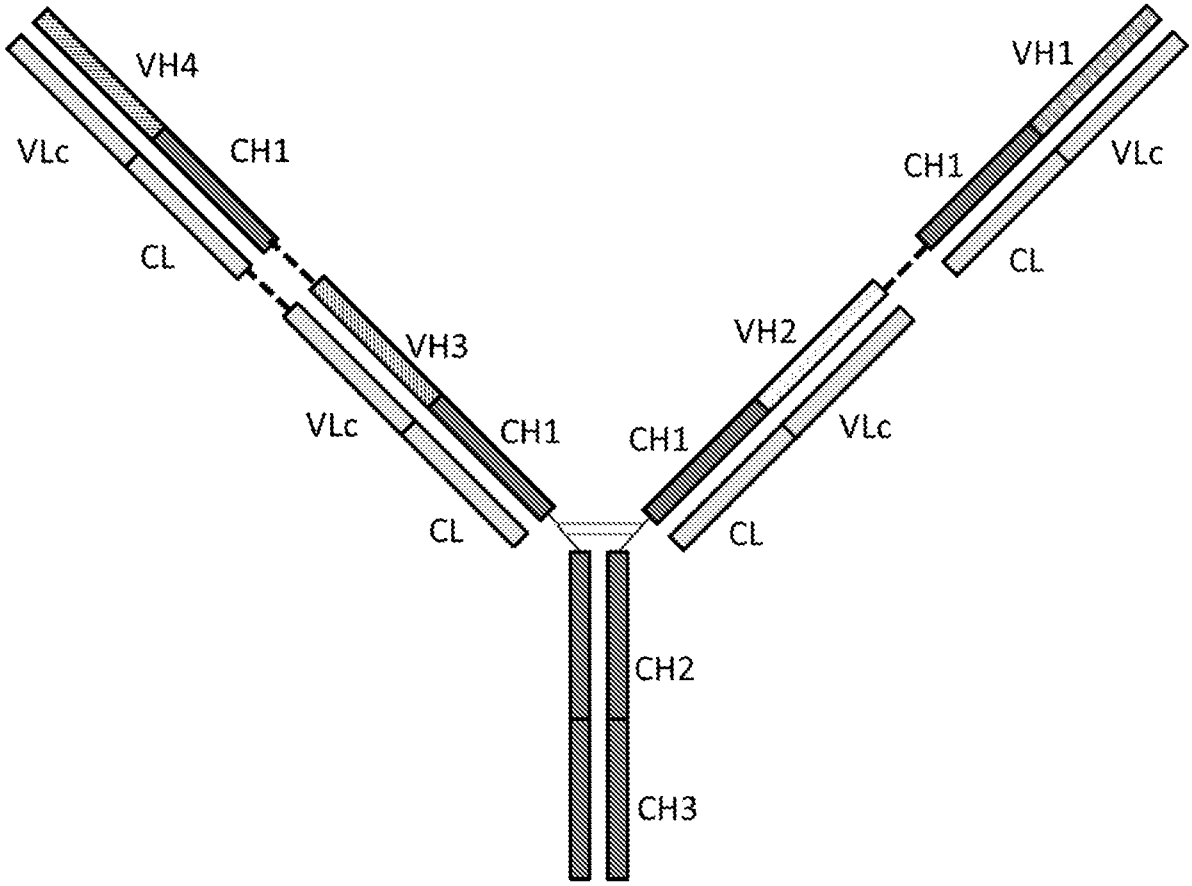

VH4
VLc
CH1
CL
VH3
VLc
CH1
CL
VH2
CH1
VLc
CL
VH1
CH1
VLc
CL
CH2
CH3

Fig. 1j

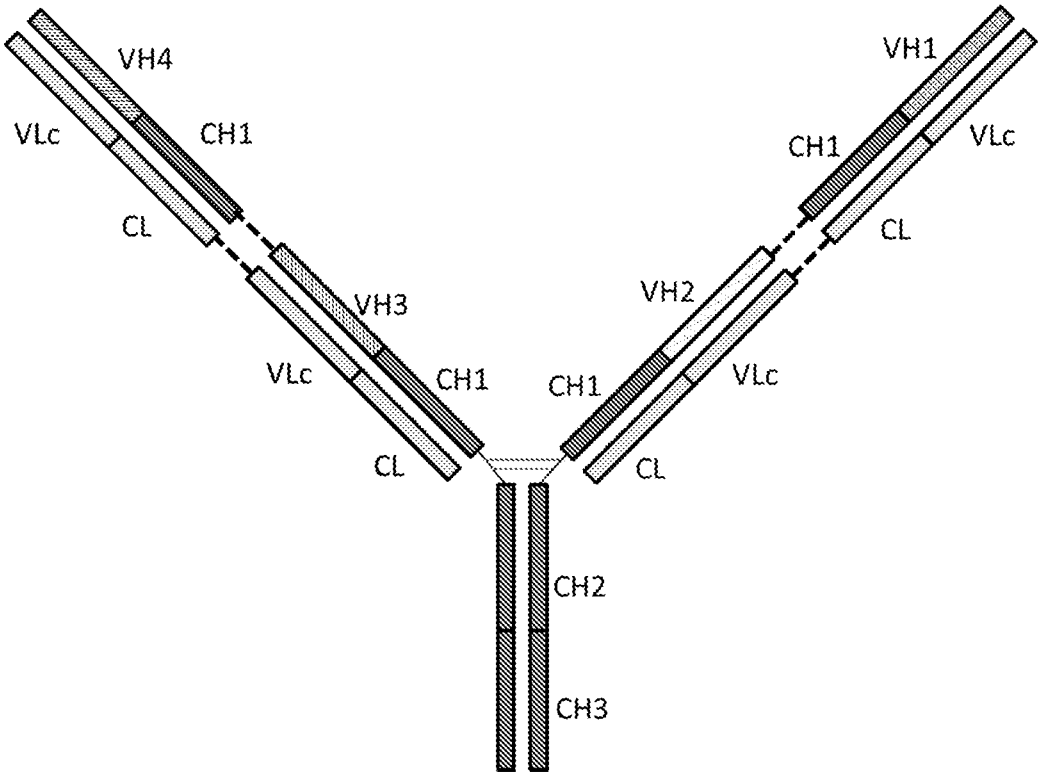

VH4
VLc
CH1
CL
VH3
VLc
CH1
CL
VH1
CH1
VLc
CL
VH2
CH1
VLc
CL
CH2
CH3

Fig. 1k

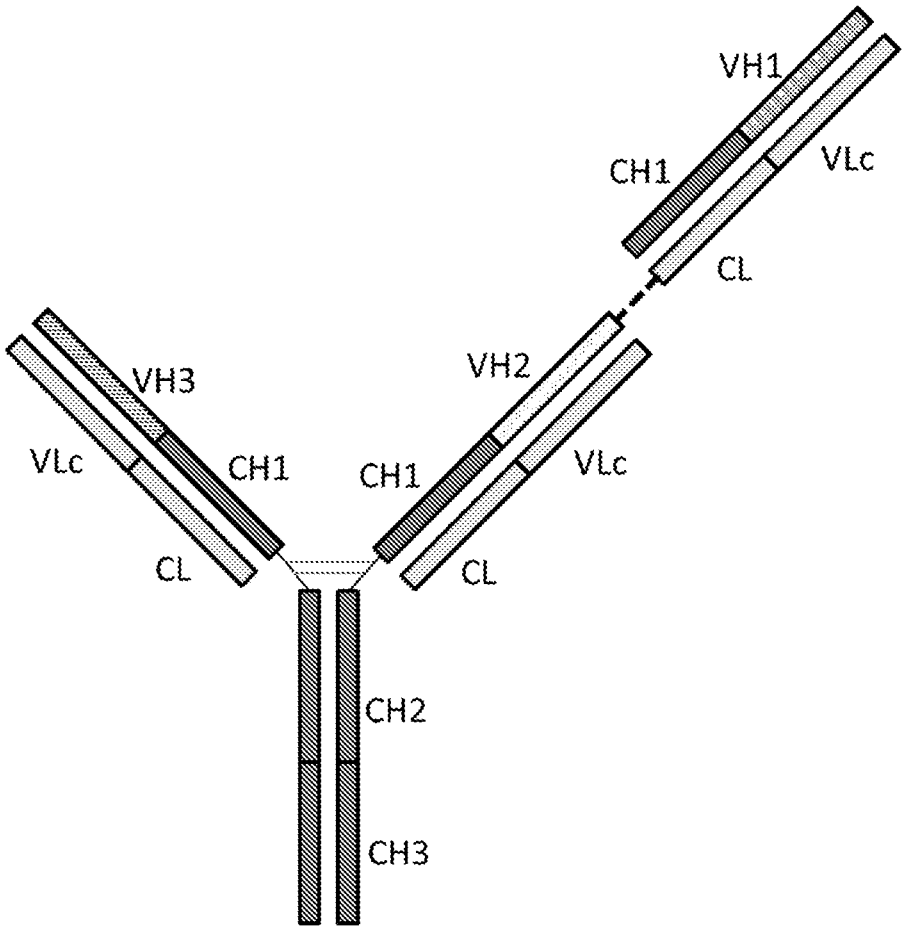



Fig. 1I

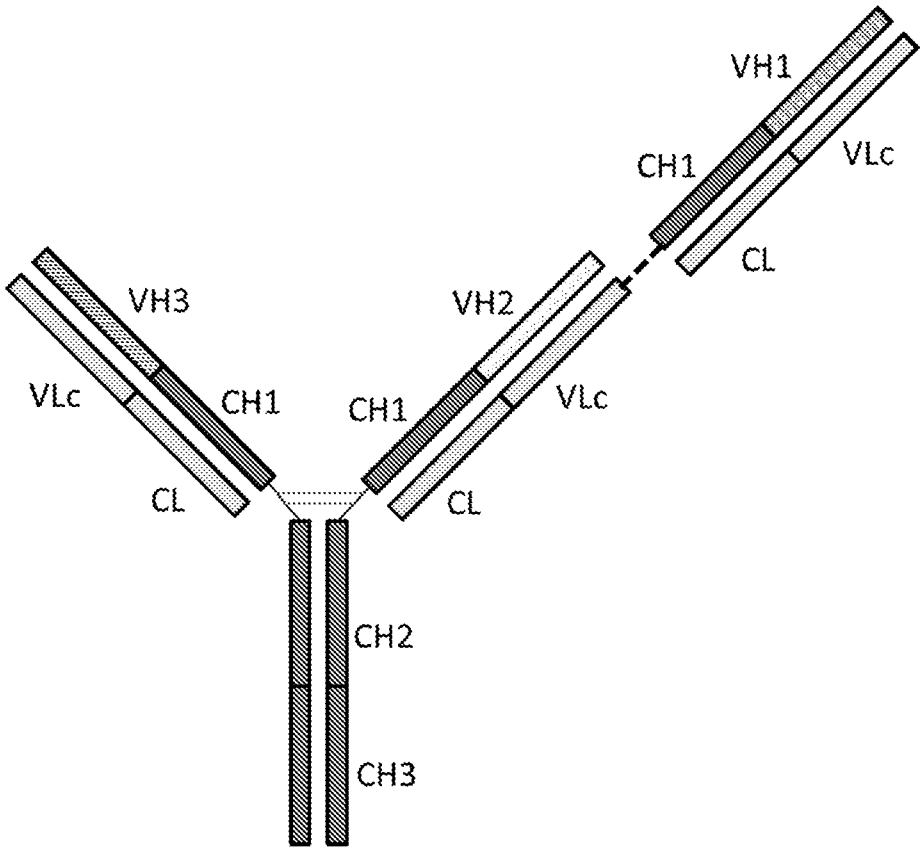



Fig. 1m

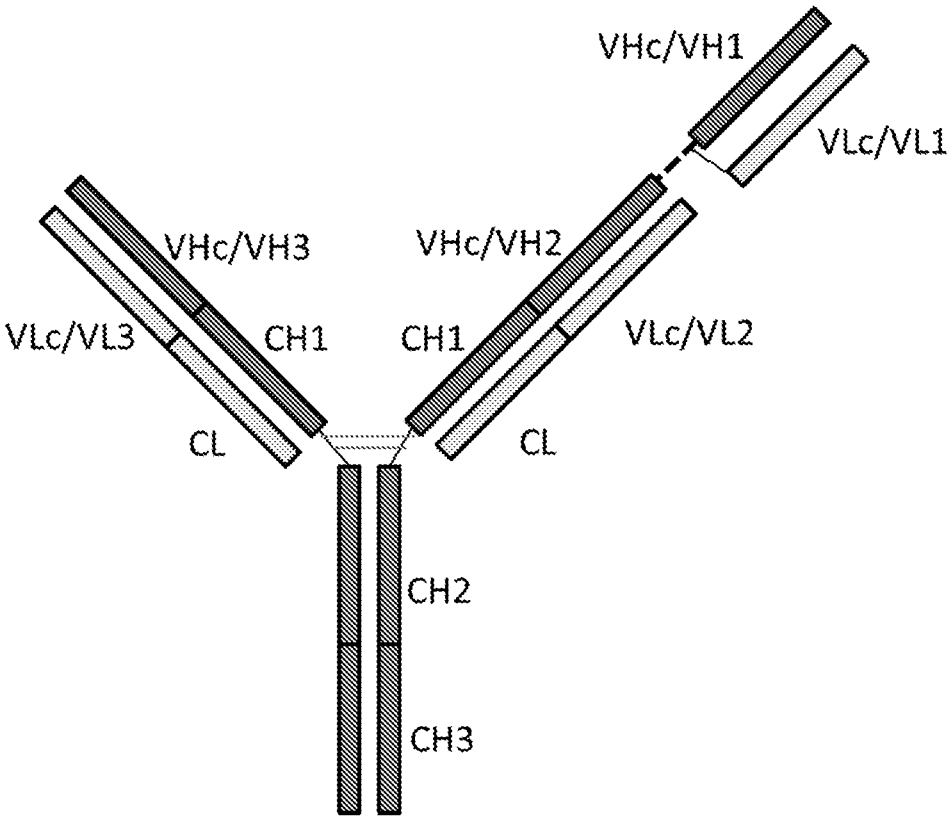

VHc/VH1
VLc/VL1
VHc/VH3
VHc/VH2
VLc/VL3
CH1
CH1
VLc/VL2
CL
CL
CH2
CH3

Fig. 1n

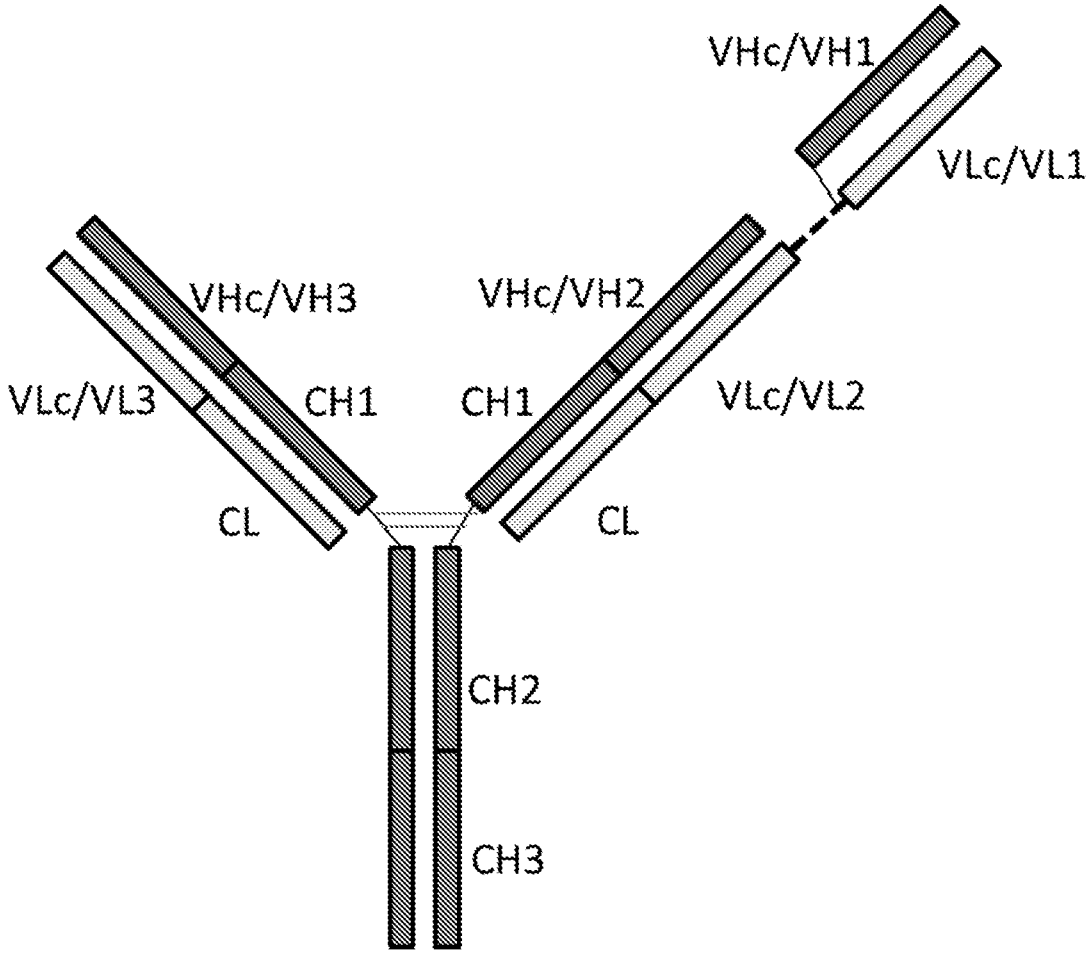

VHc/VH1
VLc/VL1
VHc/VH3
VHc/VH2
VLc/VL3
CH1
CH1
VLc/VL2
CL
CL
CH2
CH3

Fig. 1o

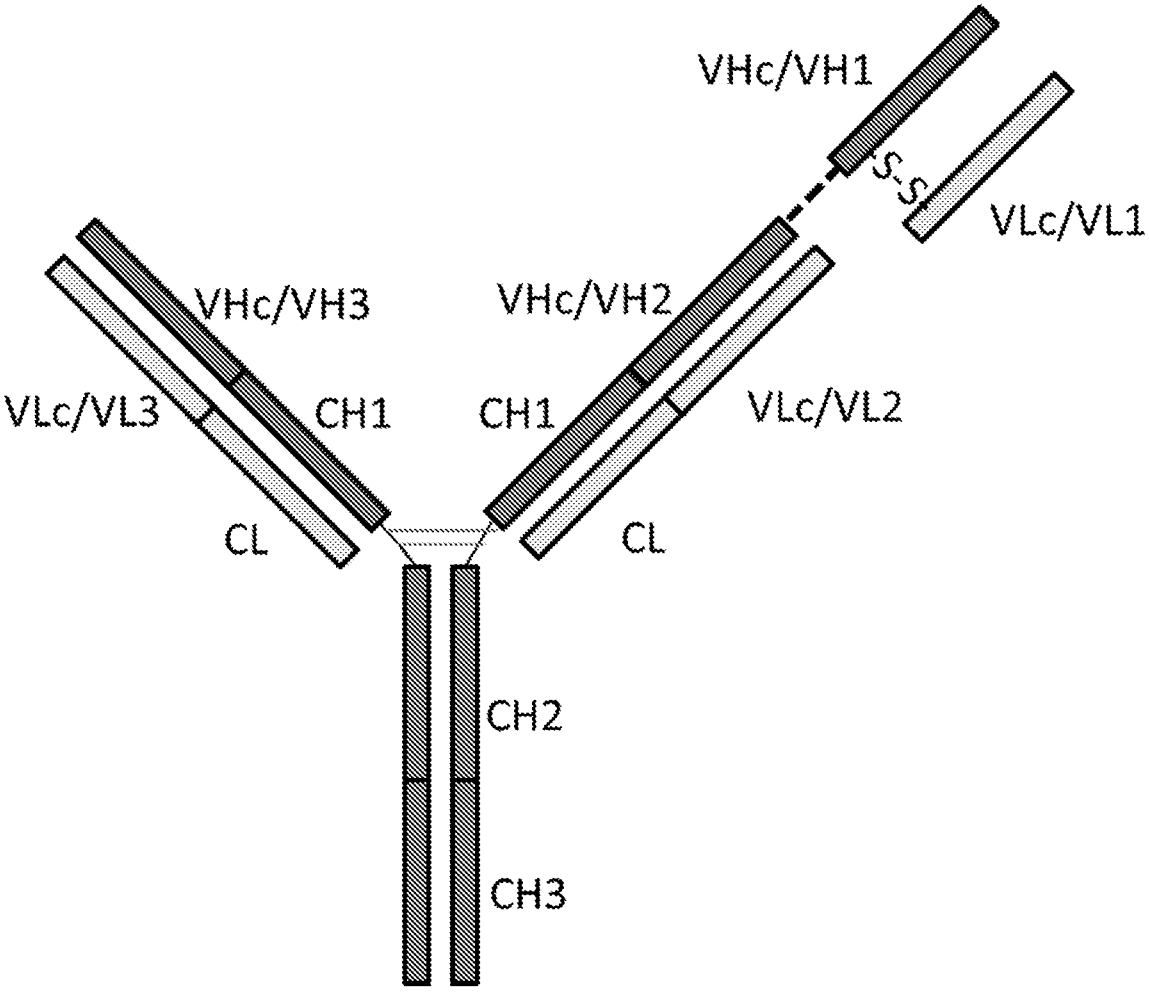

VHc/VH1
S-S
VLc/VL1
VHc/VH3
VHc/VH2
VLc/VL3
CH1
CH1
VLc/VL2
CL
CL
CH2
CH3

Fig. 1p

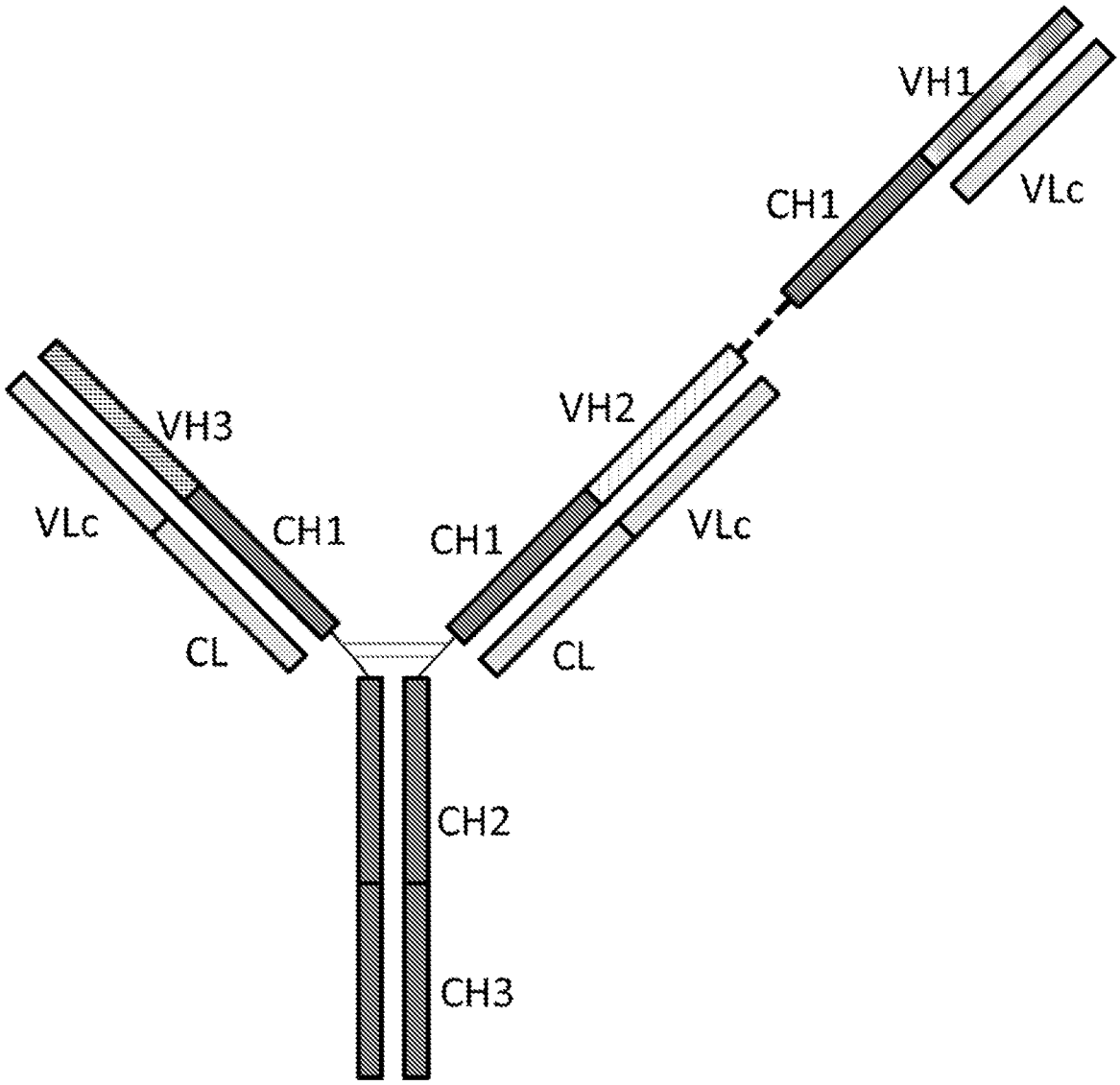



Fig. 1q

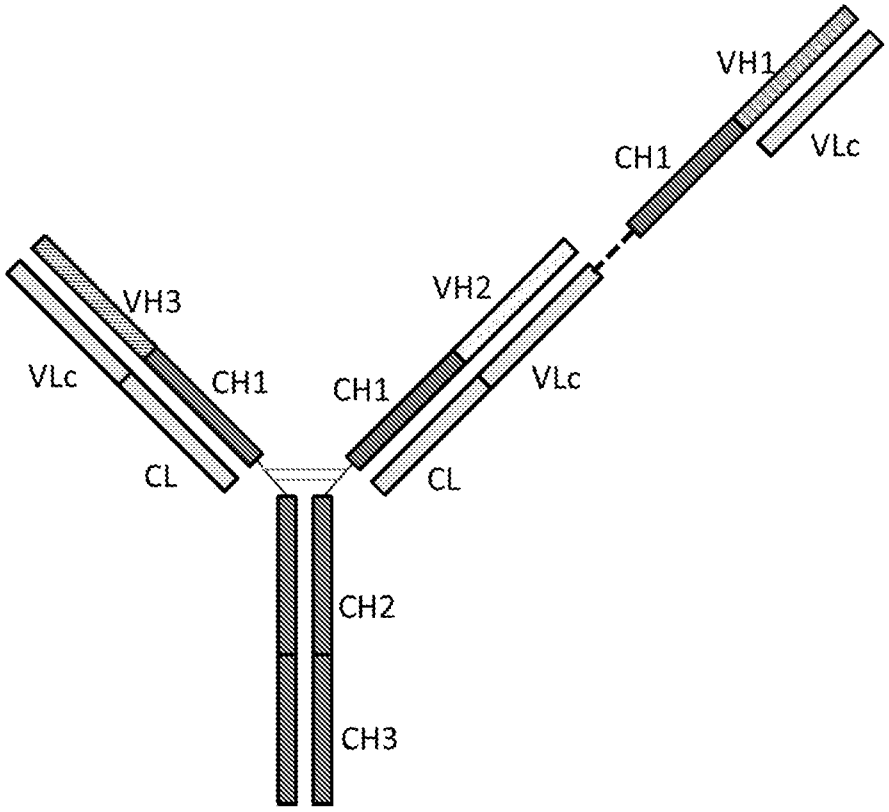



Fig. 1r

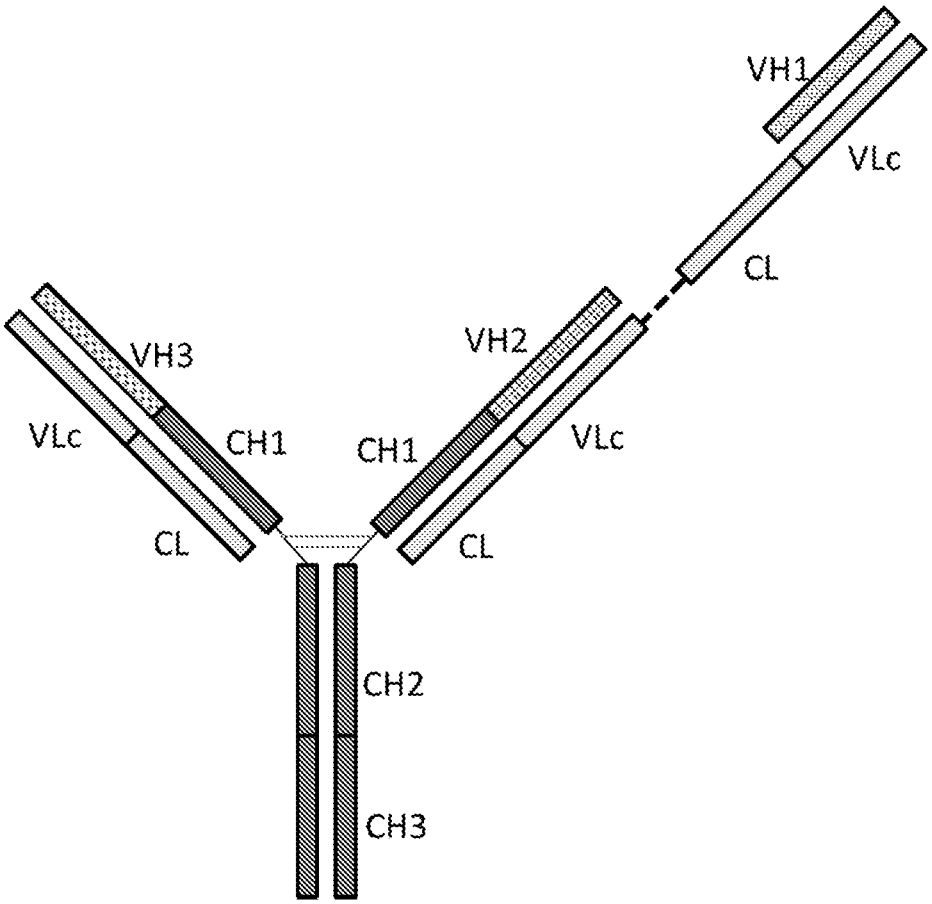



Fig. 1s

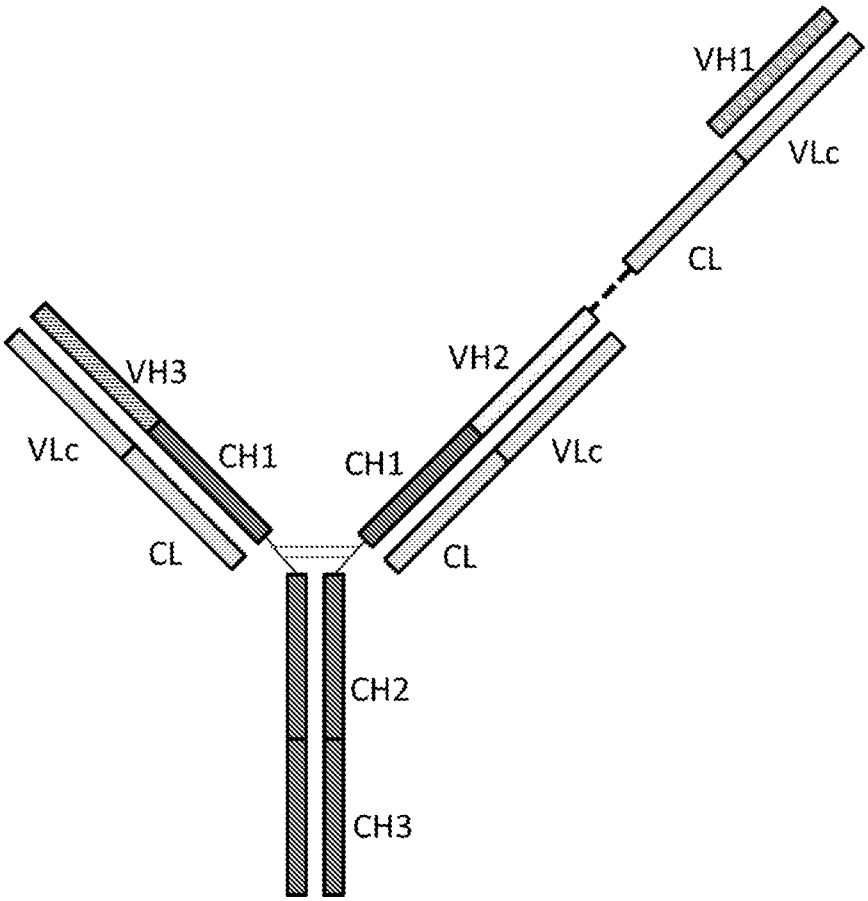



Fig. 1t

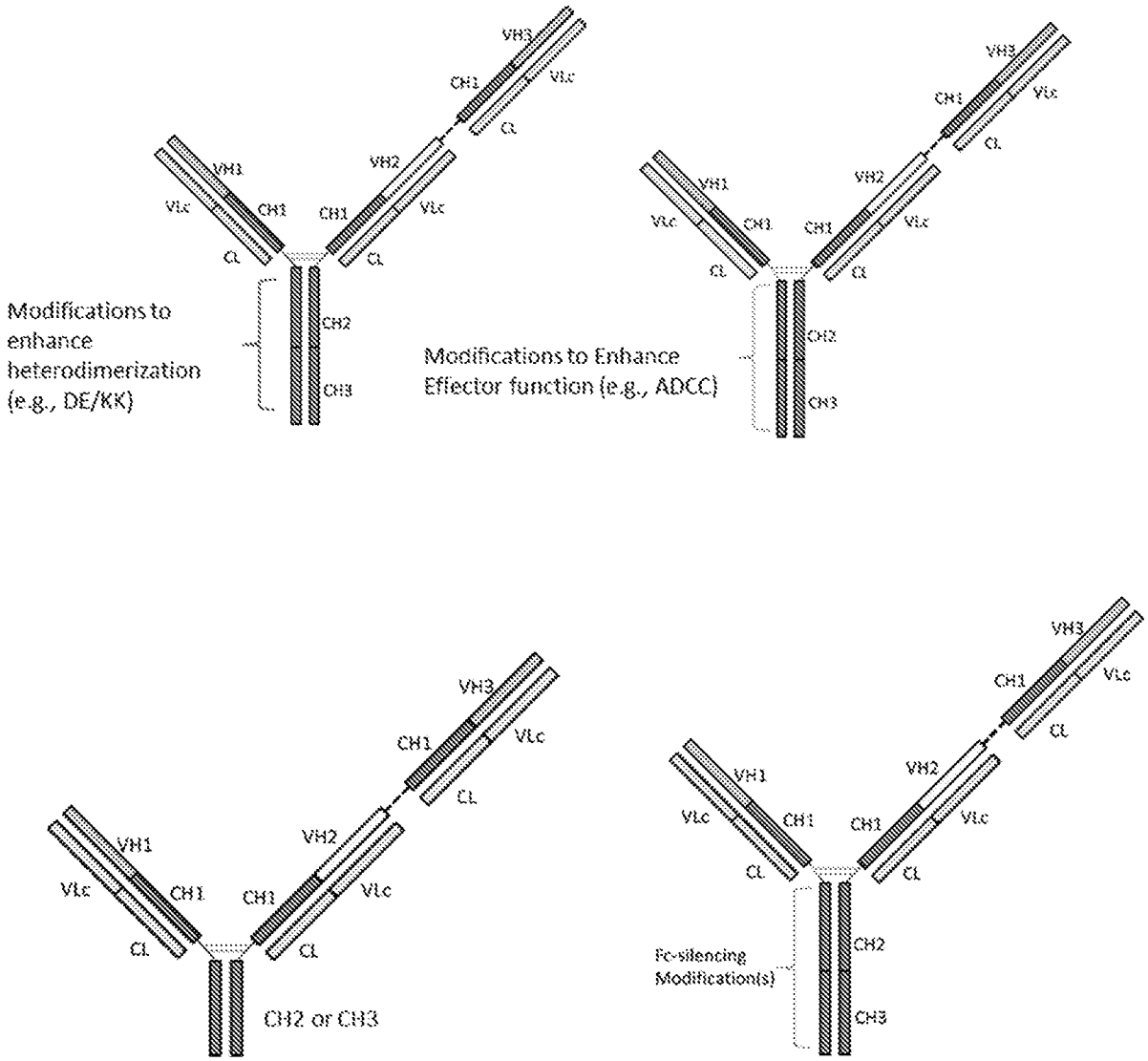
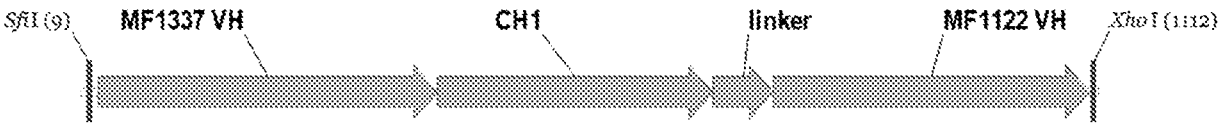

VH3
CH1
VLc
CL
VH1
VLc
CH1
VH2
CH1
VLc
CL
CL
CH2
CH3
Modifications to enhance heterodimerization (e.g., DE/KK)
Modifications to Enhance Effector function (e.g., ADCC)
CH2 or CH3
Fc-silencing Modification(s)

Fig. 1u




IgG1H

1117 bp

Fig. 2a

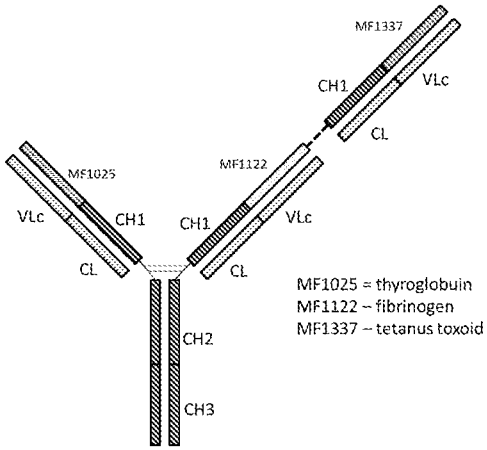



Fig. 2b

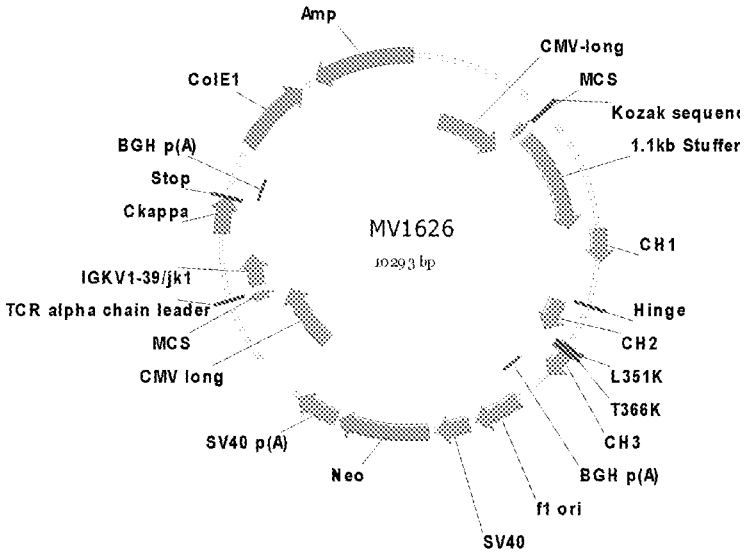

Amp
CMV-long
MCS
Kozak sequen
1.1kb Stuffer
CH1
Hinge
CH2
L351K
T366K
CH3
BGH p(A)
f1 ori
SV40
Neo
SV40 p(A)
CMV long
MCS
TCR alpha chain leader
IGKV1-39/jk1
Ckappa
Stop
BGH p(A)
ColE1
MV1626
10293 bp

Fig. 3

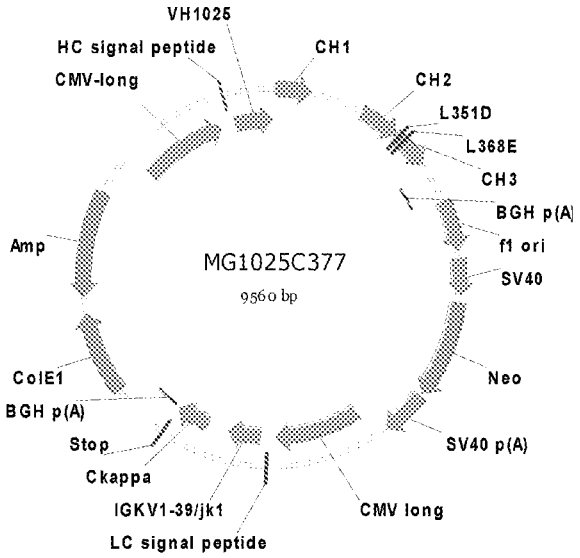

VH1025
HC signal peptide
CMV-long
CH1
CH2
L351D
L368E
CH3
BGH p(A)
f1 ori
SV40
Neo
SV40 p(A)
CMV long
LC signal peptide
IGKV1-39/jk1
Ckappa
Stop
BGH p(A)
ColE1
Amp
MG1025C377
9560 bp

Fig. 4

| | | SEQ ID NO: |
|---|---|---|
| Translation of IgG1M | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTSPPSPAPELLGG------EVQL | 259 |
| Translation of IgG1M4 | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTSPPSP----------EVQL | 260 |
| Translation of IgG1 UM | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHT----------EVQL | 261 |
| Translation of IgG1G4S | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDGGGSGGGGS----------EVQL | 263 |
| Translation of IgG1L | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTAPELLG--G----------EVQL | 263 |
| Translation of IgG1R | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTEAAAKEAAAKAPELLGG--EVQL | 264 |
| Translation of IgG2AH | ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKSSVESPPSPAPPVAG----------EVQL | 265 |
| Translation of IgG2AMR | ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKSSVESPPSP----------EVQL | 266 |
| Translation of IgG2AGL | ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKSSVEAPPVAG----------EVQL | 267 |
| Translation of IgG2AGAS | ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVGGGSGGGGS----------EVQL | 268 |
| Translation of IgG2AG4SL | ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVGGGSGGGGSAPPVAG----------EVQL | 269 |
| Translation of IgG2AR | ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKSVEEAAAKEAAAKAPPVAG----------EVQL | 270 |
| Translation of IgG2BH | ASTKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKRVELKTPLGDTTHT----------EVQL | 271 |
| Translation of IgG2BMH | ASTKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCSVESPPSP----------EVQL | 272 |
| Translation of IgG2R4L | ASTKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCSVEAPPVAG----------EVQL | 273 |
| Translation of IgG2ER | ASTKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCSVEAAAKEAAAKAPPVAG-----EVQL | 274 |
| Translation of IgG3UH | ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVELKTPLGDTTHT----------EVQL | 275 |
| Translation of IgG3UR | ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVELKTPLGDTTHTEAAAKEAAAKAPEFLGGEVQL | 277 |
| Translation of IgG4H | ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPSPSSPAPEFLGG----------EVQL | 278 |
| Translation of IgG4UH | ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPP----------EVQL | 279 |
| Translation of IgG4MH | ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPSPSSP----------EVQL | 280 |
| Translation of IgG4R | ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPEAAAKEAAAKAPEFLGG----EVQL | 281 |
| Translation of IgG4YL | ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPAPEFLGG----------EVQL | 282 |
| Consensus | ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKRVE-KS----TS----------EVQL | 283 |

Figure 5

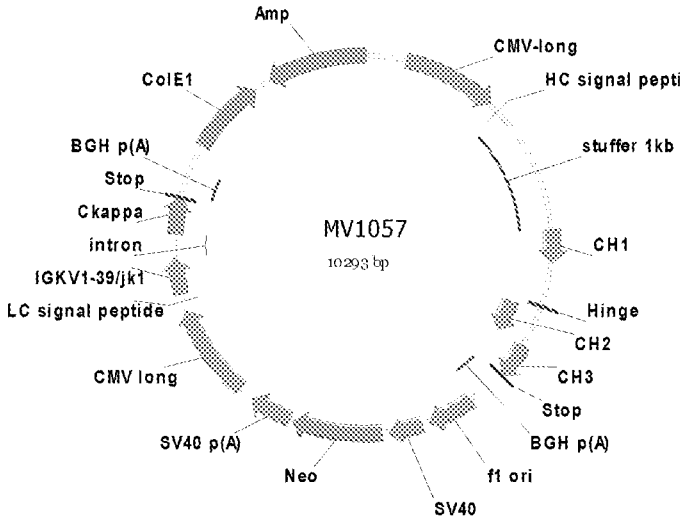



Figure 6

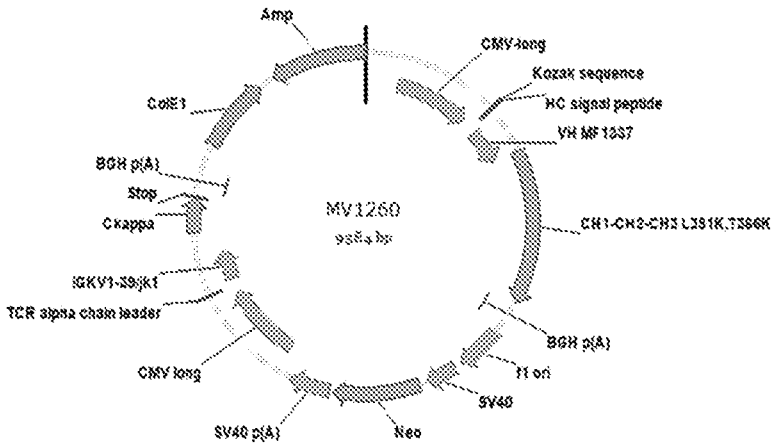



Figure 7

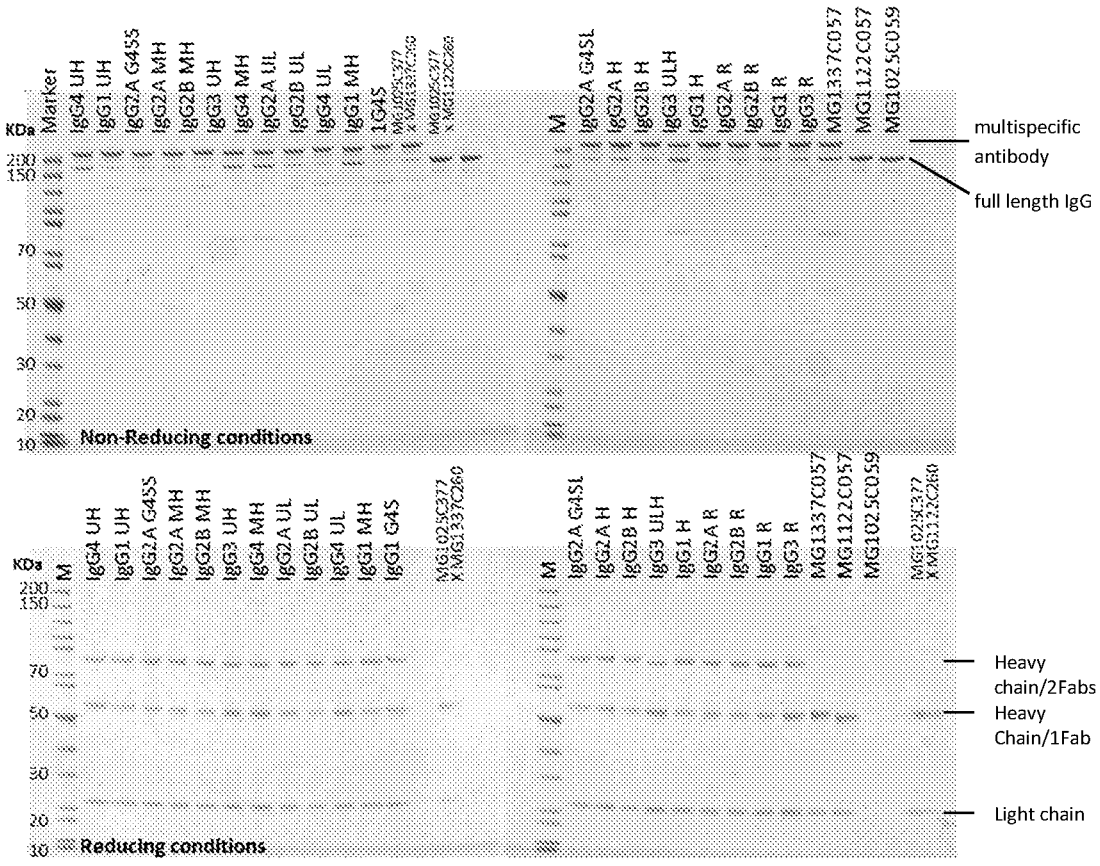
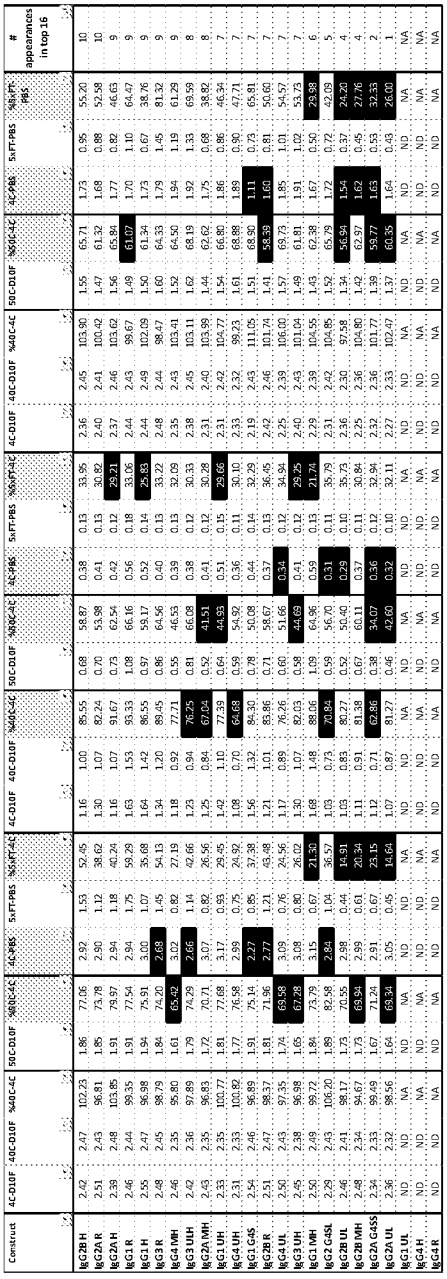

KDa
Marker
IgG4 UH
IgG1 UH
IgG2A G4SS
IgG2A MH
IgG2B MH
IgG3 UH
IgG4 MH
IgG2A UL
IgG2B UL
IgG4 UL
IgG1 MH
IgG1 G4S
MG1025C059
MG1122C057
MG1337C057
200
150
70
50
30
20
10
Non-Reducing conditions
multispecific antibody
full length IgG
M
IgG2A G4SL
IgG2A H
IgG2B H
IgG3 ULH
IgG1 H
IgG2A R
IgG2B R
IgG1 R
IgG3 R
MG1025C377 X MG1337C260
MG1025C377 X MG1122C260
Reducing conditions
Heavy chain/2Fabs
Heavy Chain/1Fab
Light chain

Fig. 8



| Construct | 4C-D10F | 40C-D10F | %40C-4C | 50C-D10F | %60C-4C | 4C-PBS | 5xFT-PBS | %5xFT-4C | 4C-D10F | 40C-D10F | %40C-4C | 50C-D10F | %50C-4C | 4C-PBS | 5xFT-PBS | %5xFT-4C | 4C-D10F | 40C-D10F | %40C-4C | 50C-D10F | %50C-4C | 4C-PBS | 5xFT-PBS | %5xFT-PBS | # appearances in top 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IgG2B H | 2.42 | 2.47 | 102.23 | 1.86 | 77.06 | 2.92 | 1.53 | 52.45 | 1.16 | 1.00 | 85.55 | 0.68 | 58.87 | 0.38 | 0.13 | 33.95 | 2.36 | 2.45 | 103.90 | 1.55 | 65.71 | 1.73 | 0.95 | 55.20 | 10 |
| IgG2A R | 2.51 | 2.43 | 96.81 | 1.85 | 73.78 | 2.90 | 1.12 | 38.62 | 1.30 | 1.07 | 82.24 | 0.70 | 53.98 | 0.41 | 0.13 | 30.82 | 2.40 | 2.41 | 100.42 | 1.47 | 61.32 | 1.68 | 0.88 | 52.58 | 10 |
| IgG2A H | 2.39 | 2.48 | 103.85 | 1.91 | 79.97 | 2.94 | 1.18 | 40.24 | 1.16 | 1.07 | 91.67 | 0.73 | 62.54 | 0.42 | 0.12 | 29.21 | 2.37 | 2.46 | 103.62 | 1.56 | 65.84 | 1.77 | 0.82 | 46.63 | 9 |
| IgG1 R | 2.46 | 2.44 | 99.35 | 1.91 | 77.54 | 2.94 | 1.75 | 59.29 | 1.63 | 1.53 | 93.33 | 1.08 | 66.16 | 0.56 | 0.18 | 33.65 | 2.44 | 2.43 | 99.67 | 1.49 | 61.07 | 1.70 | 1.10 | 64.47 | 9 |
| IgG1 H | 2.55 | 2.47 | 96.98 | 1.94 | 75.91 | 3.00 | 1.07 | 35.68 | 1.64 | 1.42 | 86.55 | 0.97 | 59.17 | 0.52 | 0.14 | 25.83 | 2.44 | 2.49 | 102.09 | 1.50 | 61.34 | 1.73 | 0.67 | 38.76 | 9 |
| IgG3 R | 2.48 | 2.45 | 98.79 | 1.84 | 74.20 | 2.68 | 1.45 | 54.13 | 1.34 | 1.20 | 89.45 | 0.86 | 64.56 | 0.40 | 0.13 | 33.22 | 2.48 | 2.44 | 98.47 | 1.60 | 64.33 | 1.79 | 1.45 | 81.32 | 9 |
| IgG4 MH | 2.46 | 2.35 | 95.80 | 1.61 | 65.42 | 3.02 | 0.82 | 27.19 | 1.18 | 0.92 | 77.71 | 0.55 | 46.53 | 0.39 | 0.13 | 32.09 | 2.35 | 2.43 | 103.41 | 1.52 | 64.50 | 1.94 | 1.19 | 61.29 | 9 |
| IgG3 UIH | 2.42 | 2.36 | 97.89 | 1.79 | 74.29 | 2.66 | 1.14 | 42.66 | 1.23 | 0.94 | 76.25 | 0.81 | 66.08 | 0.38 | 0.12 | 30.33 | 2.38 | 2.45 | 103.11 | 1.62 | 68.19 | 1.92 | 1.33 | 69.59 | 8 |
| IgG2A MH | 2.45 | 2.35 | 96.83 | 1.72 | 70.71 | 3.07 | 0.82 | 26.56 | 1.25 | 0.84 | 67.04 | 0.52 | 41.51 | 0.41 | 0.12 | 30.28 | 2.31 | 2.40 | 103.99 | 1.44 | 62.62 | 1.75 | 0.68 | 38.82 | 8 |
| IgG1 UH | 2.33 | 2.35 | 100.77 | 1.81 | 77.68 | 3.17 | 0.93 | 29.45 | 1.42 | 1.10 | 77.39 | 0.64 | 44.55 | 0.51 | 0.15 | 29.66 | 2.31 | 2.42 | 104.77 | 1.54 | 66.80 | 1.86 | 0.86 | 46.34 | 7 |
| IgG4 UH | 2.31 | 2.33 | 100.82 | 1.77 | 76.58 | 2.99 | 0.75 | 24.92 | 1.08 | 0.70 | 64.68 | 0.59 | 54.92 | 0.36 | 0.11 | 30.10 | 2.33 | 2.32 | 99.23 | 1.61 | 68.88 | 1.89 | 0.90 | 47.71 | 7 |
| IgG1 G4S | 2.54 | 2.46 | 96.89 | 1.91 | 75.14 | 2.27 | 0.85 | 37.38 | 1.56 | 1.32 | 84.30 | 0.78 | 50.08 | 0.44 | 0.14 | 32.29 | 2.19 | 2.43 | 111.05 | 1.51 | 68.90 | 1.11 | 0.73 | 65.81 | 7 |
| IgG2B R | 2.51 | 2.47 | 98.37 | 1.81 | 71.96 | 2.77 | 1.21 | 43.48 | 1.21 | 1.01 | 83.86 | 0.71 | 58.67 | 0.37 | 0.13 | 36.45 | 2.42 | 2.46 | 101.74 | 1.41 | 58.39 | 1.60 | 0.81 | 50.60 | 7 |
| IgG4 UL | 2.50 | 2.43 | 97.35 | 1.74 | 69.58 | 3.09 | 0.76 | 24.56 | 1.17 | 0.89 | 70.46 | 0.60 | 51.66 | 0.34 | 0.12 | 34.94 | 2.25 | 2.39 | 106.00 | 1.57 | 69.73 | 1.95 | 1.01 | 54.57 | 7 |
| IgG3 UH | 2.45 | 2.38 | 96.98 | 1.65 | 67.28 | 3.08 | 0.80 | 26.02 | 1.30 | 1.07 | 82.03 | 0.98 | 64.69 | 0.41 | 0.12 | 29.25 | 2.40 | 2.43 | 101.04 | 1.49 | 61.81 | 1.91 | 1.02 | 53.73 | 7 |
| IgG1 MH | 2.50 | 2.49 | 99.72 | 1.84 | 73.79 | 3.15 | 0.67 | 21.30 | 1.68 | 1.48 | 88.06 | 1.09 | 64.96 | 0.59 | 0.13 | 21.74 | 2.29 | 2.39 | 104.55 | 1.43 | 62.38 | 1.67 | 0.90 | 29.98 | 6 |
| IgG2 G4SL | 2.29 | 2.43 | 106.20 | 1.89 | 82.58 | 2.84 | 1.04 | 36.57 | 1.03 | 0.73 | 70.84 | 0.59 | 56.70 | 0.31 | 0.11 | 35.79 | 2.31 | 2.42 | 104.85 | 1.52 | 65.79 | 1.72 | 0.72 | 42.09 | 5 |
| IgG2B UL | 2.46 | 2.41 | 98.17 | 1.73 | 70.55 | 2.98 | 0.44 | 14.91 | 1.03 | 0.83 | 80.27 | 0.52 | 50.40 | 0.29 | 0.10 | 35.73 | 2.36 | 2.30 | 97.58 | 1.34 | 56.94 | 1.54 | 0.37 | 24.20 | 4 |
| IgG2B MH | 2.48 | 2.34 | 94.67 | 1.73 | 69.94 | 2.99 | 0.61 | 20.34 | 1.11 | 0.91 | 81.38 | 0.67 | 60.11 | 0.37 | 0.11 | 30.84 | 2.25 | 2.36 | 104.80 | 1.42 | 62.97 | 1.62 | 0.45 | 27.76 | 4 |
| IgG2A G4SS | 2.34 | 2.33 | 99.49 | 1.67 | 71.24 | 2.91 | 0.67 | 23.15 | 1.12 | 0.71 | 62.86 | 0.38 | 34.07 | 0.36 | 0.12 | 32.94 | 2.32 | 2.36 | 101.77 | 1.39 | 59.77 | 1.63 | 0.53 | 32.33 | 2 |
| IgG2A UL | 2.36 | 2.32 | 98.56 | 1.64 | 69.34 | 3.05 | 0.45 | 14.64 | 1.07 | 0.87 | 81.27 | 0.46 | 42.60 | 0.32 | 0.10 | 32.11 | 2.27 | 2.33 | 102.47 | 1.37 | 60.35 | 1.64 | 0.43 | 26.00 | 1 |
| IgG1 UL | ND | ND | NA | ND | NA | ND | ND | NA | ND | ND | NA | ND | NA | ND | ND | NA | ND | ND | NA | ND | NA | ND | ND | NA | NA |
| IgG4 H | ND | ND | NA | ND | NA | ND | ND | NA | ND | ND | NA | ND | NA | ND | ND | NA | ND | ND | NA | ND | NA | ND | ND | NA | NA |
| IgG4 R | ND | ND | NA | ND | NA | ND | ND | NA | ND | ND | NA | ND | NA | ND | ND | NA | ND | ND | NA | ND | NA | ND | ND | NA | NA |

Fig. 9

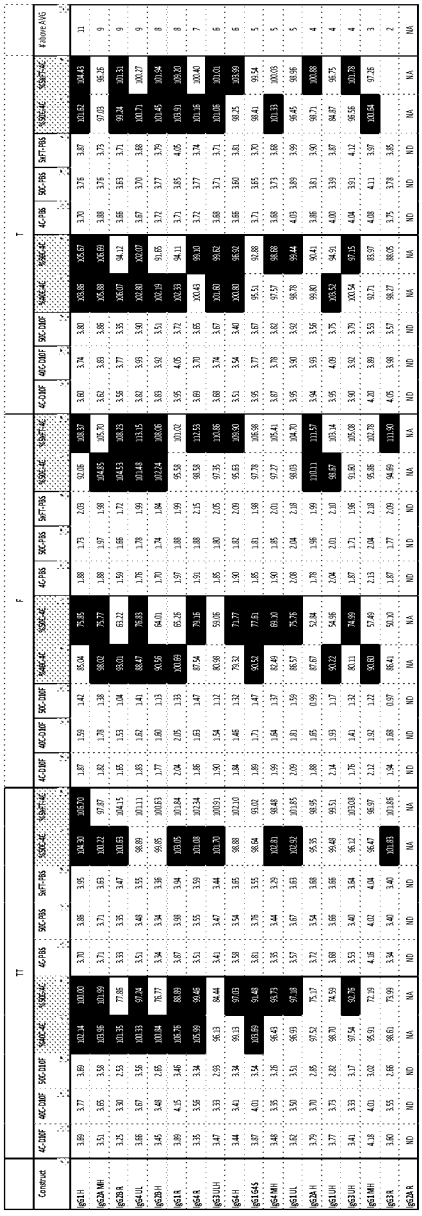


Fig. 10

**Figure 11A (SEQ ID NO: 35)**

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKVEIK
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE
QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

**Figure 11B (SEQ ID NOs: 36 and 37)**

gacatccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcacc
D I Q M T Q S P S S L S A S V G D R V T
atcacttgccgggcaagtcagagcattagcagctacttaaattggtatcagcagaaacca
I T C R A S Q S I S S Y L N W Y Q Q K P
gggaaagcccctaagctcctgatctatgctgcatccagtttgcaaagtggggtcccatca
G K A P K L L I Y A A S S L Q S G V P S
aggttcagtggcagtggatctgggacagatttcactctcaccatcagcagtctgcaacct
R F S G S G S G T D F T L T I S S L Q P
gaagattttgcaacttactactgtcaacagagttacagtacccctccaacgttcggccaa
E D F A T Y Y C Q Q S Y S T P P T F G Q
gggaccaaggtggagatcaaa
G T K V E I K

**Figure 11C (SEQ ID NOs: 38 and 39)**

cgaactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatct
R T V A A P S V F I F P P S D E Q L K S
ggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacag
G T A S V V C L L N N F Y P R E A K V Q
tggaaggtggataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggac
W K V D N A L Q S G N S Q E S V T E Q D

**Figure 11C (continued)**

agcaaggacagcacctacagcctcagcagcaccctgacgctgagcaaagcagactacgag
S K D S T Y S L S S T L T L S K A D Y E
aaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaag
K H K V Y A C E V T H Q G L S S P V T K
agcttcaacaggggagagtgttag
S F N R G E C -

**Figure 11D (SEQ ID NO: 40)**

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPITFGQGTRLEIK

**Figure 11E (SEQ ID NO: 41)**

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTP

Fig 12.

| | | | Absorbance 405 nm | | | 90° light scatter intensity | | | Trp fluo % Change | | | ANS fluo % Change | | | Nile Red fluo % Change | | | Nile Red microscopy particles/fld | | | | DLS % monomer (intensity) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | T1 | Shd | 50 | 5F1 | T1 | Shd | 50 | 5F1 | Shd | 50 | 5F1 | Shd | 50 | 5F1 | T1 | Shd | 50 | 5F1 | T1 | Shd | 50 | 5F1 |
| PT231709d01 | tg01 1* | Tecbiotec 1122x1337x6744 | 0.0363 | 0.0338 | 0.0365 | 0.0408 | 1.1 | 1.3 | 1.3 | 1.5 | -7.2 | 2.8 | -1.2 | 0.7 | 4.2 | -1.8 | -30 | 9.3 | -5.0 | 5 | 14 | 7 | 24 | 100 | 93 | 86 | 80 |
| PT231709d02 | tg02A MH* | Tecbiotec 1122x1337x6744 | 0.0375 | 0.040 | 0.038 | 0.049 | 1.0 | 1.7 | 1.4 | 1.4 | -7.7 | 3.5 | -3.0 | 0.6 | 9.3 | -3.3 | -2.9 | -0.1 | -1.7 | 18 | 5 | 13 | 37 | 100 | 99 | 83 | 83 |
| PT231709d03 | tg04 UL* | Tecbiotec 1122x1337x6744 | 0.0373 | 0.0365 | 0.0374 | 0.0373 | 1.0 | 1.0 | 1.1 | 1.2 | -9.8 | 6.2 | -2.1 | 0.5 | 7.7 | -6.3 | -4.5 | 5.1 | -2.1 | 26 | 5 | 6 | 22 | 100 | 76 | 49 | 72 |
| PT231709d04 | tg01 G4S | Tecbiotec 1122x1337x6744 | 0.0375 | 0.0363 | 0.037 | 0.041 | 1.0 | 1.0 | 1.1 | 1.5 | -1.8 | 1.6 | -3.1 | -1.1 | 4.7 | -6.2 | -2.3 | -2.6 | -2.4 | 4 | 87 | 14 | 25 | 100 | 73 | 103 | 93 |
| PT231709d05 | tg01 MH | Tecbiotec 1122x1337x6744 | 0.0370 | 0.0363 | 0.037 | 0.0415 | 1.0 | 1.6 | 1.6 | 2.3 | -6.5 | 0.2 | -1.9 | 0.9 | 5.2 | -5.1 | -7.6 | 3.5 | -5.5 | 4 | 7 | 11 | 69 | 100 | 103 | 105 | 100 |
| PT231709d06 | tg01 R | Tecbiotec 1122x1337x6744 | 0.0362 | 0.1128 | 0.038 | 0.041 | 1.1 | 13.1 | 1.3 | 1.8 | -6.3 | 0.6 | -2.5 | 14.9 | -8.3 | -5.3 | -1.6 | 0.9 | -4.4 | 3 | 1160 | 13 | 73 | 100 | 57 | 103 | 88 |
| PT231709d07 | tg01 UH | Tecbiotec 1122x1337x6744 | 0.0363 | 0.0378 | 0.0376 | 0.041 | 1.0 | 1.2 | 1.4 | 1.4 | -2.2 | 0.6 | -3.0 | -0.5 | 8.3 | -8.5 | -1.6 | -0.5 | -0.6 | 2 | 6 | 8 | 34 | 100 | 93 | 98 | 58 |
| PT231709d08 | tg01 UL | Tecbiotec 1122x1337x6744 | 0.0365 | 0.0383 | 0.037 | 0.038 | 1.1 | 2.2 | 1.1 | 1.3 | -4.6 | 3.6 | -5.7 | 0.3 | 3.1 | -1.0 | -2.5 | -0.4 | 0.6 | 4 | 31 | 8 | 58 | 100 | 103 | 99 | 79 |
| PT231709d09 | tg02A R | Tecbiotec 1122x1337x6744 | 0.0365 | 0.0384 | 0.037 | 0.0383 | 1.0 | 1.0 | 1.4 | 1.5 | -7.8 | 3.1 | -3.6 | 2.1 | 8.4 | -8.8 | 1.0 | -6.1 | 2.2 | 2 | 3 | 14 | 44 | 100 | 97 | 92 | 92 |
| PT231709d10 | tg02A R | Tecbiotec 1122x1337x6744 | 0.0368 | 0.0385 | 0.0378 | 0.038 | 1.0 | 1.6 | 1.5 | 1.3 | -13.4 | 9.6 | -5.5 | -4.8 | 17.2 | -13.2 | -3.5 | -2.0 | -0.2 | 5 | 6 | 26 | 34 | 100 | 99 | 97 | 96 |
| PT231709d11 | tg02B H | Tecbiotec 1122x1337x6744 | 0.0364 | 0.0382 | 0.0375 | 0.039 | 1.1 | 3.4 | 1.2 | 1.4 | -5.5 | 5.1 | -4.5 | 3.0 | 5.4 | -5.3 | -1.1 | -0.9 | -3.0 | 7 | 37 | 31 | 52 | 100 | 84 | 94 | 93 |
| PT231709d12 | tg02B R | Tecbiotec 1122x1337x6744 | 0.0373 | 0.043 | 0.0395 | 0.0368 | 1.0 | 2.3 | 1.1 | 1.5 | -14.8 | 16.3 | -3.9 | -4.3 | 3.0 | -6.6 | -2.2 | -1.5 | -0.7 | 7 | 18 | 14 | 31 | 100 | 63 | 98 | 75 |
| PT231709d13 | tg03 R | Tecbiotec 1122x1337x6744 | 0.0373 | 0.051 | 0.0378 | 0.038 | 0.7 | 1.8 | 0.9 | 0.9 | -5.4 | 1.5 | -1.1 | 3.7 | 1.8 | 2.3 | -2.7 | 0.1 | 1.6 | 7 | 184 | 8 | 19 | 97 | 64 | 86 | 83 |
| PT231709d14 | tg03 UH | Tecbiotec 1122x1337x6744 | 0.0378 | 0.0373 | 0.0378 | 0.0374 | 1.0 | 1.0 | 1.1 | 1.2 | -7.3 | 5.5 | -3.0 | 1.3 | 16.1 | -4.0 | -1.7 | 6.7 | -3.8 | 20 | 6 | 3 | 36 | 100 | 89 | 97 | 81 |
| PT231709d15 | tg03 ULH | Tecbiotec 1122x1337x6744 | 0.0373 | 0.2693 | 0.1328 | 0.0373 | 1.1 | 25.1 | 1.1 | 1.2 | -0.6 | -1.7 | -1.5 | 43.7 | 224.1 | -0.6 | -6.2 | -0.2 | -0.2 | 5 | 2228 | 7 | 49 | 39 | 37 | 93 | 72 |
| PT231709p16 | tg04 H | Tecbiotec 1122x1337x6744 | 0.0373 | 0.046 | 0.039 | 0.0392 | 1.0 | 2.6 | 1.2 | 1.8 | -6.4 | 6.5 | -8.1 | 3.5 | -1.1 | -0.3 | 1.6 | -4.7 | 2.3 | 4 | 61 | 26 | 39 | 100 | 78 | 91 | 95 |
| PT231709p17 | tg04 MH | Tecbiotec 1122x1337x6744 | 0.0365 | 0.059 | 0.0377 | 0.0378 | 1.0 | 1.0 | 1.1 | 1.2 | -9.2 | 5.6 | -4.8 | 2.8 | 4.4 | -7.0 | 2.1 | 5.2 | -5.6 | 5 | 4 | 3 | 39 | 100 | 78 | 95 | 91 |
| PT231709p18 | tg04 R | Tecbiotec 1122x1337x6744 | 0.0373 | 0.059 | 0.0373 | 0.0378 | 1.0 | 8.8 | 1.6 | 1.4 | -10.8 | 5.6 | -5.3 | 5.6 | 0.3 | -8.7 | 0.1 | 0.1 | 2.4 | 31 | 622 | 13 | 49 | 100 | 63 | 95 | 95 |
| PG67344p63 | NA | control tg5 | 0.038 | 0.0373 | 0.0368 | 0.0375 | 0.8 | 0.8 | 0.8 | 0.9 | 4.3 | 1.7 | 3.3 | 3.3 | 7.8 | 4.8 | -2.1 | 1.5 | 4.4 | 1 | 1 | 2 | 16 | 100 | 98 | 100 | 80 |
| PG1122p55 | NA | control tg5 | 0.0368 | 0.0358 | 0.0378 | 0.0373 | 0.8 | 0.8 | 0.8 | 0.8 | -3.8 | 0.2 | 4.4 | 4.1 | -3.5 | 15.3 | 6.3 | -8.3 | -1.5 | 2 | 2 | 2 | 16 | 100 | 71 | 95 | 82 |
| PG1331p350 | NA | control tg5 | 0.0375 | 0.0483 | 0.0368 | 0.048 | 0.8 | 2.9 | 0.8 | 1.3 | 4.6 | 3.6 | 3.9 | 0.9 | 0.8 | 4.9 | -1.9 | 2.5 | 5.4 | 19 | 126 | 12 | 119 | 100 | 62 | 93 | 75 |
| PG1886p67 | NA | control Bicinate 1122x1337 | 0.037 | 0.0475 | 0.0363 | 0.0378 | 0.8 | 2.8 | 0.8 | 1.0 | 4.7 | 2.8 | 3.2 | 2.2 | 9.1 | 1.6 | -4.9 | 7.4 | -1.6 | 8 | 111 | 8 | 38 | 100 | 71 | 100 | 81 |

Fig 13.

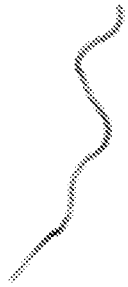
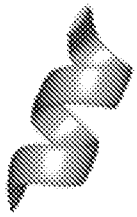
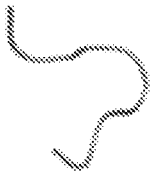
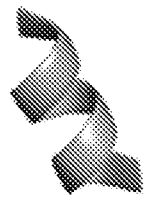
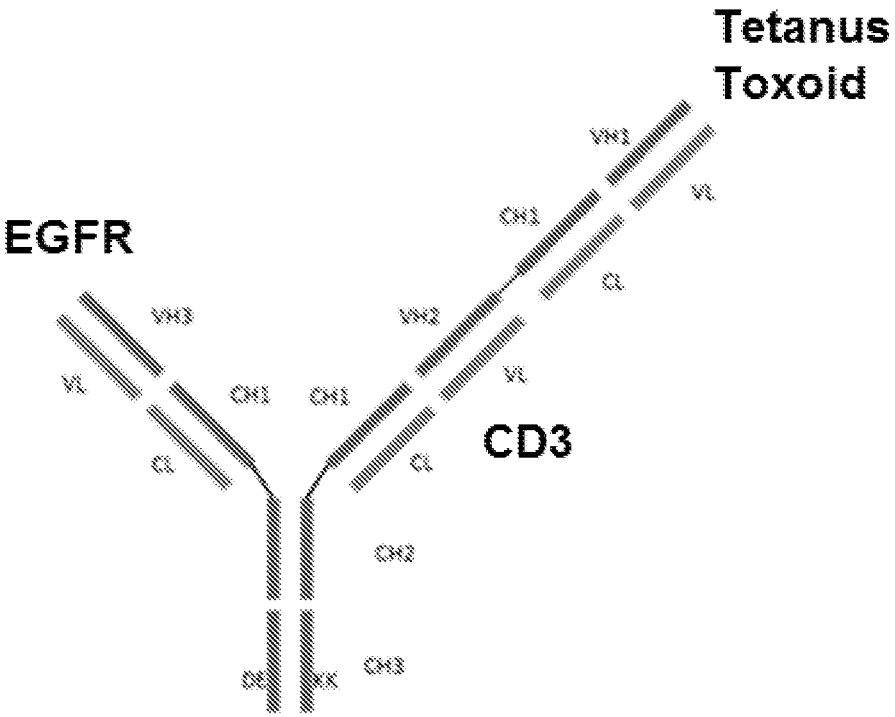
| | | | |
|---|---|---|---|
| IgG1 UH | SEQ ID NO: 2 | IgG2A H | SEQ ID NO: 15 |
|  | |  | |
| IgG2A MH | SEQ ID NO: 4 | IgG2B H | SEQ ID NO: 16 |
|  | |  | |
| IgG1 MH | SEQ ID NO: 11 | IgG1 H | SEQ ID NO:19 |
|  | |  | |
| IgG1G4 | SEQ ID NO: 12 | IgG2B R | SEQ ID NO: 21 |
|  | |  | |

Fig. 14

14a




14b

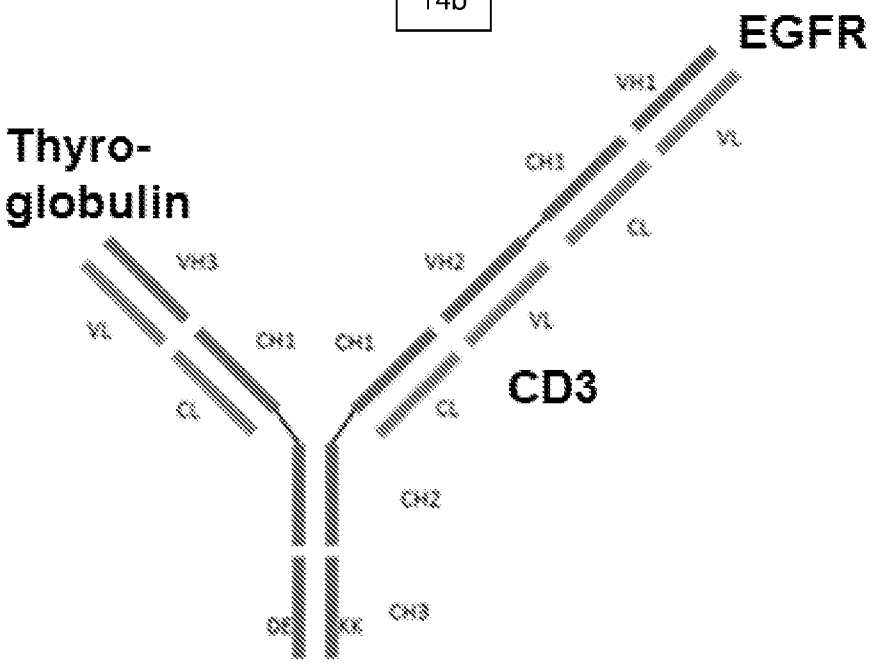


Fig. 15a

T cell Activation:

EGFRxCD3:TT

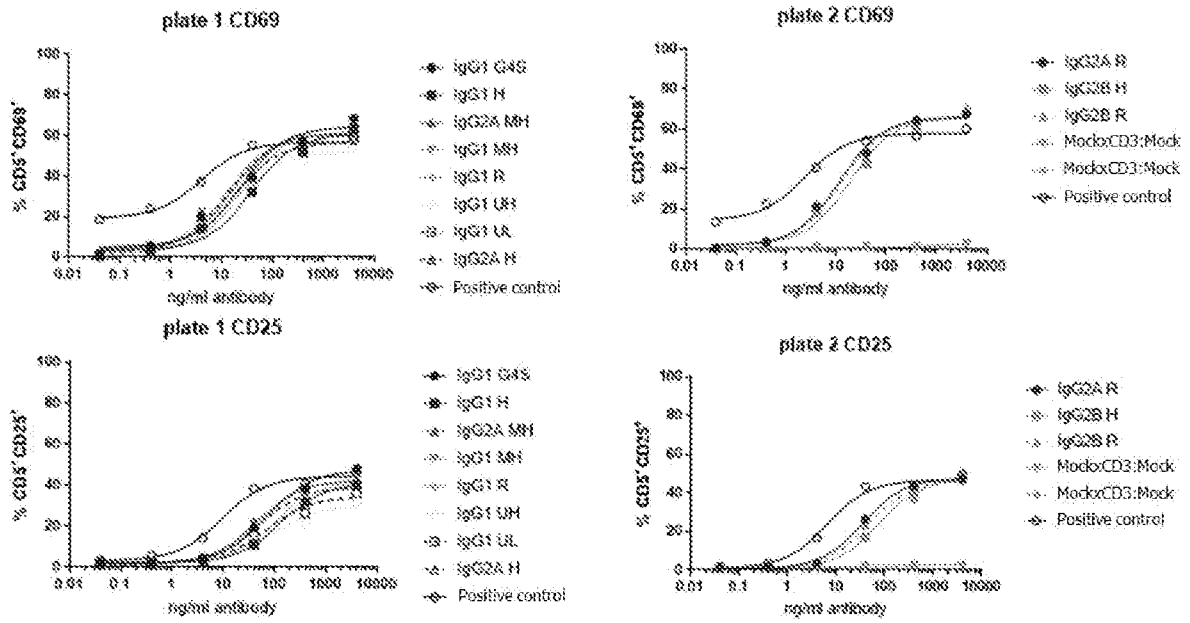


Fig. 15b

T cell Activation:

THYROxCD3:EGFR

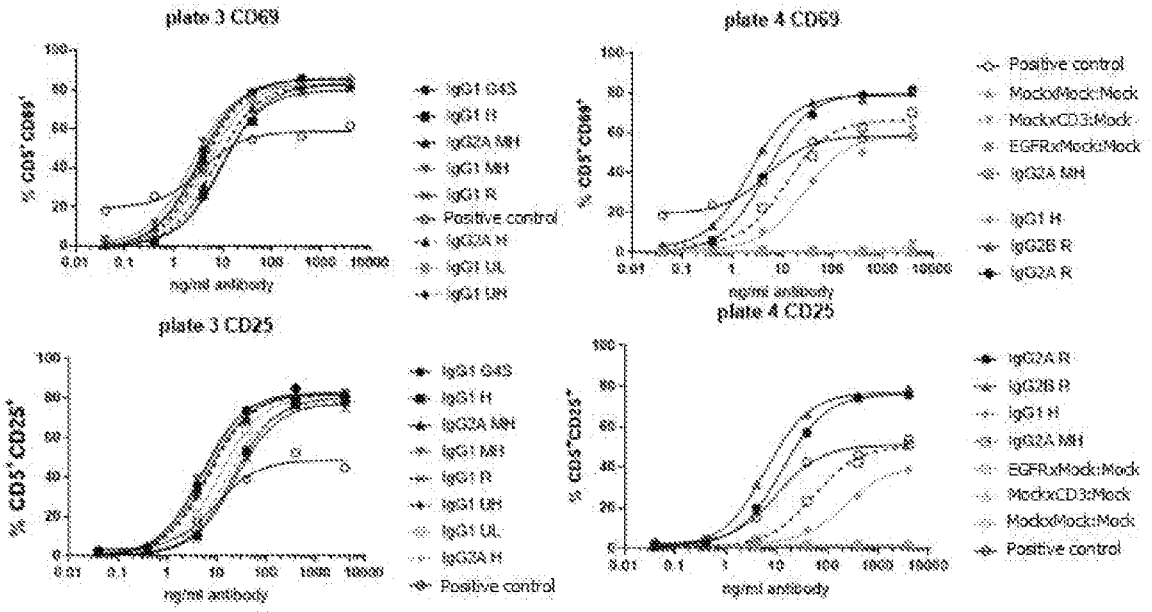


Fig. 16a

T cell Cytotoxicity:

EGFRxCD3:TT

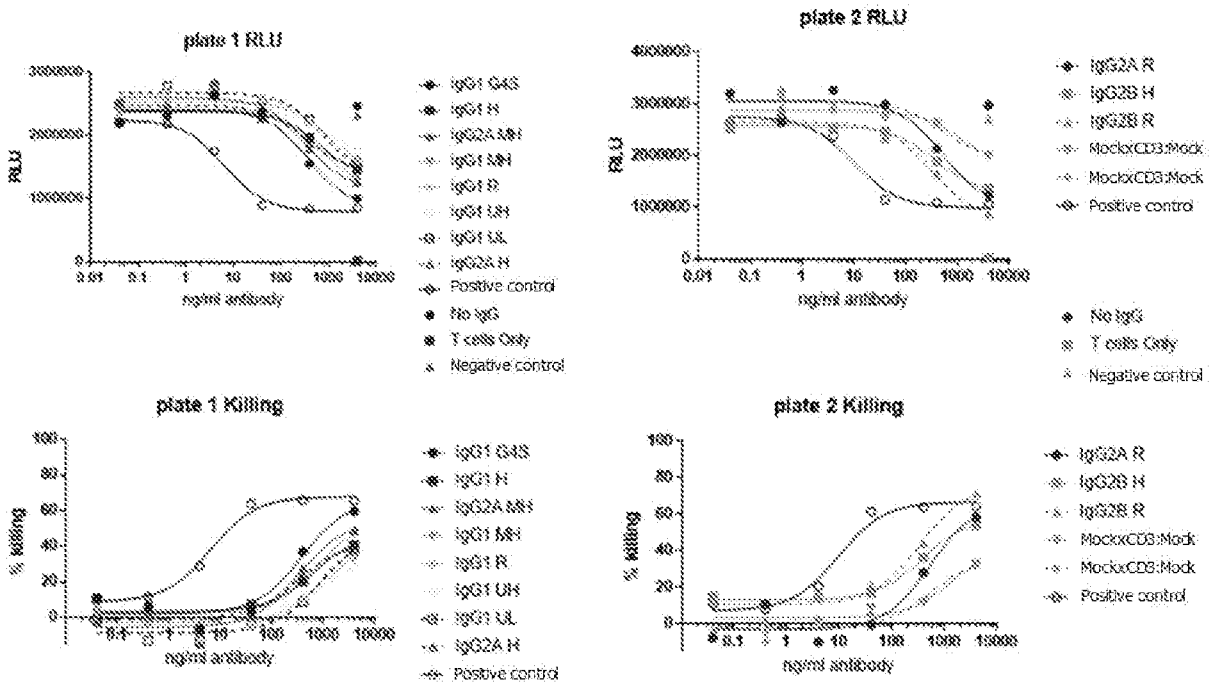


Fig. 16b

T cell Cytotoxicity:

THYROxCD3:EGFR

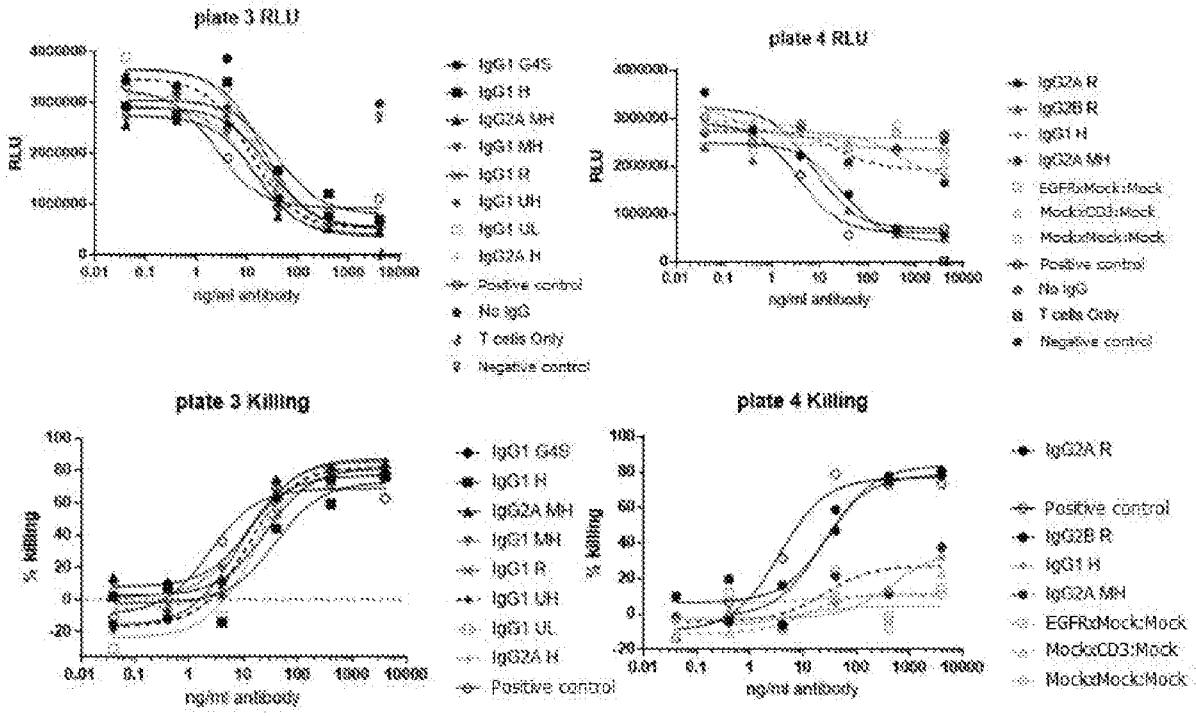


Fig. 17a

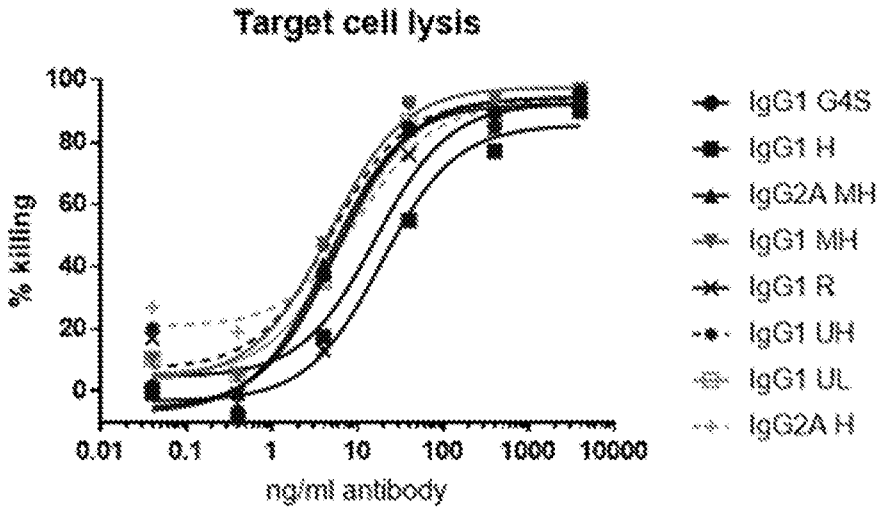



Fig. 17b

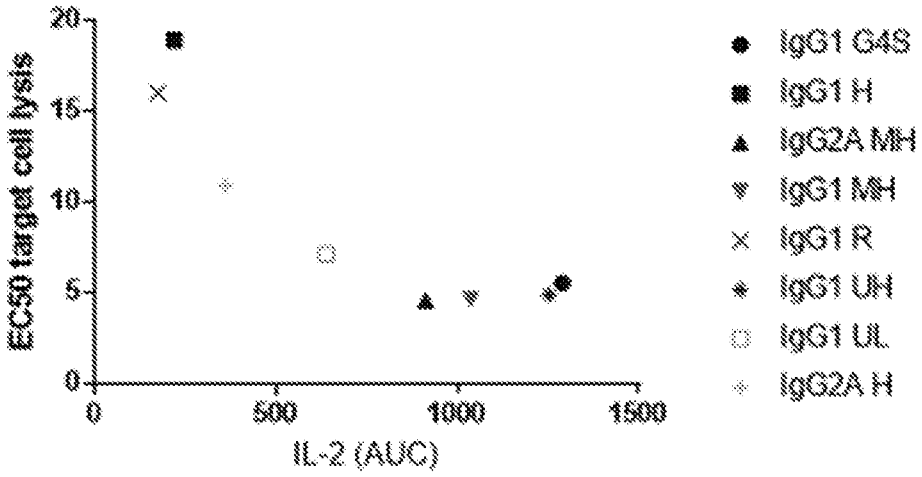


Fig. 17c

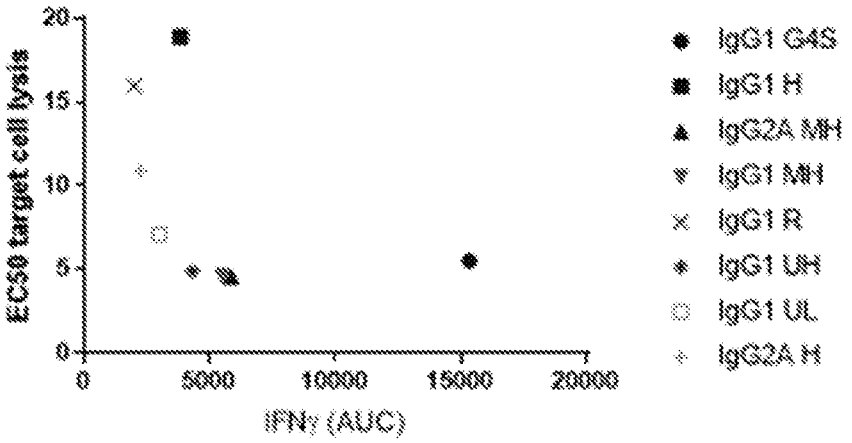



Fig. 17d

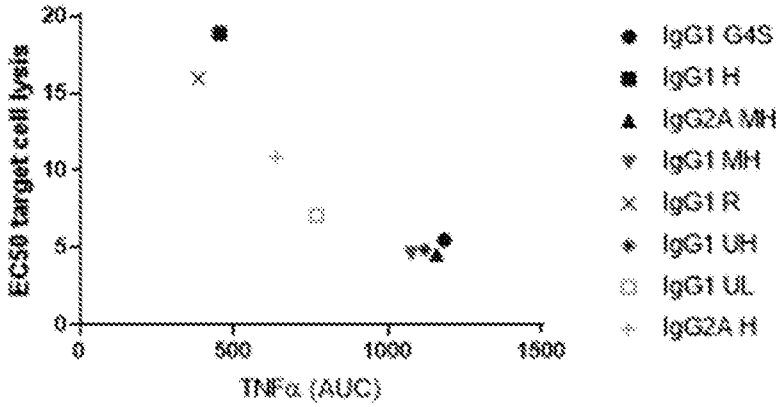


Fig. 18

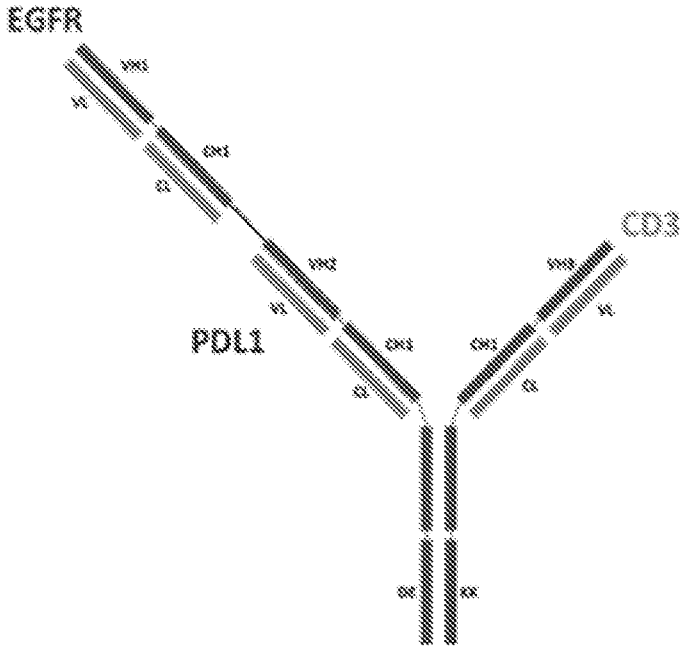


Fig. 19

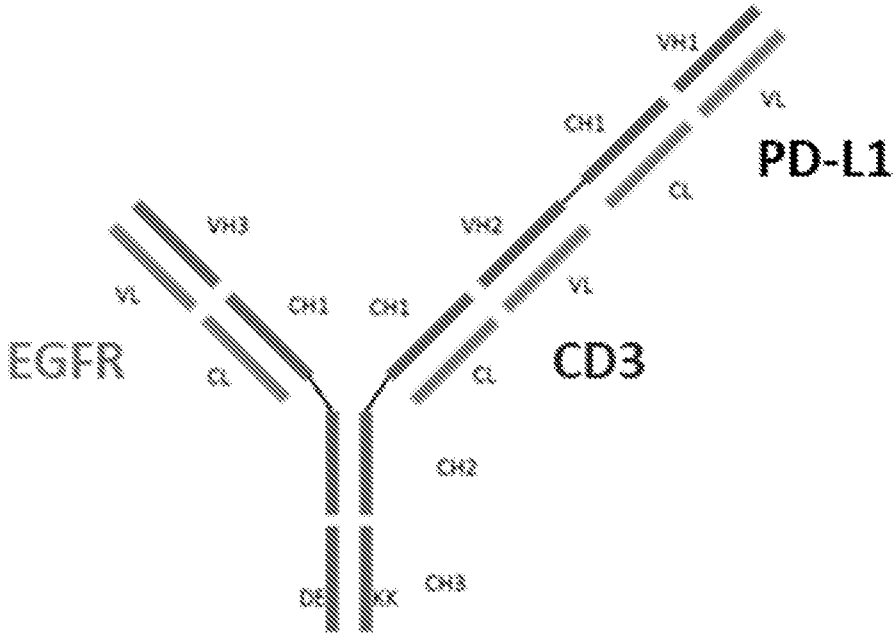


Fig. 20

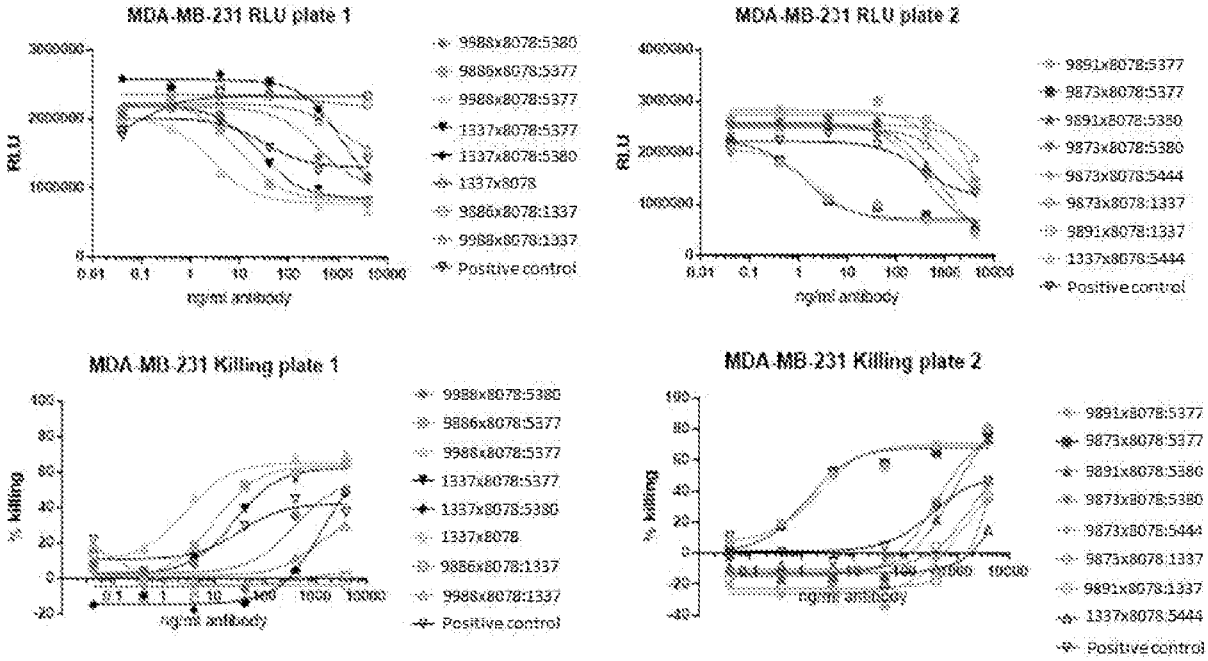

MDA-MB-231 RLU plate 1
RLU
ng/ml antibody
9988x8078:5380
9886x8078:5377
9988x8078:5377
1337x8078:5377
1337x8078:5380
1337x8078
9886x8078:1337
9988x8078:1337
Positive control
MDA-MB-231 RLU plate 2
RLU
ng/ml antibody
9891x8078:5377
9873x8078:5377
9891x8078:5380
9873x8078:5380
9873x8078:5444
9873x8078:1337
9891x8078:1337
1337x8078:5444
Positive control
MDA-MB-231 Killing plate 1
% killing
ng/ml antibody
9988x8078:5380
9886x8078:5377
9988x8078:5377
1337x8078:5377
1337x8078:5380
1337x8078
9886x8078:1337
9988x8078:1337
Positive control
MDA-MB-231 Killing plate 2
% killing
ng/ml antibody
9891x8078:5377
9873x8078:5377
9891x8078:5380
9873x8078:5380
9873x8078:5444
9873x8078:1337
9891x8078:1337
1337x8078:5444
Positive control

Fig. 21

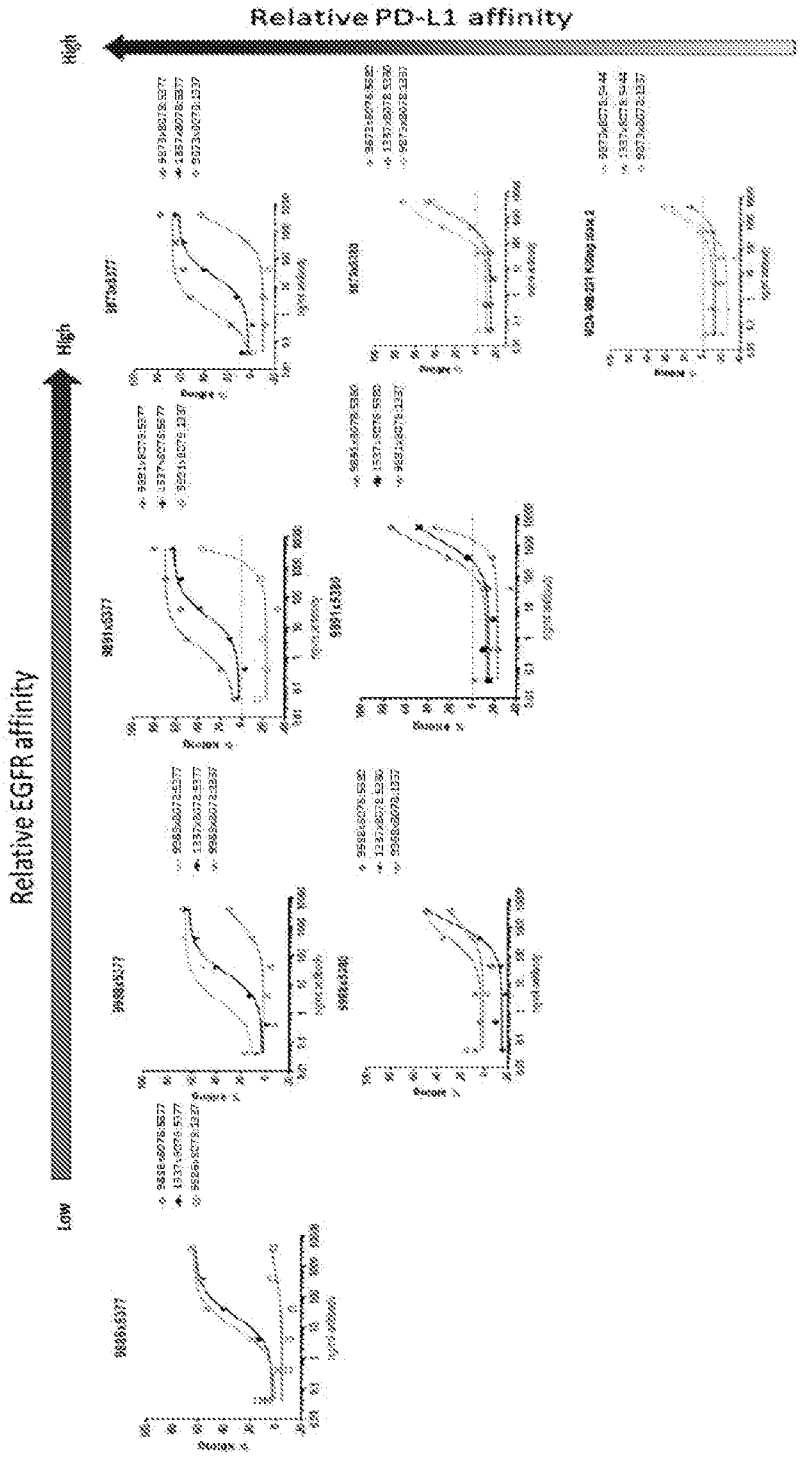

Relative PD-L1 affinity
High
Relative EGFR affinity
High
Low

Fig. 22

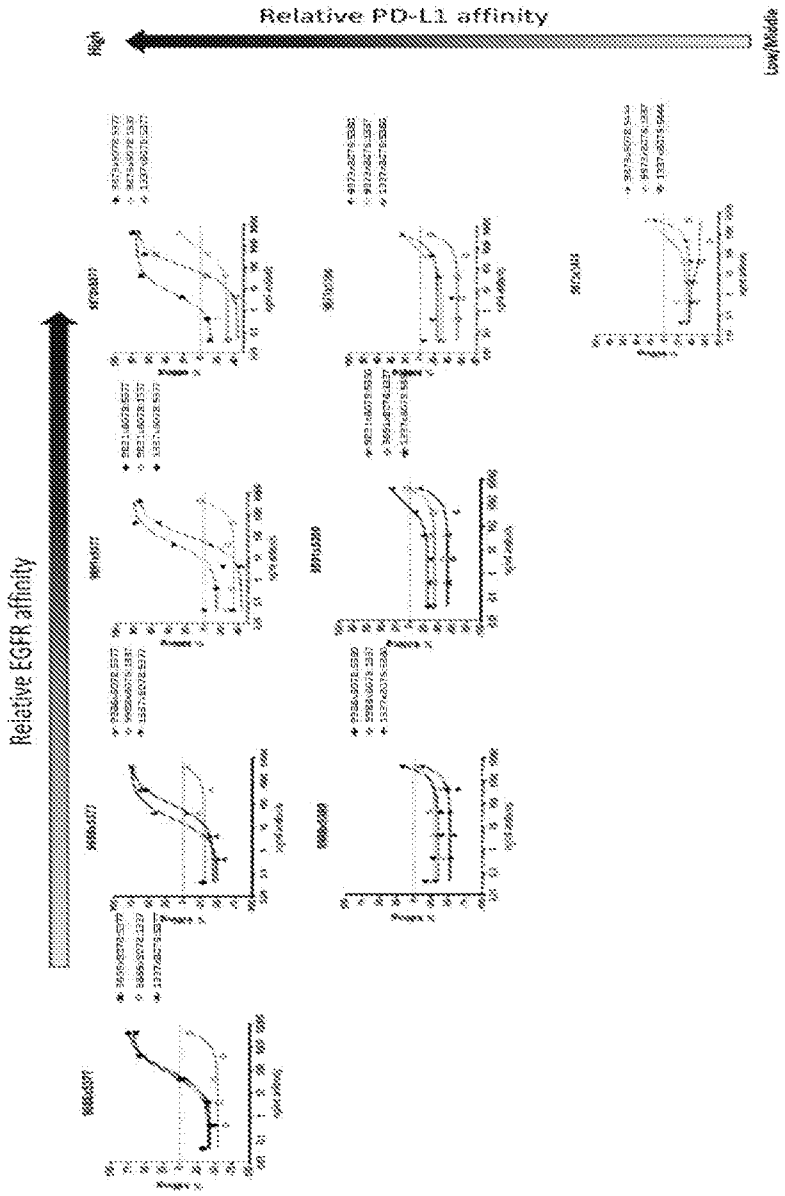
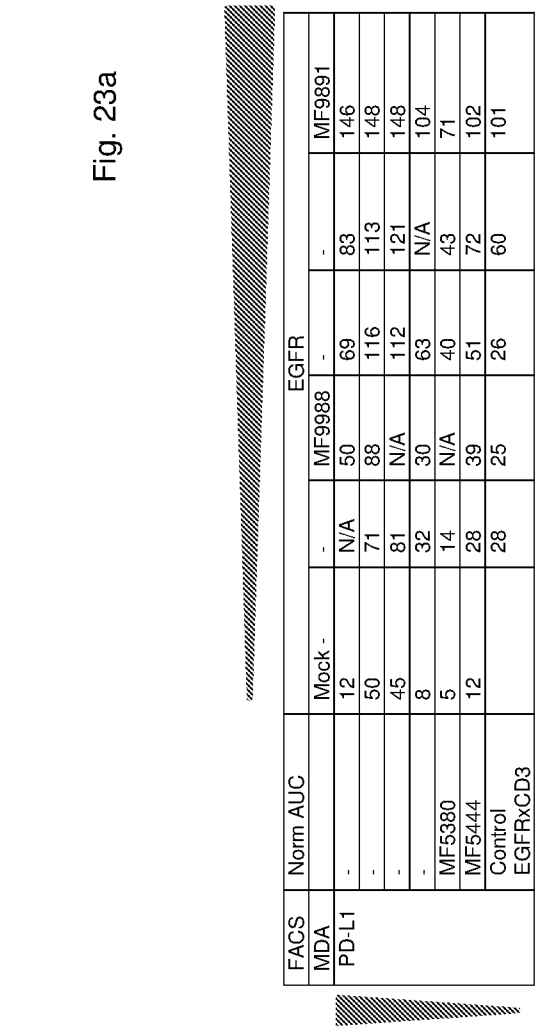

Relative PD-L1 affinity
High
Low/Middle
Relative EGFR affinity

Fig. 23a



| FACS | Norm AUC | EGFR | | | | | |
|---|---|---|---|---|---|---|---|
| MDA | | Mock - | - | MF9988 | - | - | MF9891 |
| PD-L1 | - | 12 | N/A | 50 | 69 | 83 | 146 |
| | - | 50 | 71 | 88 | 116 | 113 | 148 |
| | - | 45 | 81 | N/A | 112 | 121 | 148 |
| | - | 8 | 32 | 30 | 63 | N/A | 104 |
| | MF5380 | 5 | 14 | N/A | 40 | 43 | 71 |
| | MF5444 | 12 | 28 | 39 | 51 | 72 | 102 |
| | Control EGFRxCD3 | | 28 | 25 | 26 | 60 | 101 |

Fig. 23b

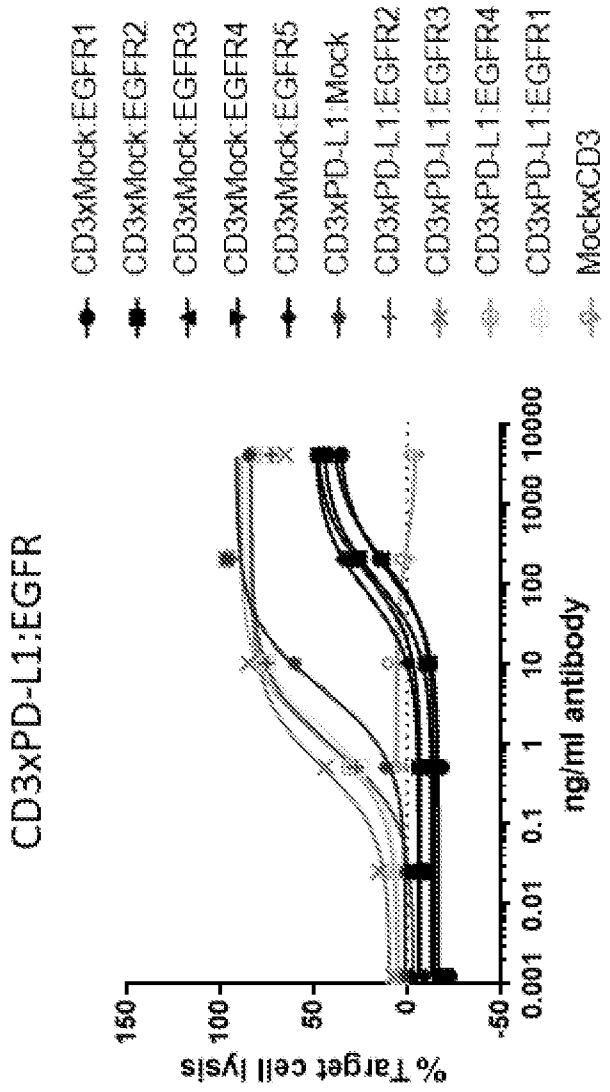

CD3xPD-L1:EGFR
CD3xMock:EGFR1
CD3xMock:EGFR2
CD3xMock:EGFR3
CD3xMock:EGFR4
CD3xMock:EGFR5
CD3xPD-L1:Mock
CD3xPD-L1:EGFR2
CD3xPD-L1:EGFR3
CD3xPD-L1:EGFR4
CD3xPD-L1:EGFR1
MockxCD3
% Target cell lysis
150
100
50
0
-50
0.001
0.01
0.1
1
10
100
1000
10000
ng/ml antibody

Fig. 24

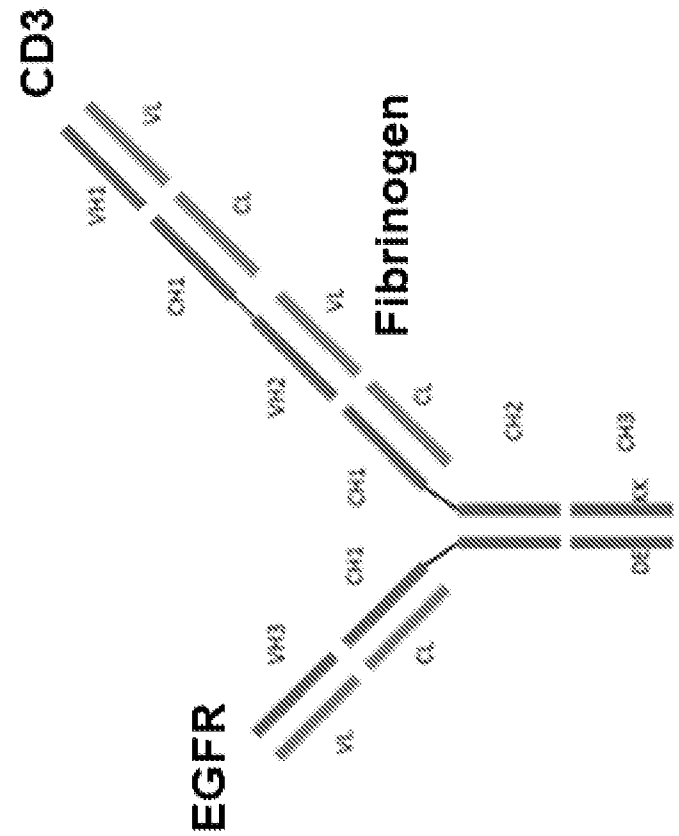


Fig. 25

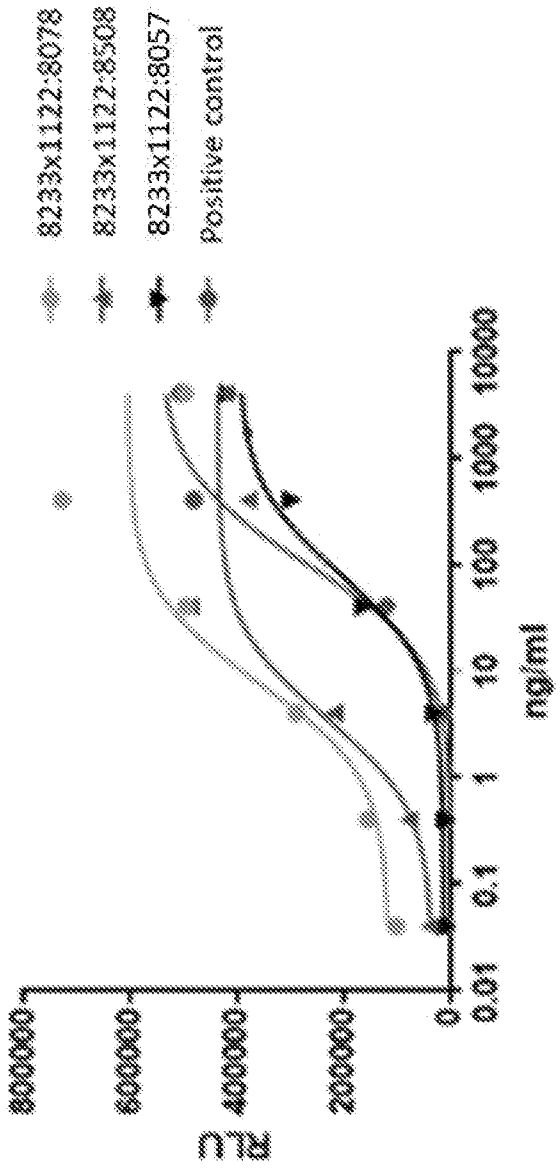

8233x1122:8078
8233x1122:8508
8233x1122:8057
Positive control
RLU
800000
600000
400000
200000
0
0.01
0.1
1
10
100
1000
10000
ng/ml

Fig. 26

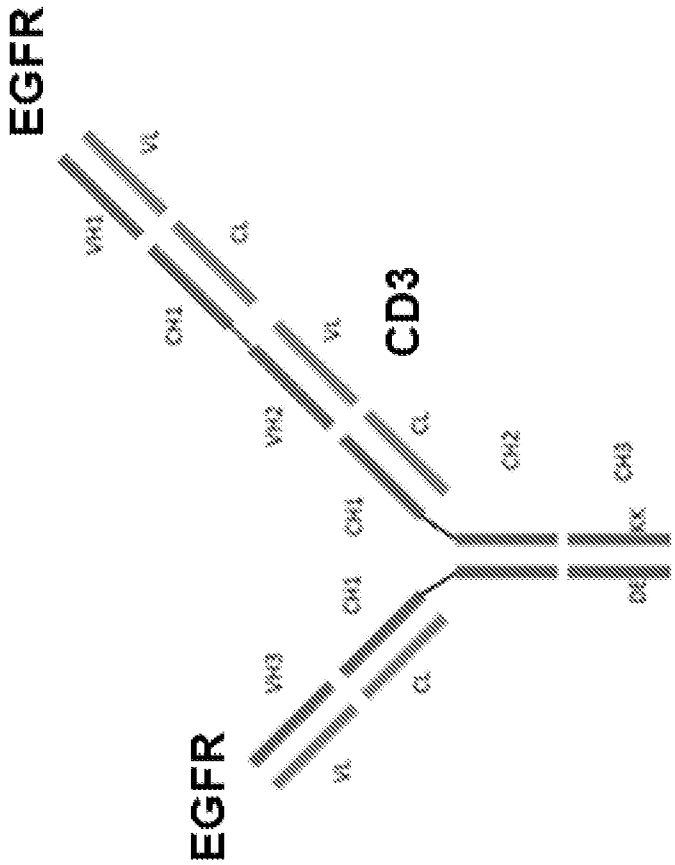


Fig. 27

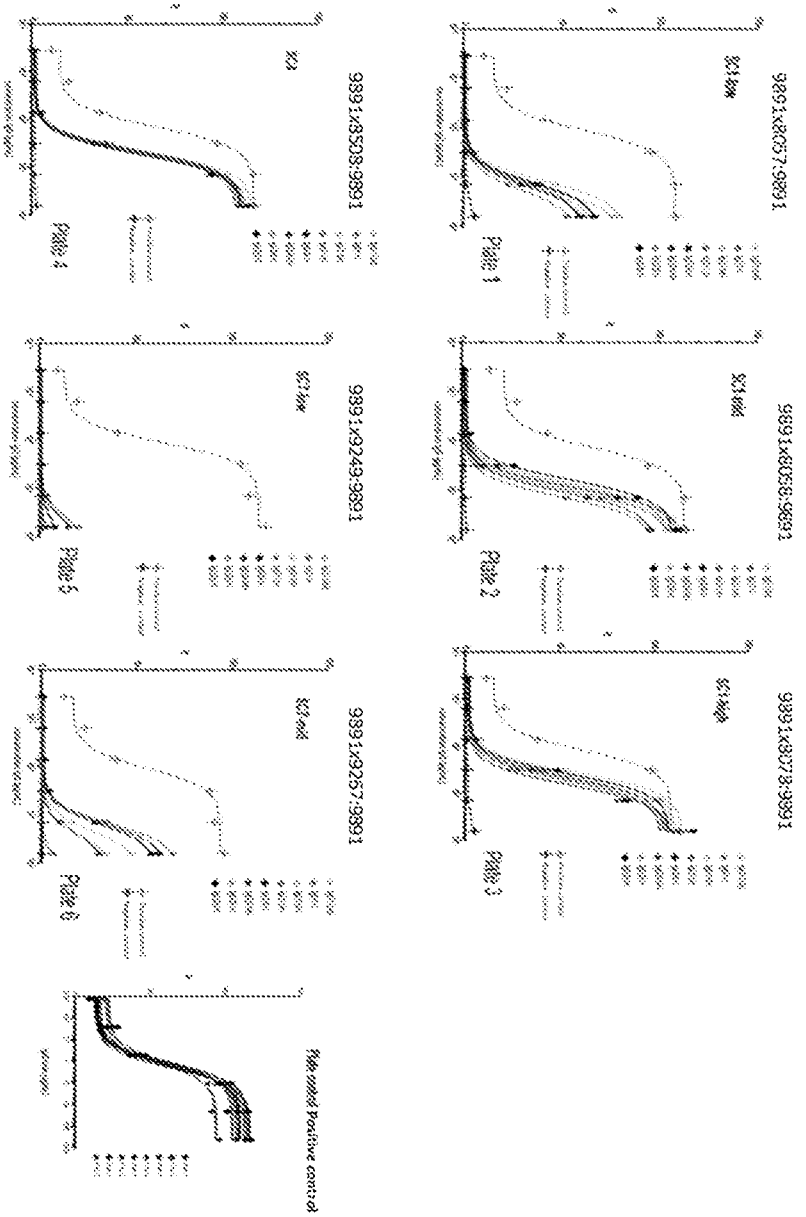


Plate 1
Plate 2
Plate 3
Plate 4
Plate 5
Plate 6
Non-related Positive control

Fig. 28

