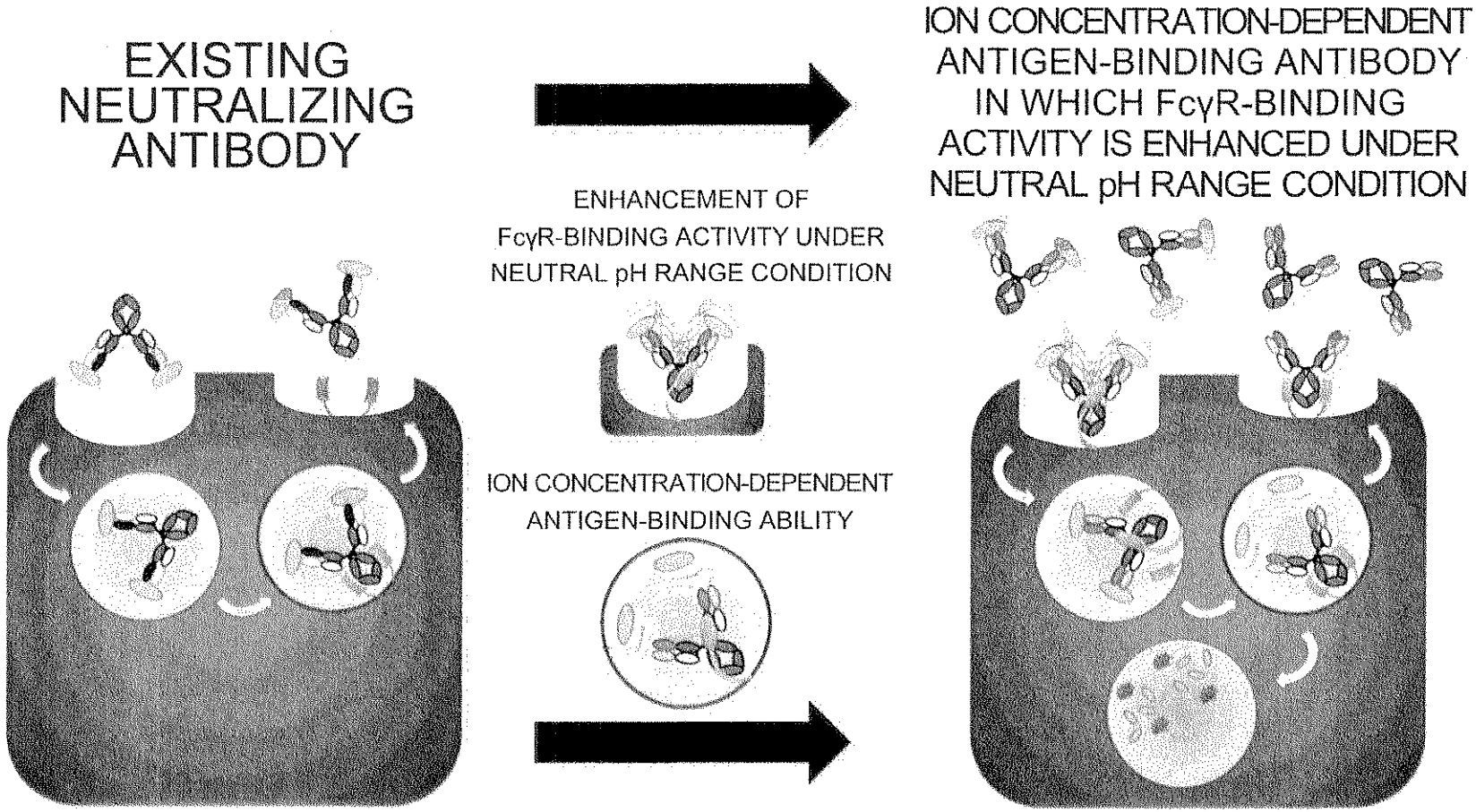

EXISTING NEUTRALIZING ANTIBODY
ENHANCEMENT OF FcγR-BINDING ACTIVITY UNDER NEUTRAL pH RANGE CONDITION
ION CONCENTRATION-DEPENDENT ANTIGEN-BINDING ABILITY
ION CONCENTRATION-DEPENDENT ANTIGEN-BINDING ANTIBODY IN WHICH FcγR-BINDING ACTIVITY IS ENHANCED UNDER NEUTRAL pH RANGE CONDITION

FIG. 1

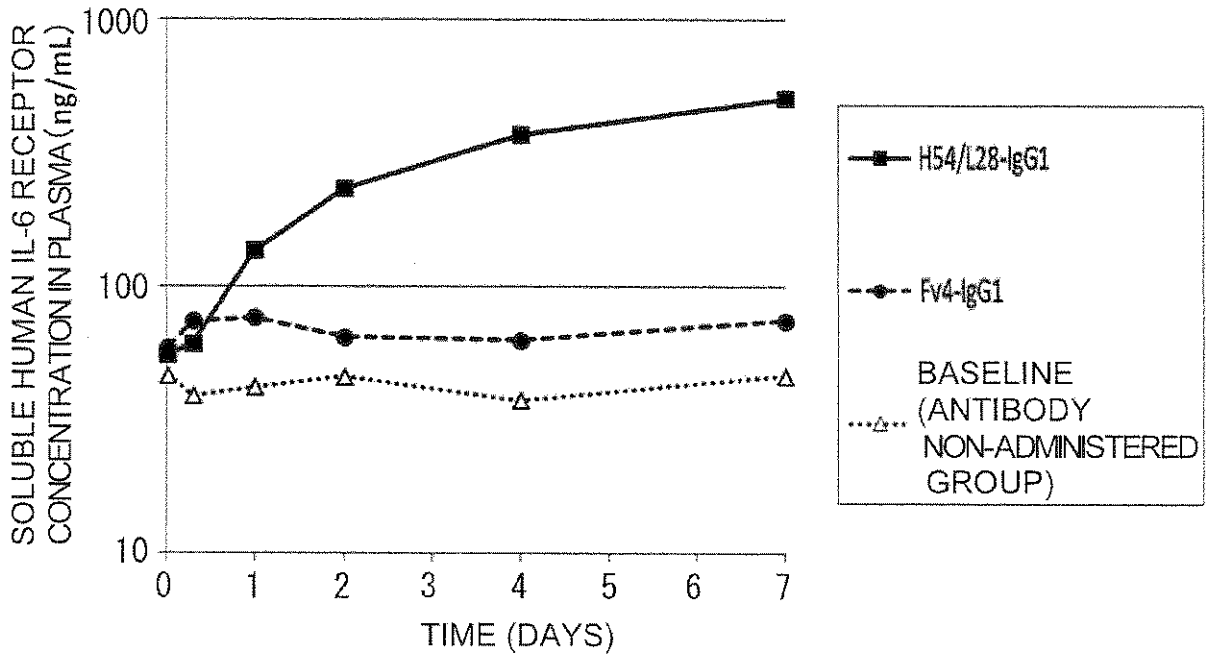

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA (ng/mL)
1000
100
10
0
1
2
3
4
5
6
7
TIME (DAYS)
H54/L28-IgG1
Fv4-IgG1
BASELINE (ANTIBODY NON-ADMINISTERED GROUP)

FIG. 2

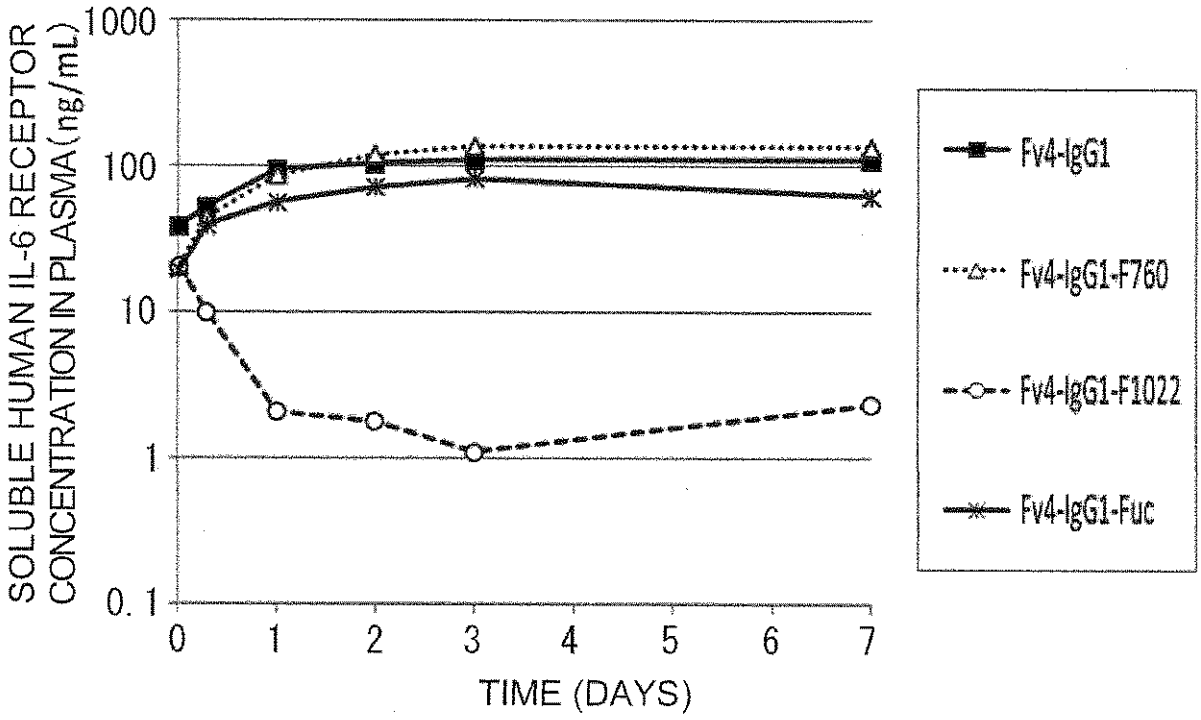

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA(ng/mL)
1000
100
10
1
0.1
0
1
2
3
4
5
6
7
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F760
Fv4-IgG1-F1022
Fv4-IgG1-Fuc

FIG. 3

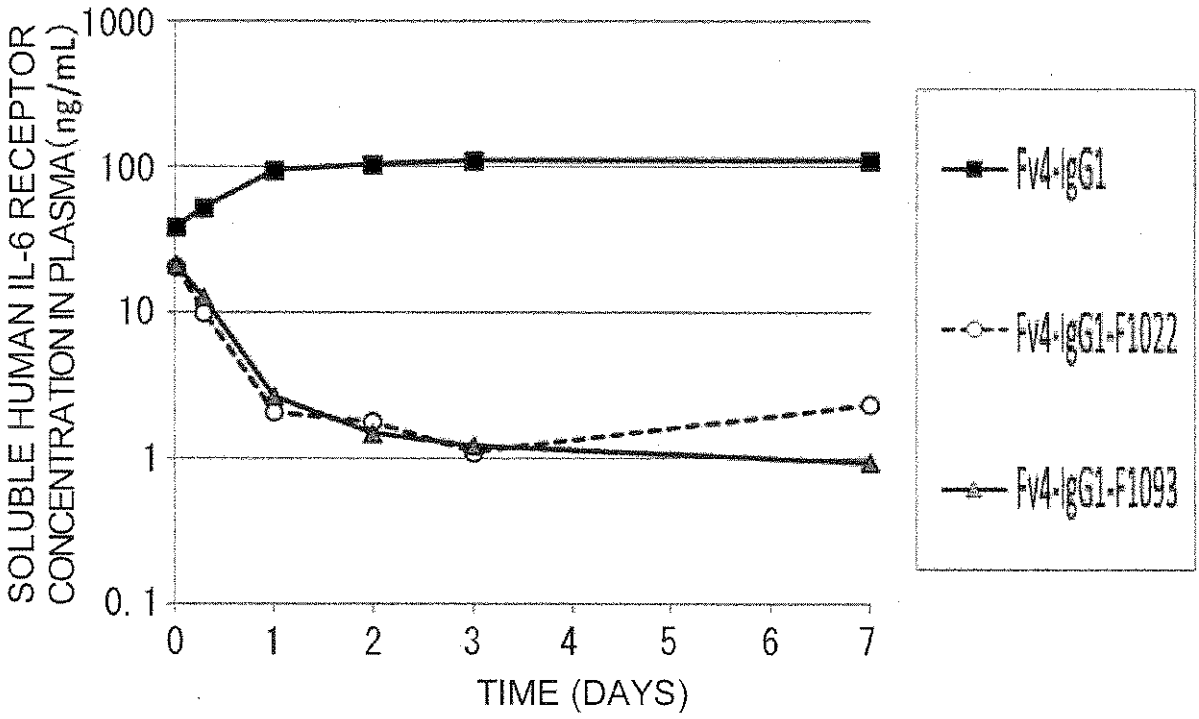

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA (ng/mL)
1000
100
10
1
0.1
0
1
2
3
4
5
6
7
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1022
Fv4-IgG1-F1093

FIG. 4

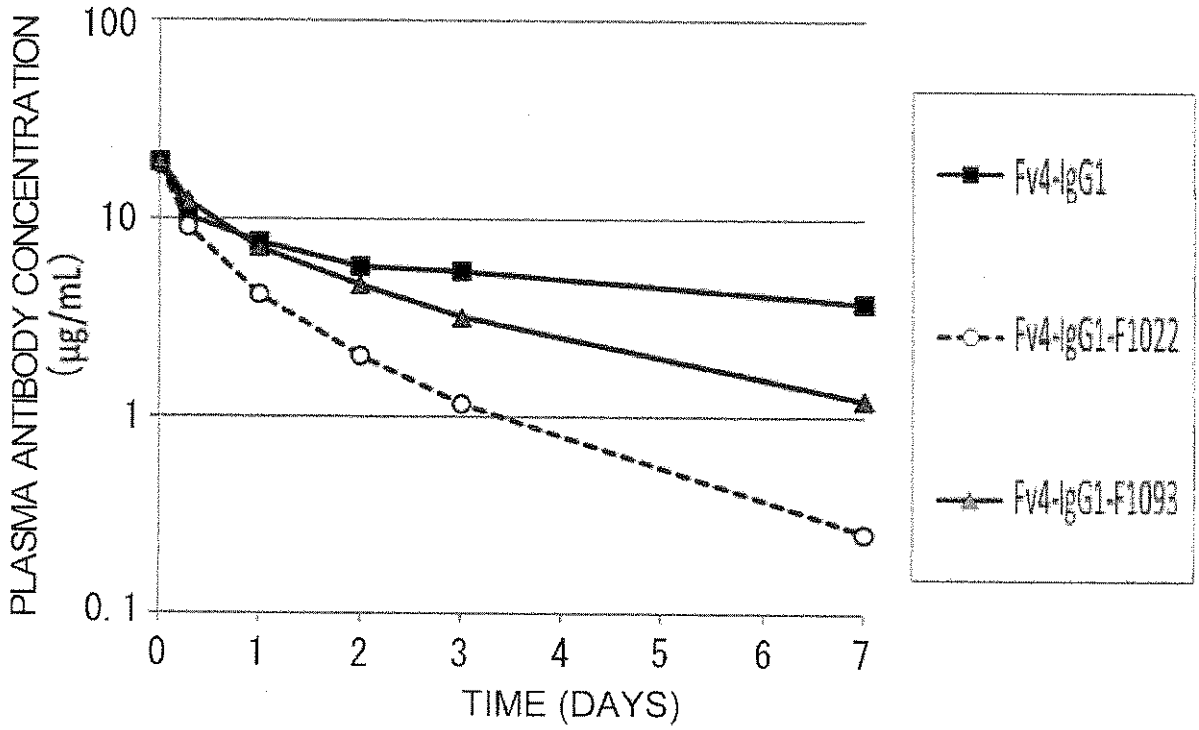

PLASMA ANTIBODY CONCENTRATION (μg/mL)
100
10
1
0.1
0
1
2
3
4
5
6
7
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1022
Fv4-IgG1-F1093

FIG. 5

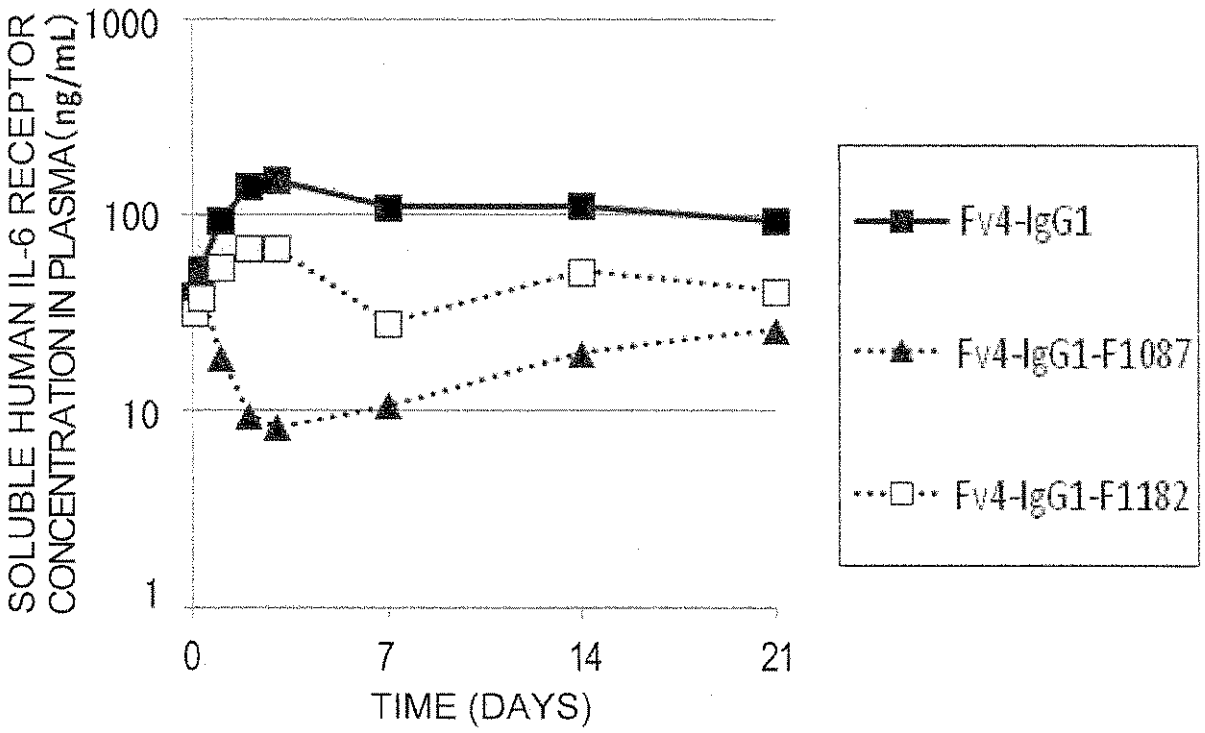

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA(ng/mL)
1000
100
10
1
0
7
14
21
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1087
Fv4-IgG1-F1182

FIG. 6

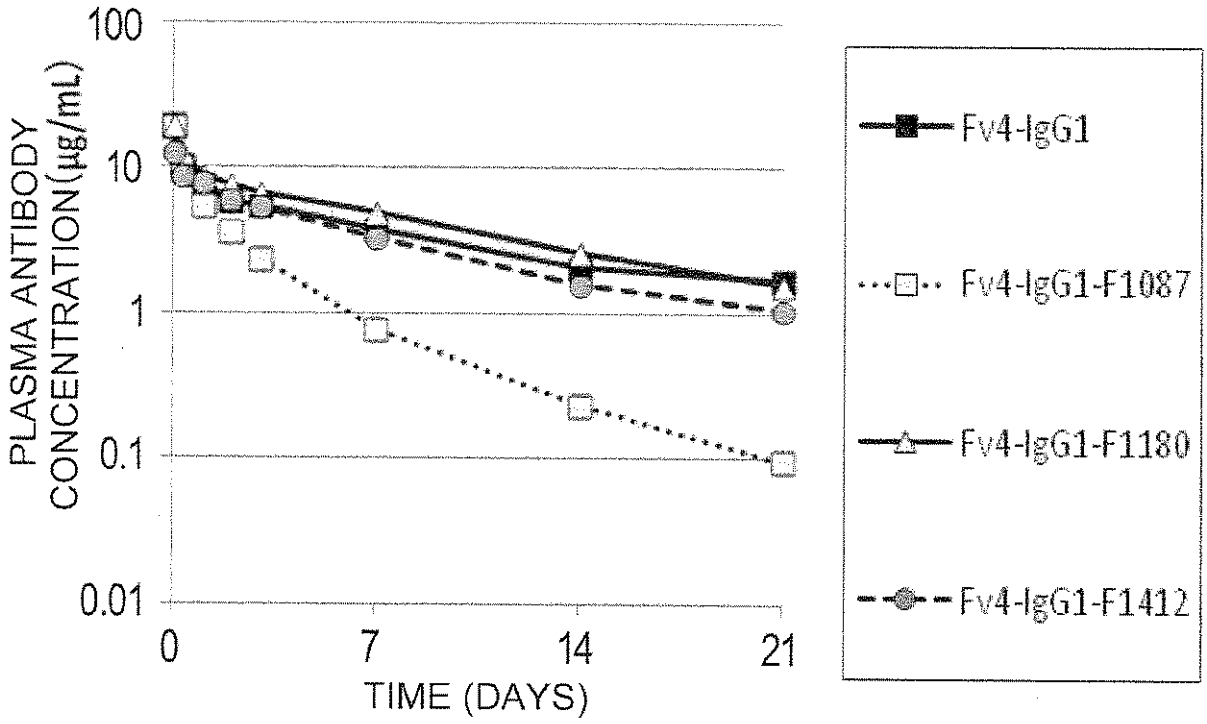

100
10
1
0.1
0.01
PLASMA ANTIBODY CONCENTRATION(μg/mL)
0
7
14
21
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1087
Fv4-IgG1-F1180
Fv4-IgG1-F1412

FIG. 7

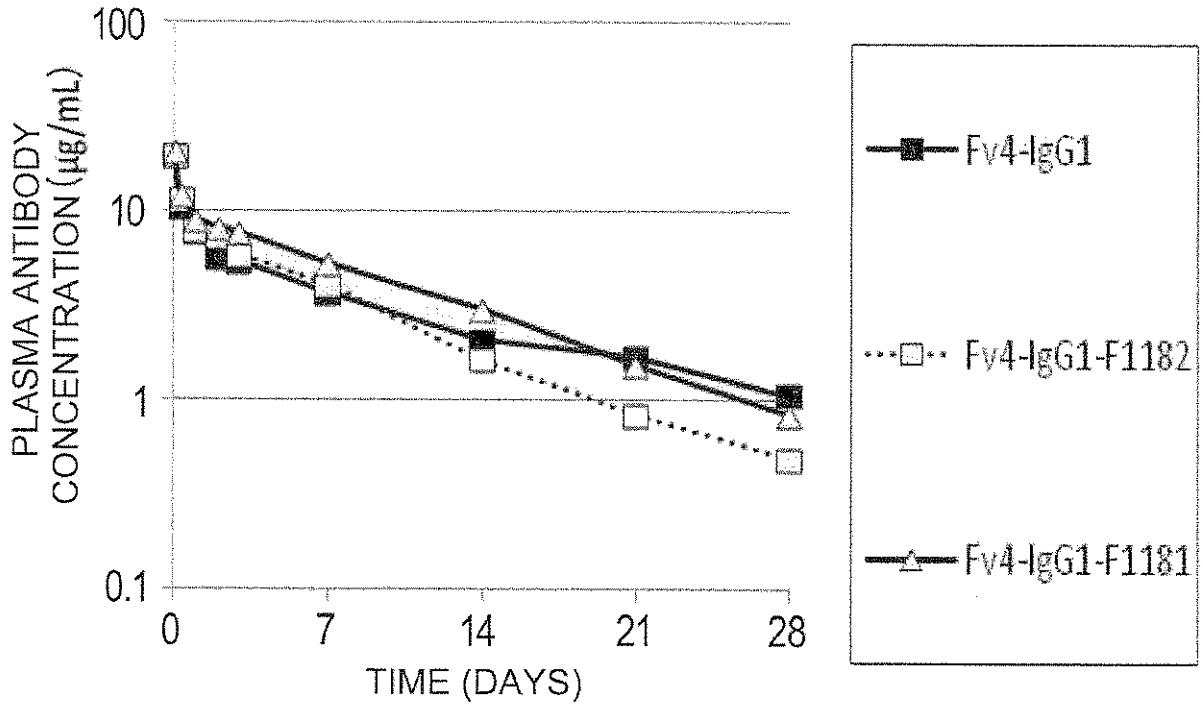

100
10
1
0.1
PLASMA ANTIBODY CONCENTRATION (μg/mL)
0
7
14
21
28
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1182
Fv4-IgG1-F1181

FIG. 8

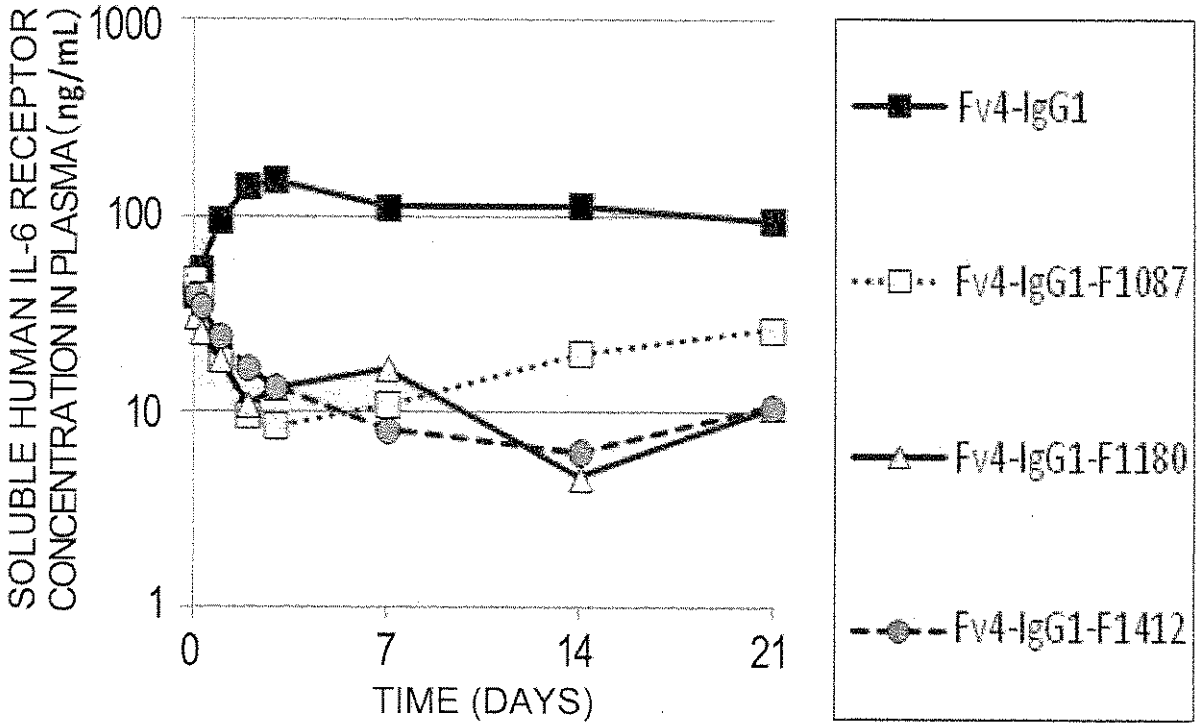

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA (ng/mL)
1000
100
10
1
0
7
14
21
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1087
Fv4-IgG1-F1180
Fv4-IgG1-F1412

FIG. 9

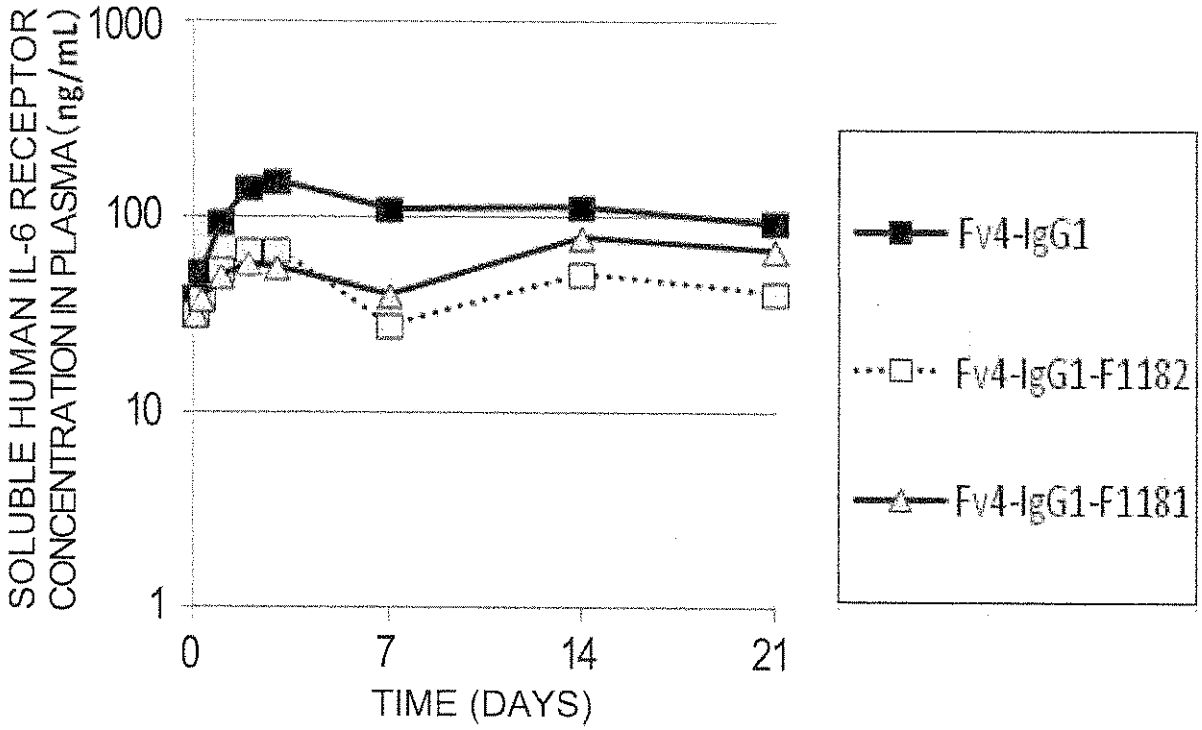

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA (ng/mL)
1000
100
10
1
0
7
14
21
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1182
Fv4-IgG1-F1181

FIG. 10

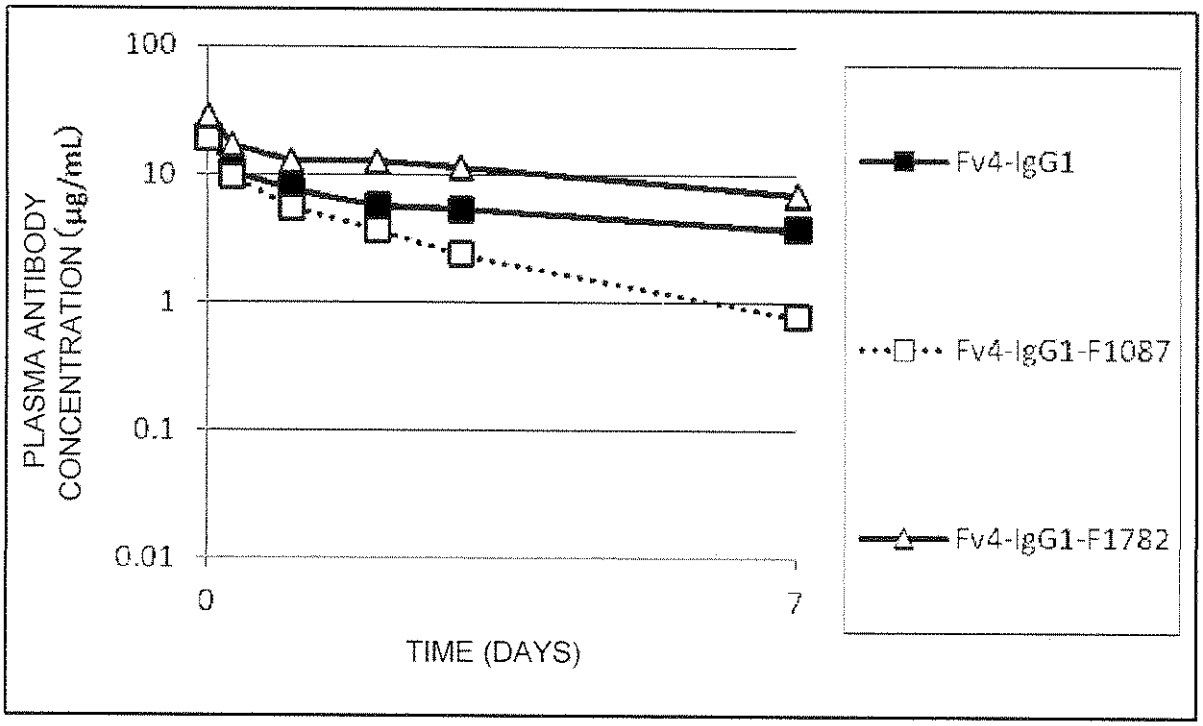

100
10
1
0.1
0.01
PLASMA ANTIBODY CONCENTRATION (μg/mL)
0
7
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1087
Fv4-IgG1-F1782

FIG. 11

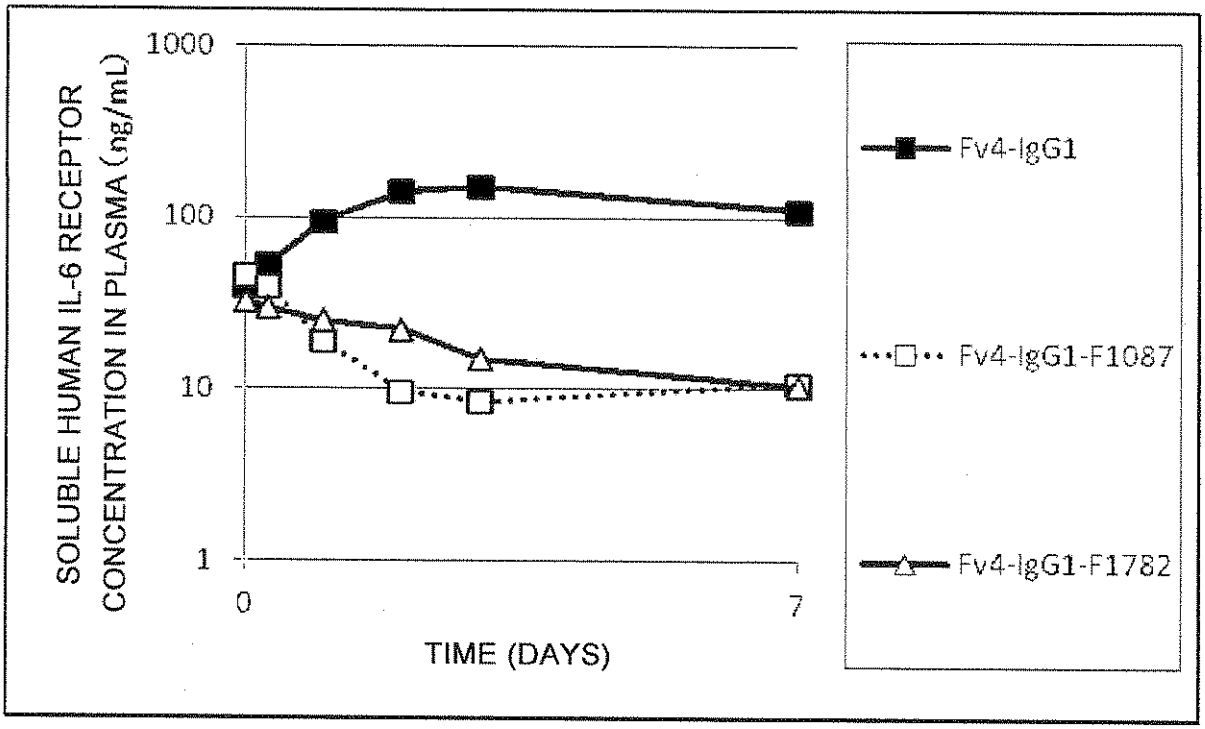

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA (ng/mL)
1000
100
10
1
0
7
TIME (DAYS)
Fv4-IgG1
Fv4-IgG1-F1087
Fv4-IgG1-F1782

FIG. 12

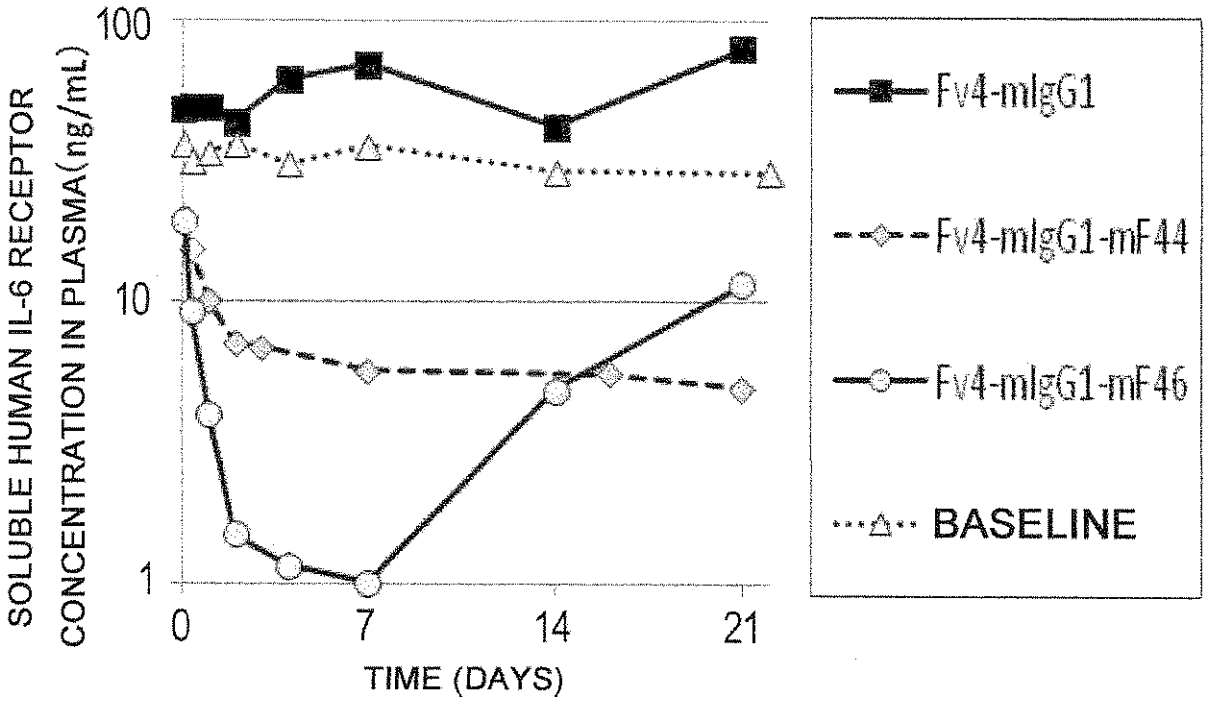

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA(ng/mL)
100
10
1
0
7
14
21
TIME (DAYS)
Fv4-mIgG1
Fv4-mIgG1-mF44
Fv4-mIgG1-mF46
BASELINE

FIG. 13

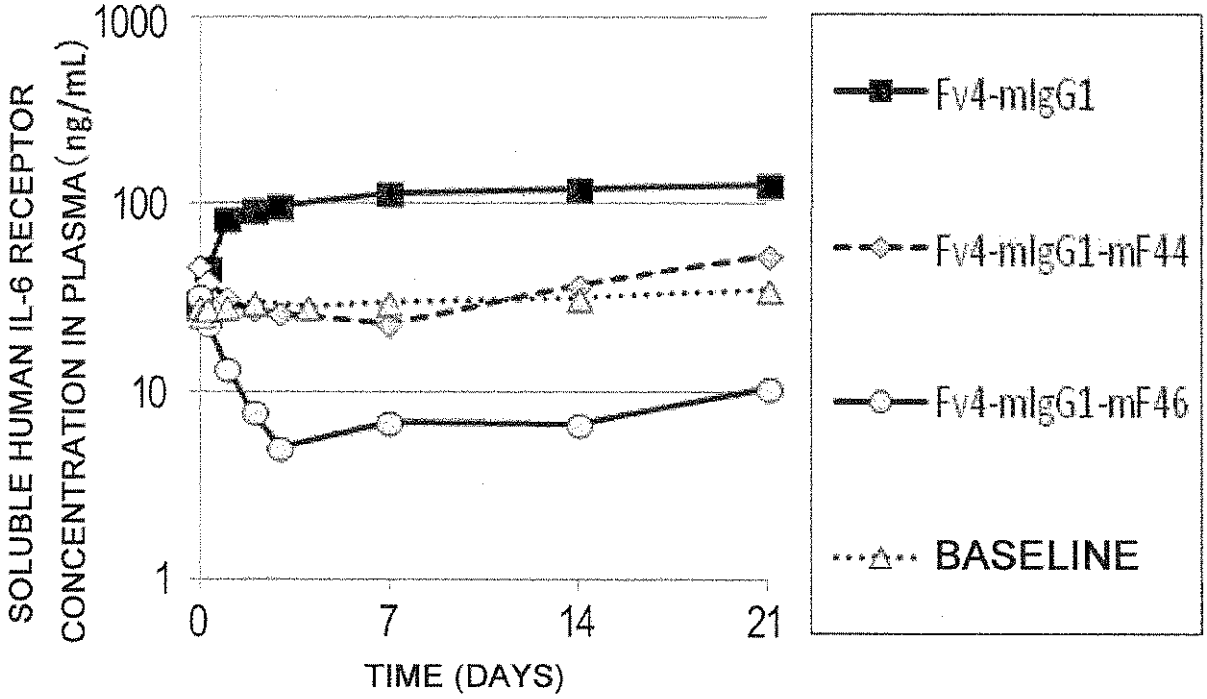

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA (ng/mL)
1000
100
10
1
0
7
14
21
TIME (DAYS)
Fv4-mIgG1
Fv4-mIgG1-mF44
Fv4-mIgG1-mF46
BASELINE

FIG. 14

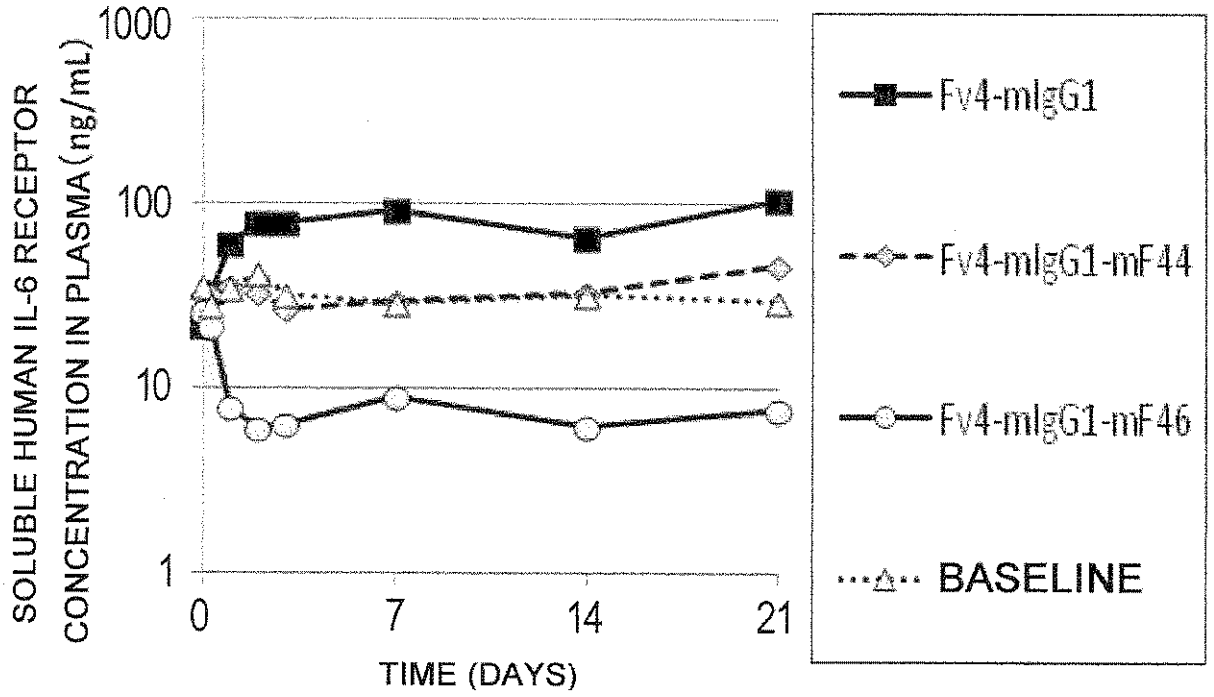

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA (ng/mL)
1000
100
10
1
0
7
14
21
TIME (DAYS)
Fv4-mIgG1
Fv4-mIgG1-mF44
Fv4-mIgG1-mF46
BASELINE

FIG. 15

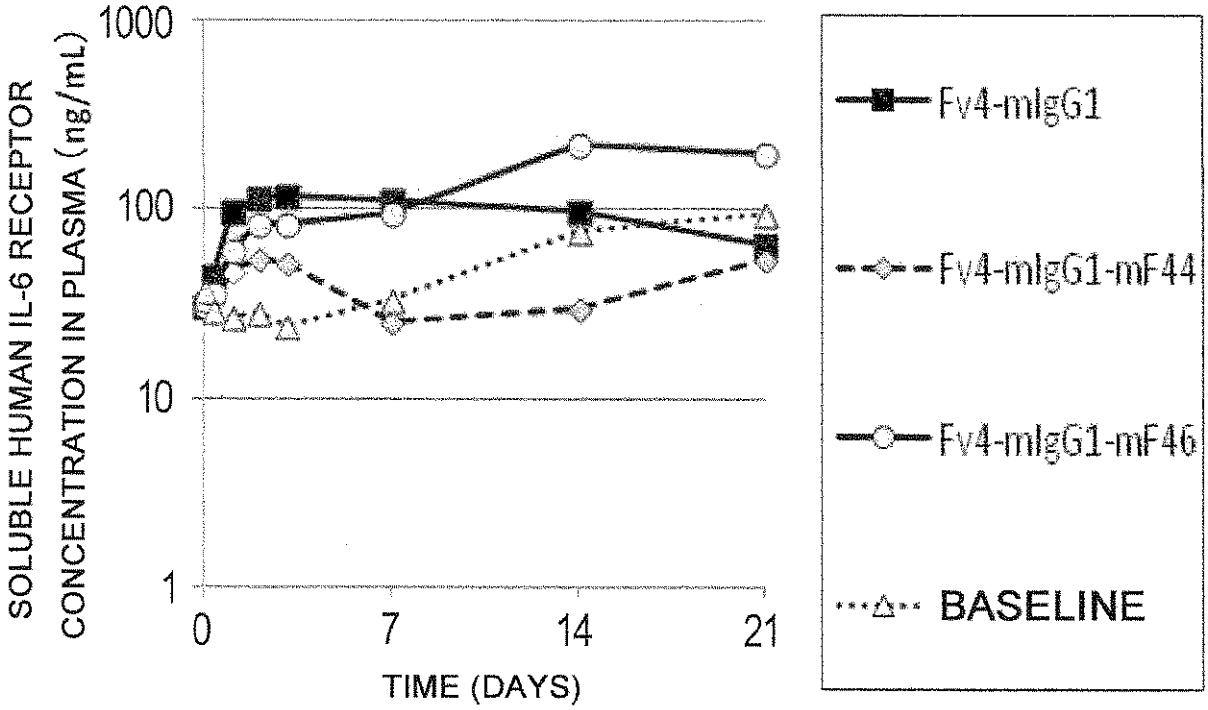

SOLUBLE HUMAN IL-6 RECEPTOR CONCENTRATION IN PLASMA (ng/mL)
1000
100
10
1
0
7
14
21
TIME (DAYS)
Fv4-mIgG1
Fv4-mIgG1-mF44
Fv4-mIgG1-mF46
BASELINE

FIG. 16

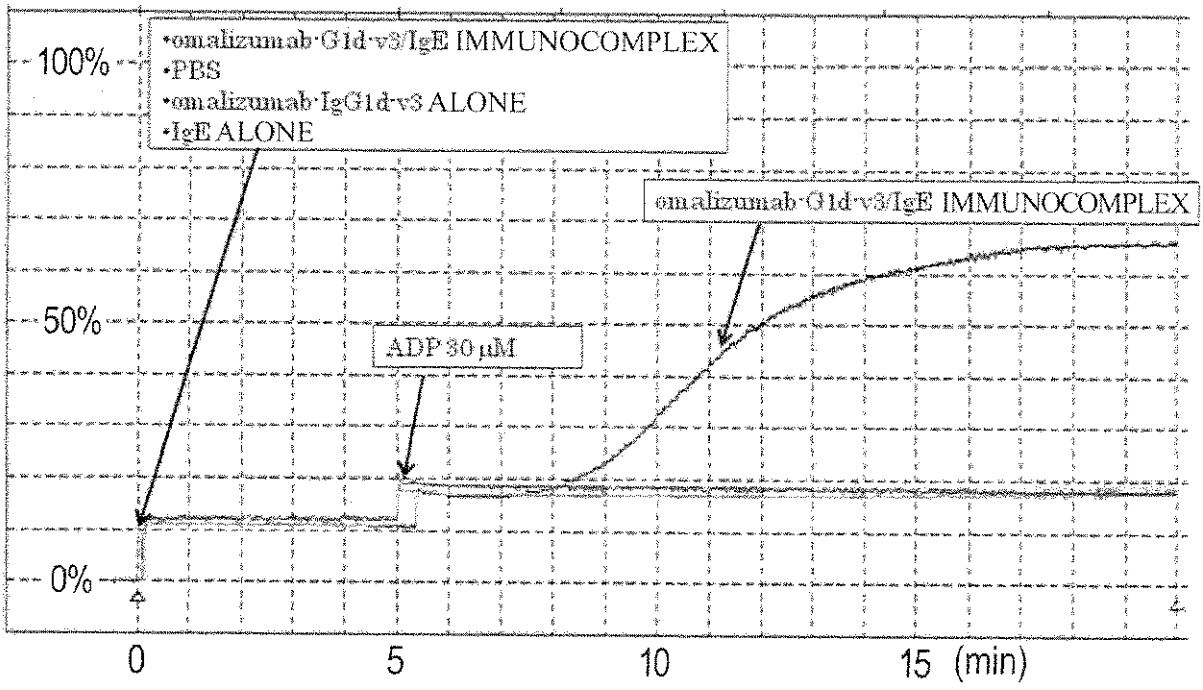

•omalizumab·G1d·v3/IgE IMMUNOCOMPLEX
•PBS
•omalizumab·IgG1d·v3 ALONE
•IgE ALONE
omalizumab·G1d·v3/IgE IMMUNOCOMPLEX
ADP 30 μM
100%
50%
0%
0
5
10
15 (min)

FIG. 17

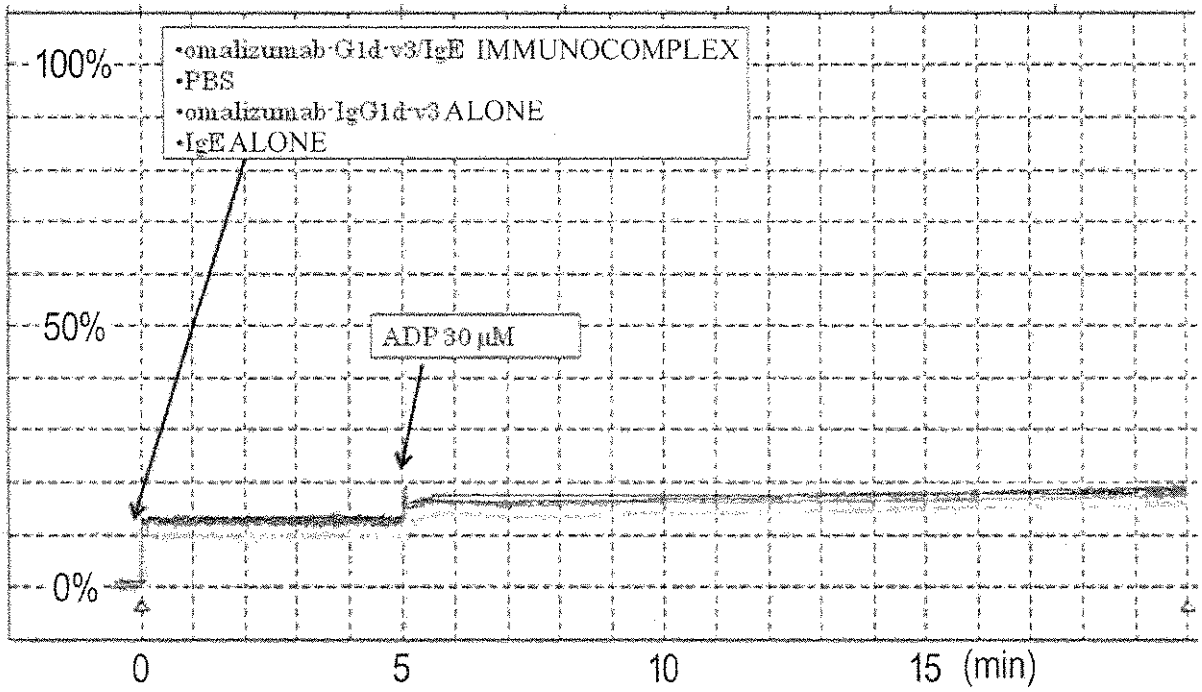

•omalizumab-G1d-v3/IgE IMMUNOCOMPLEX
•PBS
•omalizumab-IgG1d-v3 ALONE
•IgE ALONE
100%
50%
0%
ADP 30 μM
0
5
10
15 (min)

FIG. 18

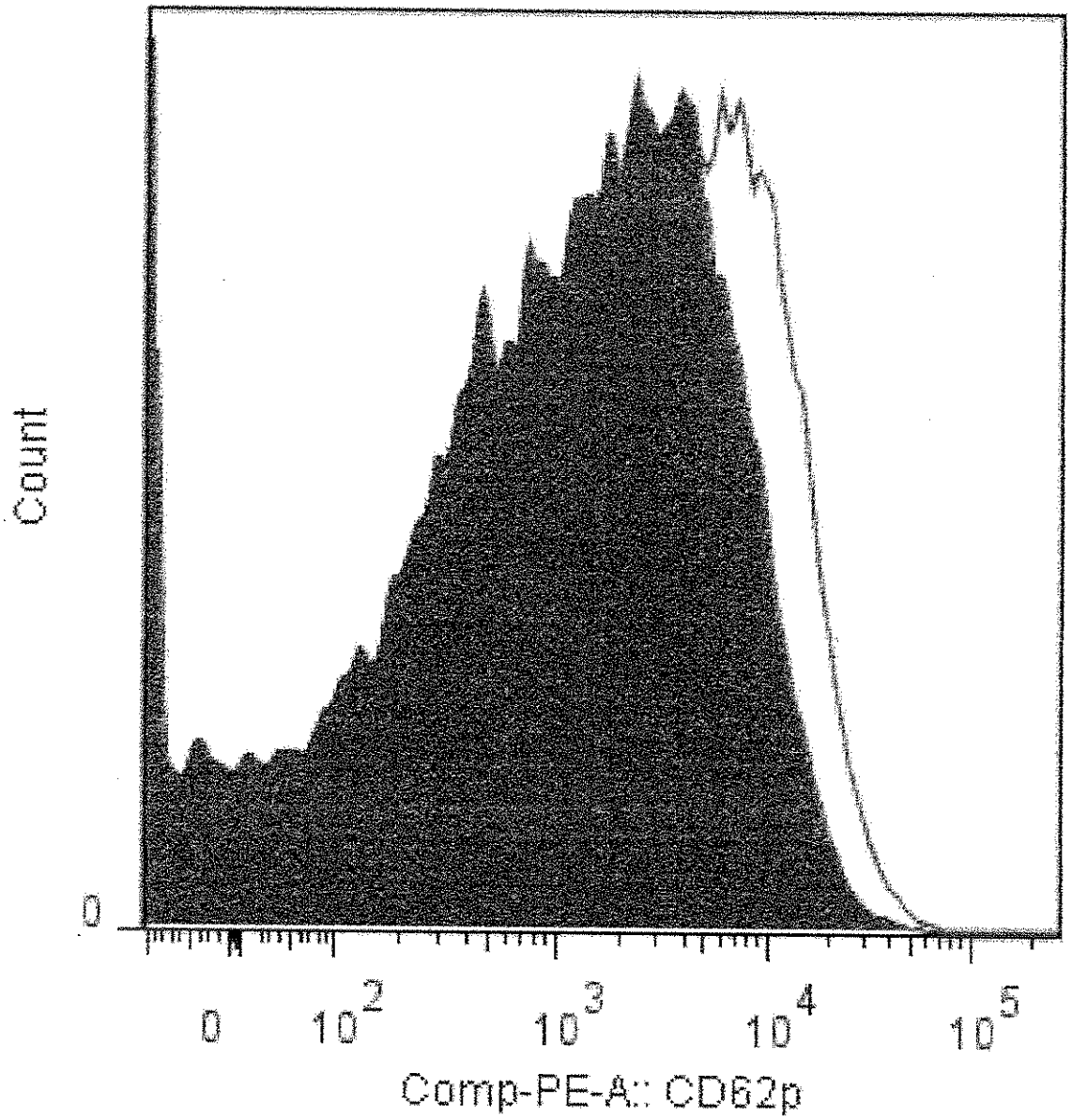


FIG. 19

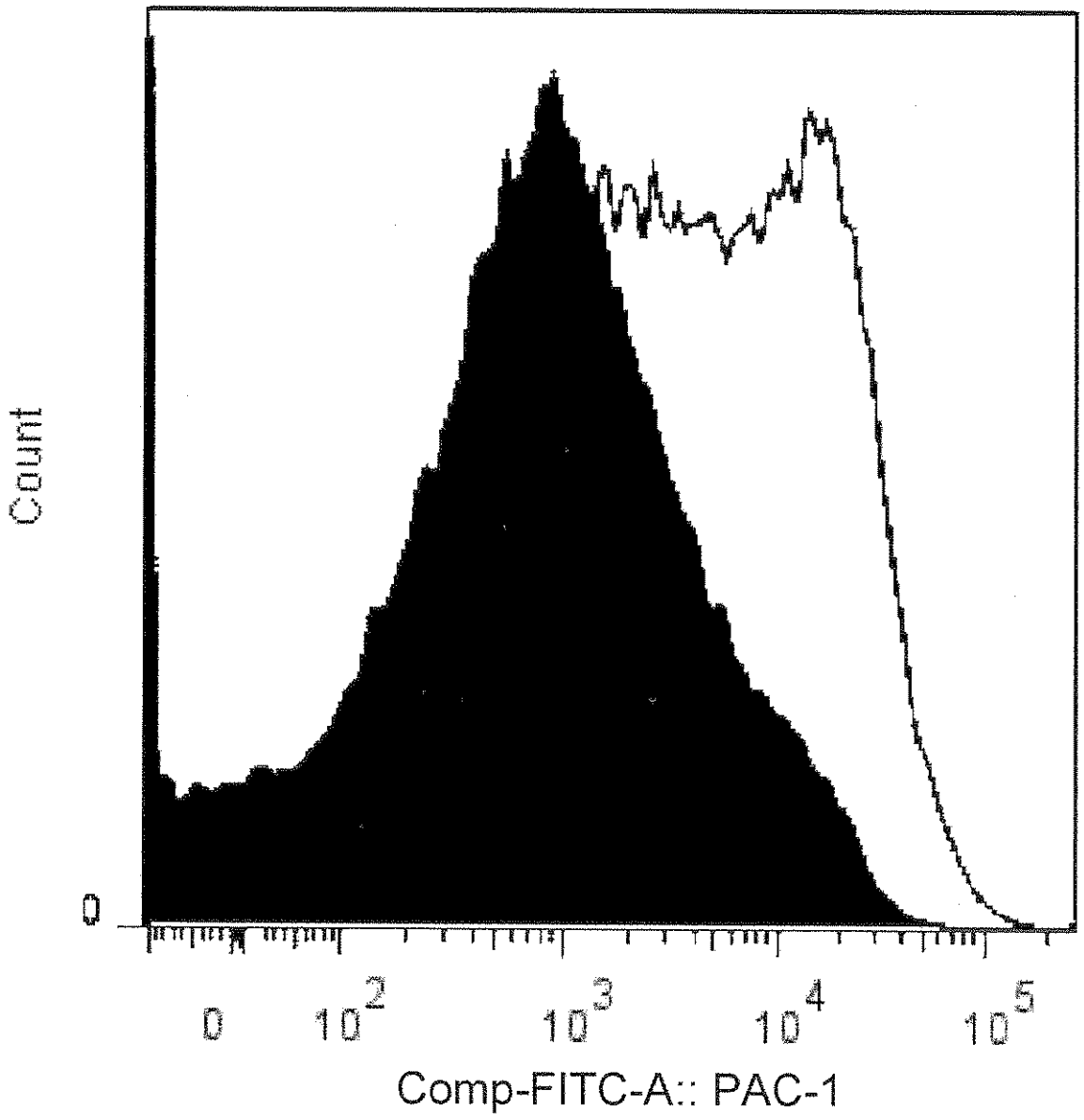


FIG. 20

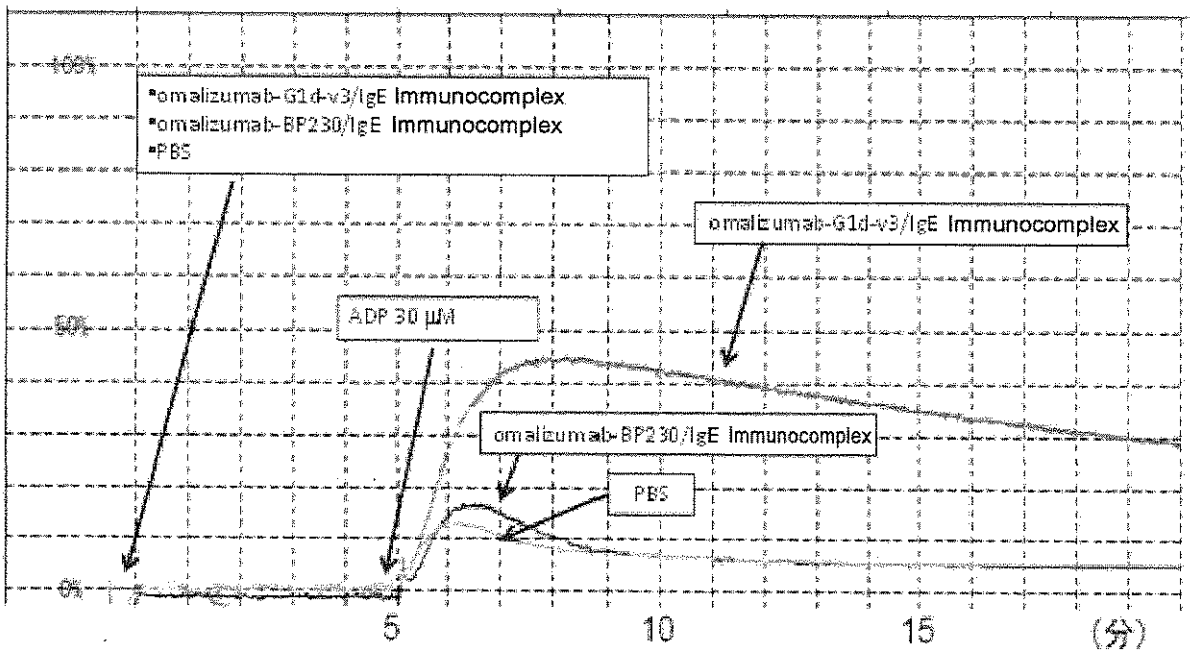

•omalizumab-G1d-v3/IgE Immunocomplex
•omalizumab-BP230/IgE Immunocomplex
•PBS
ADP 30 μM
omalizumab-G1d-v3/IgE Immunocomplex
omalizumab-BP230/IgE Immunocomplex
PBS
5
10
15
(分)

FIG. 21

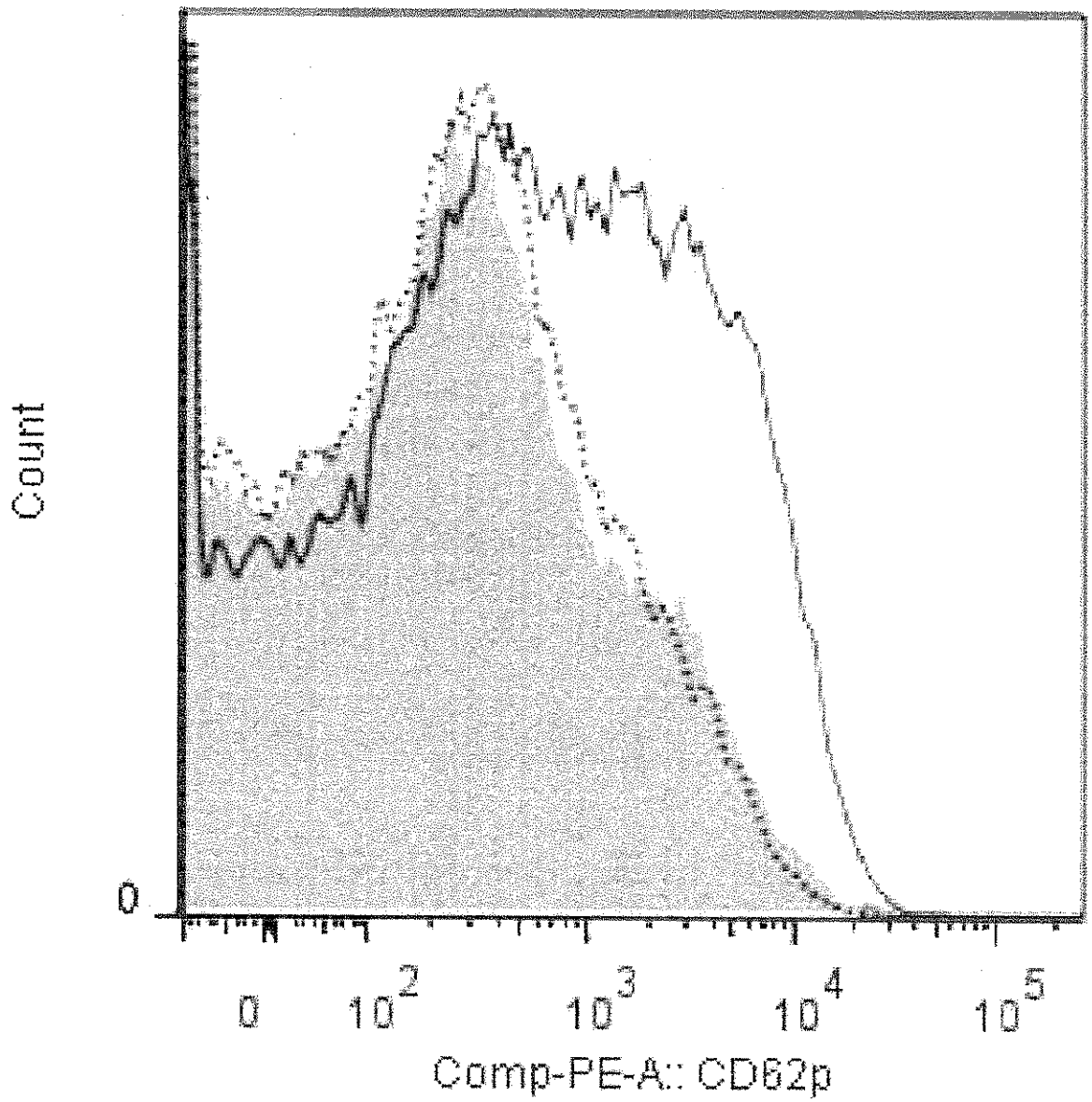


FIG. 22

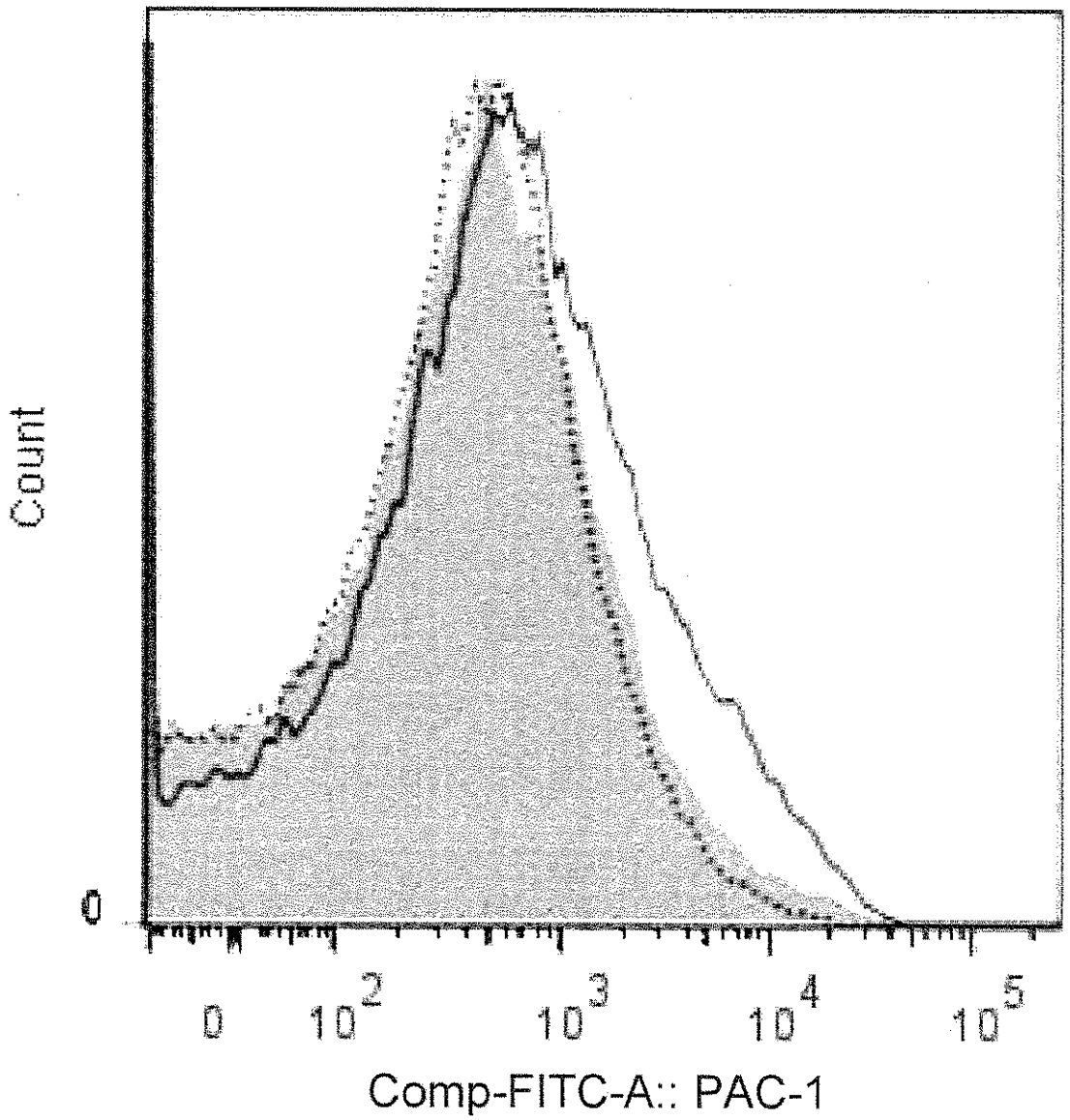


FIG. 23

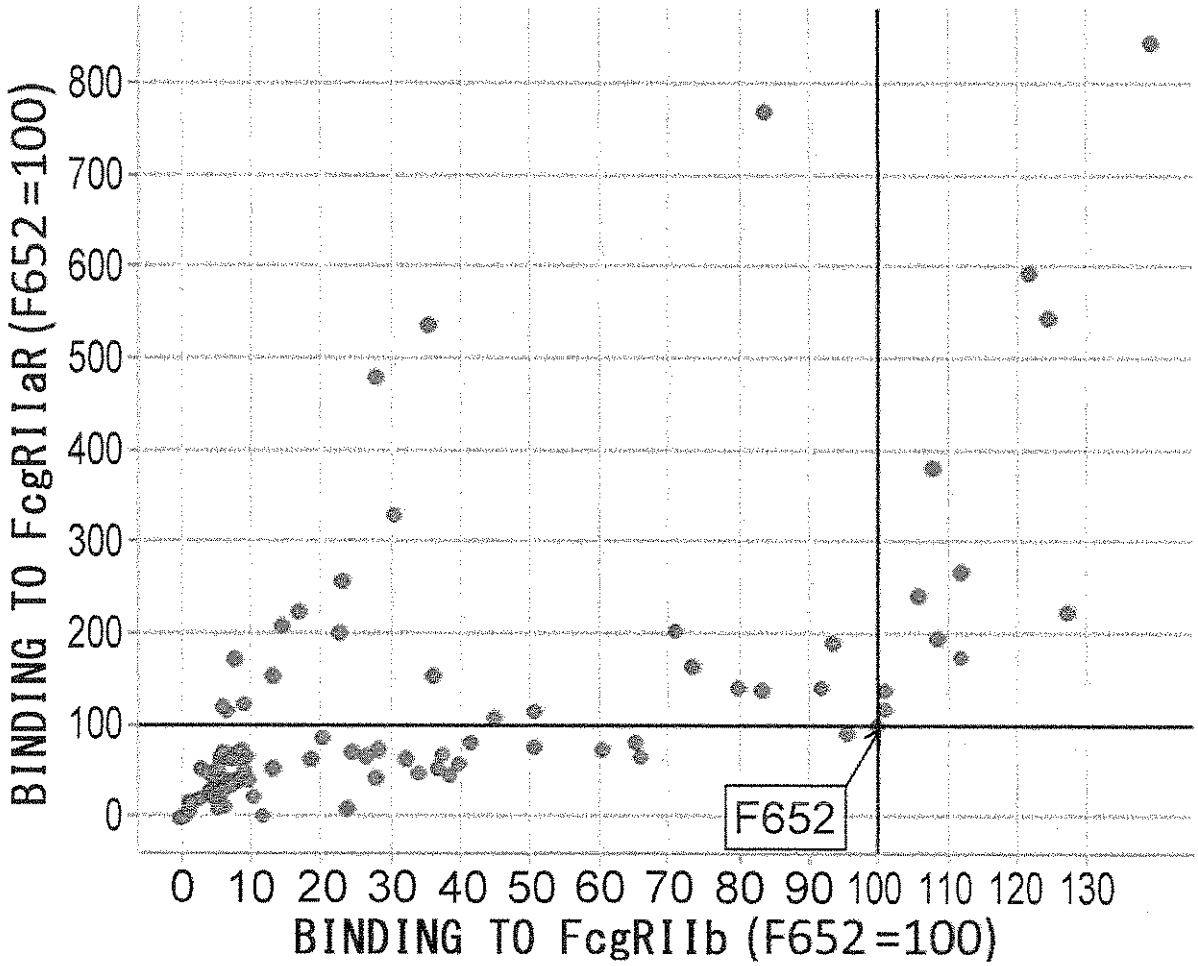

BINDING TO FcgRIIaR (F652=100)
800
700
600
500
400
300
200
100
0
F652
0 10 20 30 40 50 60 70 80 90 100 110 120 130
BINDING TO FcgRIIb (F652=100)

FIG. 24

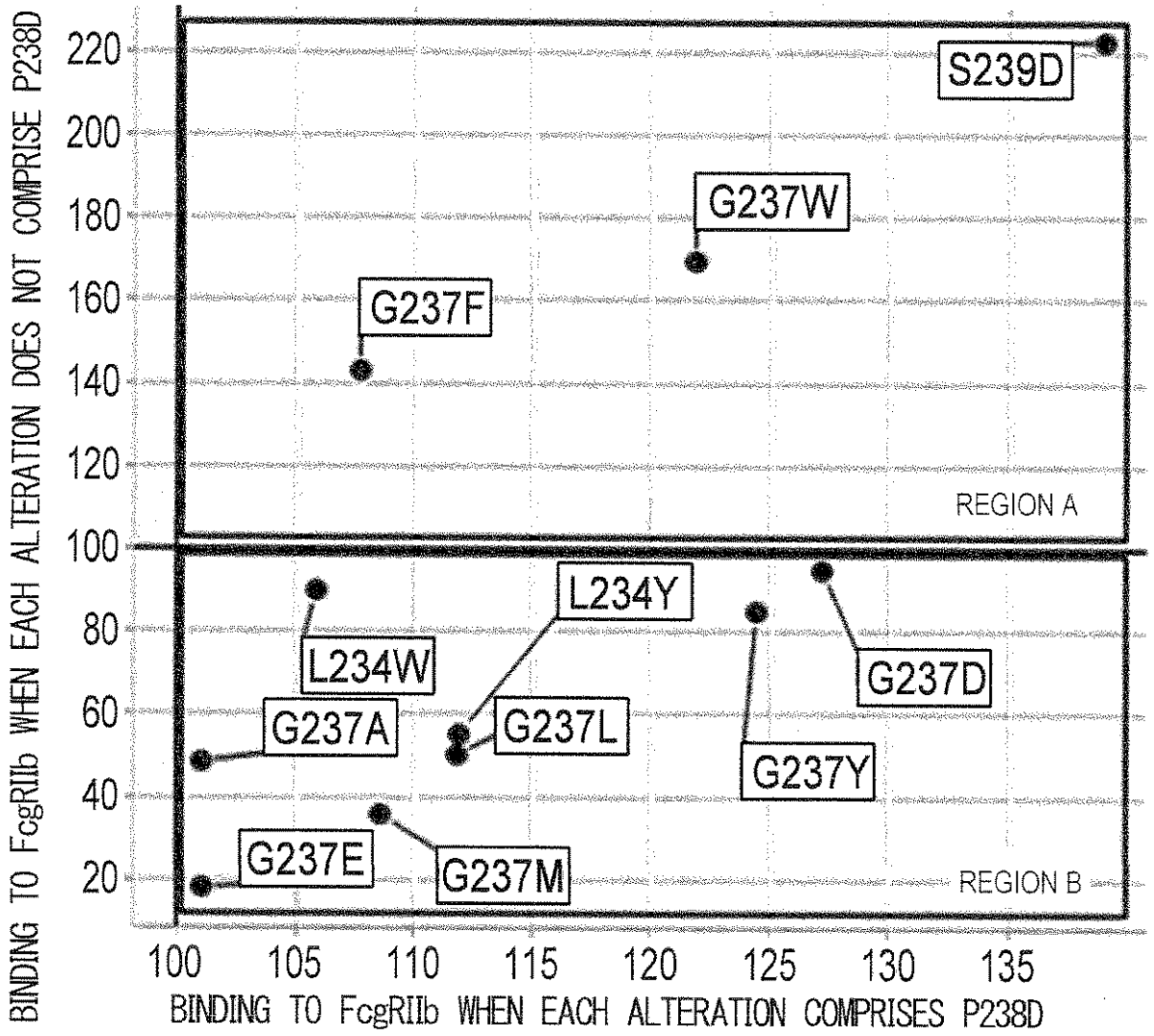

BINDING TO FcgRIIb WHEN EACH ALTERATION DOES NOT COMPRISE P238D
220
200
180
160
140
120
100
80
60
40
20
S239D
G237W
G237F
REGION A
L234Y
L234W
G237A
G237L
G237D
G237Y
G237E
G237M
REGION B
100
105
110
115
120
125
130
135
BINDING TO FcgRIIb WHEN EACH ALTERATION COMPRISES P238D

FIG. 25

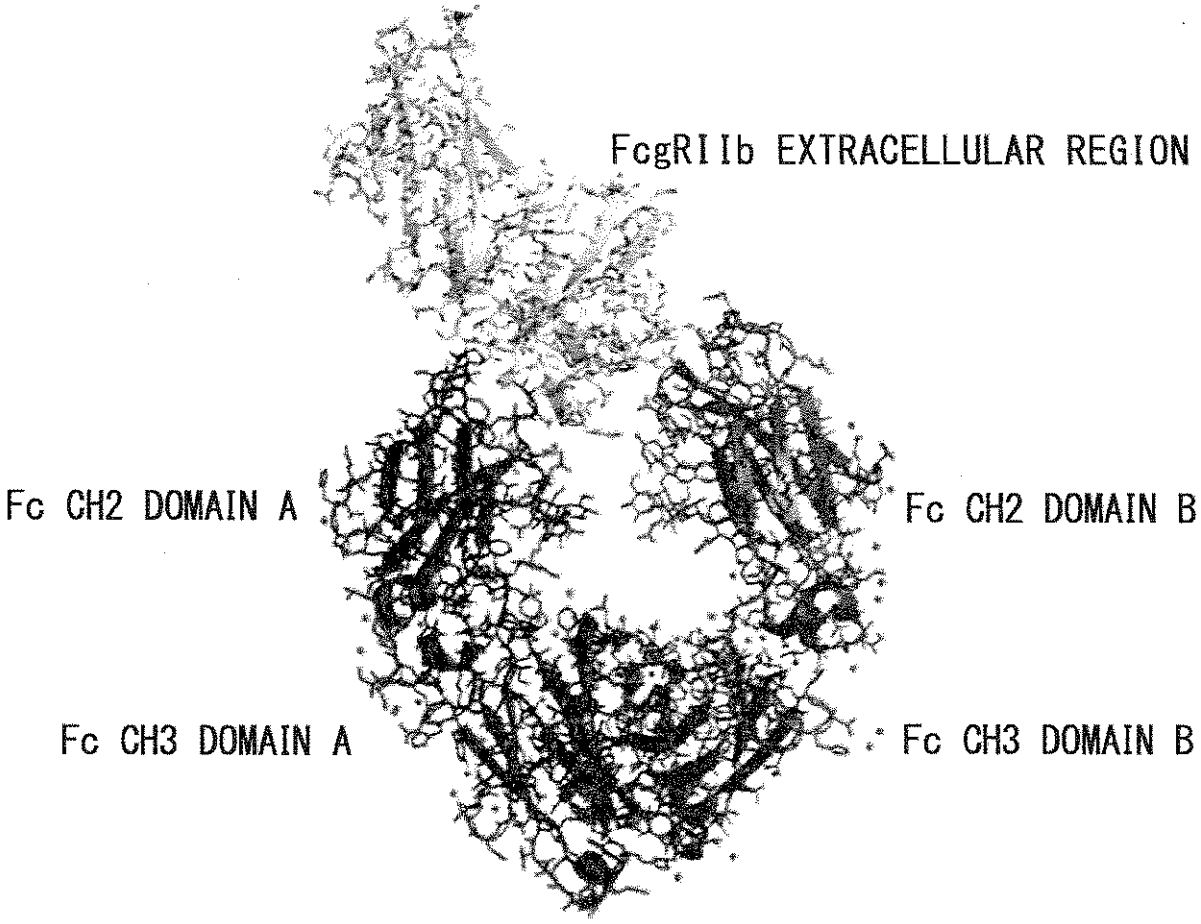
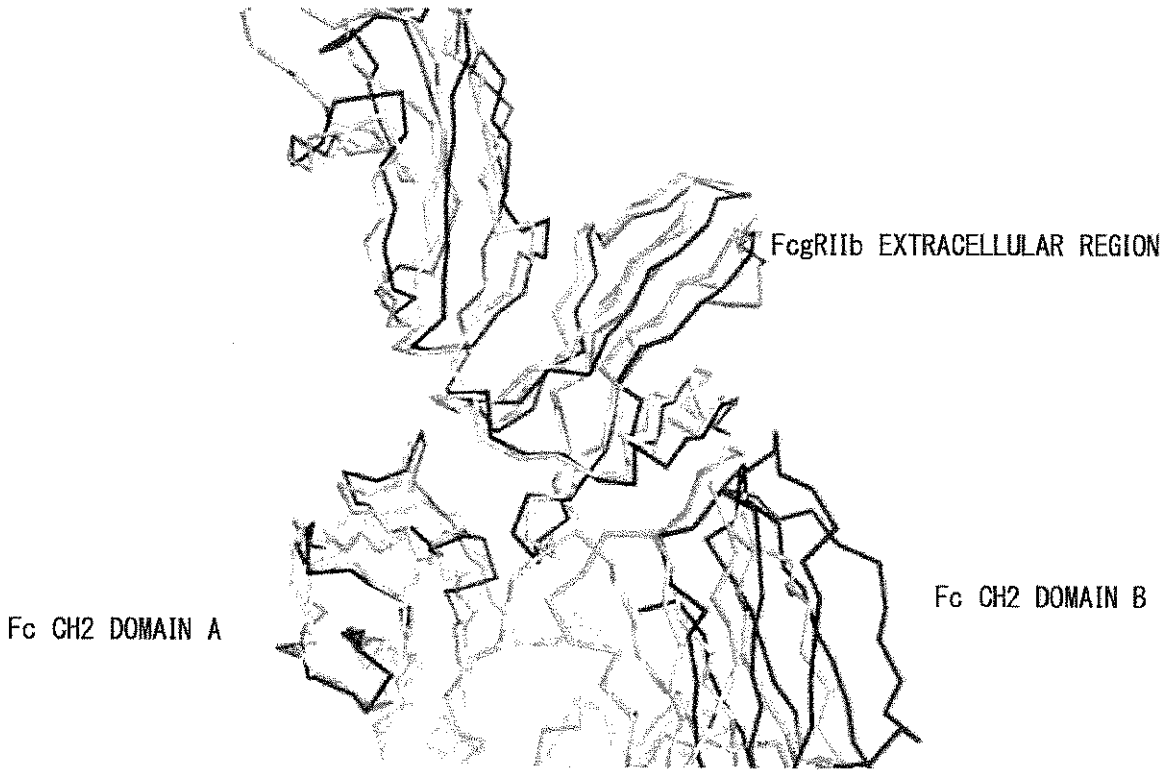
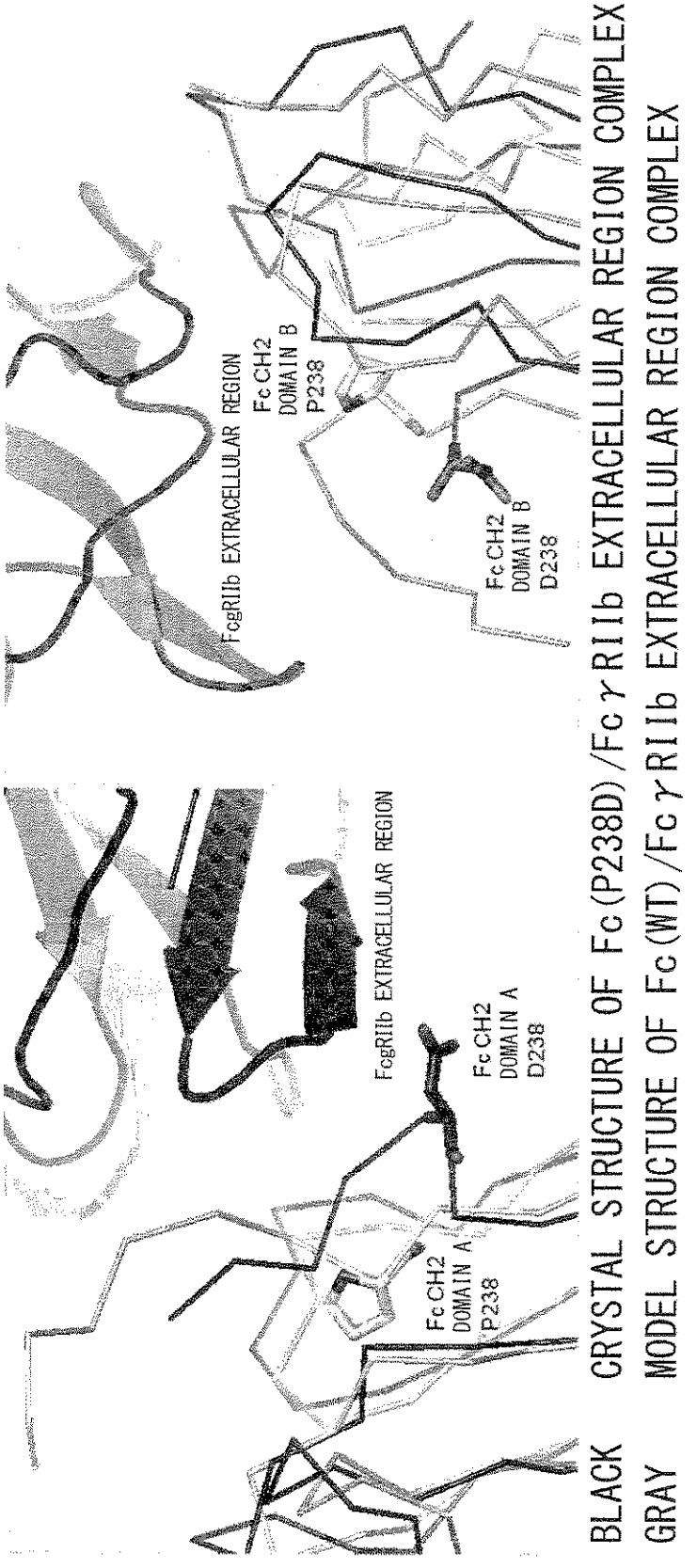

FcgRIIb EXTRACELLULAR REGION
Fc CH2 DOMAIN A
Fc CH2 DOMAIN B
Fc CH3 DOMAIN A
Fc CH3 DOMAIN B

FIG. 26



BLACK CRYSTAL STRUCTURE OF Fc(P238D)/FcγRIIb EXTRACELLULAR REGION COMPLEX
GRAY MODEL STRUCTURE OF Fc(WT)/FcγRIIb EXTRACELLULAR REGION COMPLEX

FIG. 27



BLACK CRYSTAL STRUCTURE OF Fc(P238D)/FcγRIIb EXTRACELLULAR REGION COMPLEX
GRAY MODEL STRUCTURE OF Fc(WT)/FcγRIIb EXTRACELLULAR REGION COMPLEX

FIG. 28

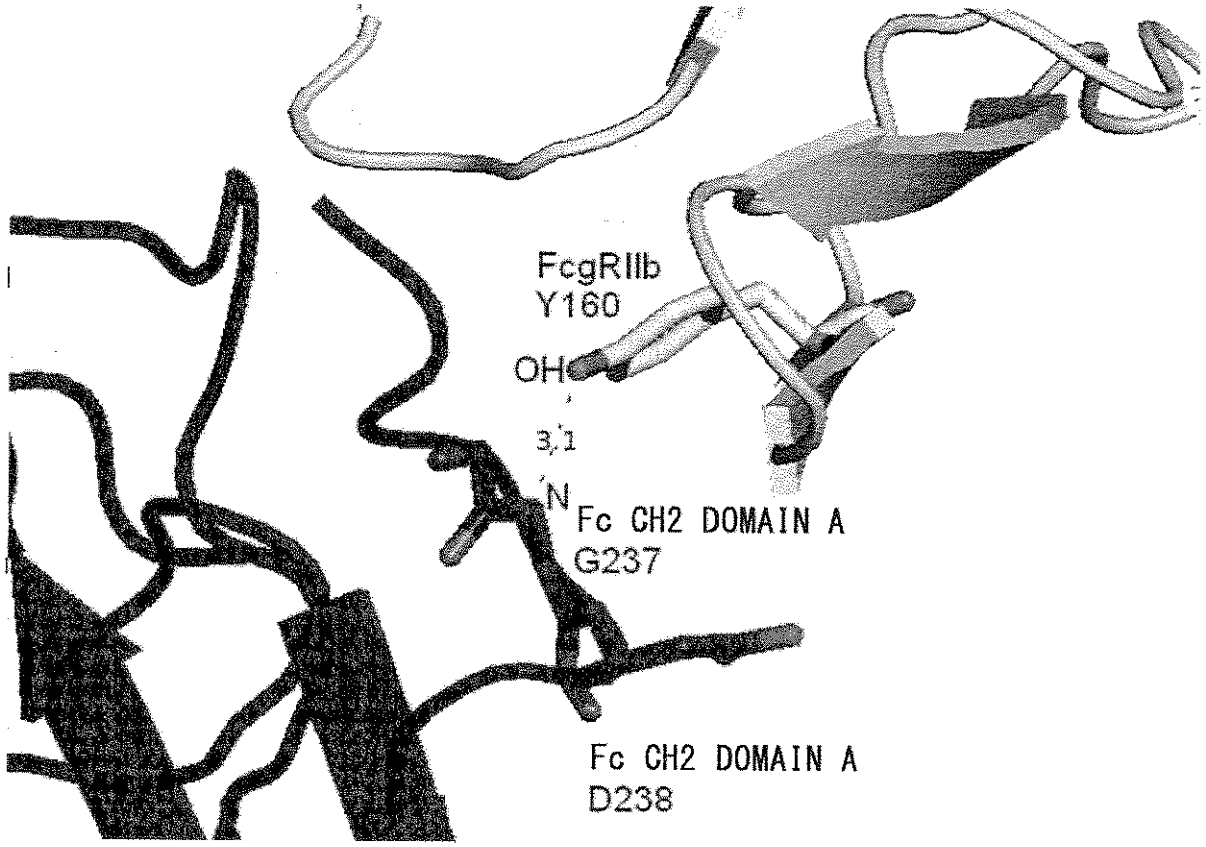


FIG. 29

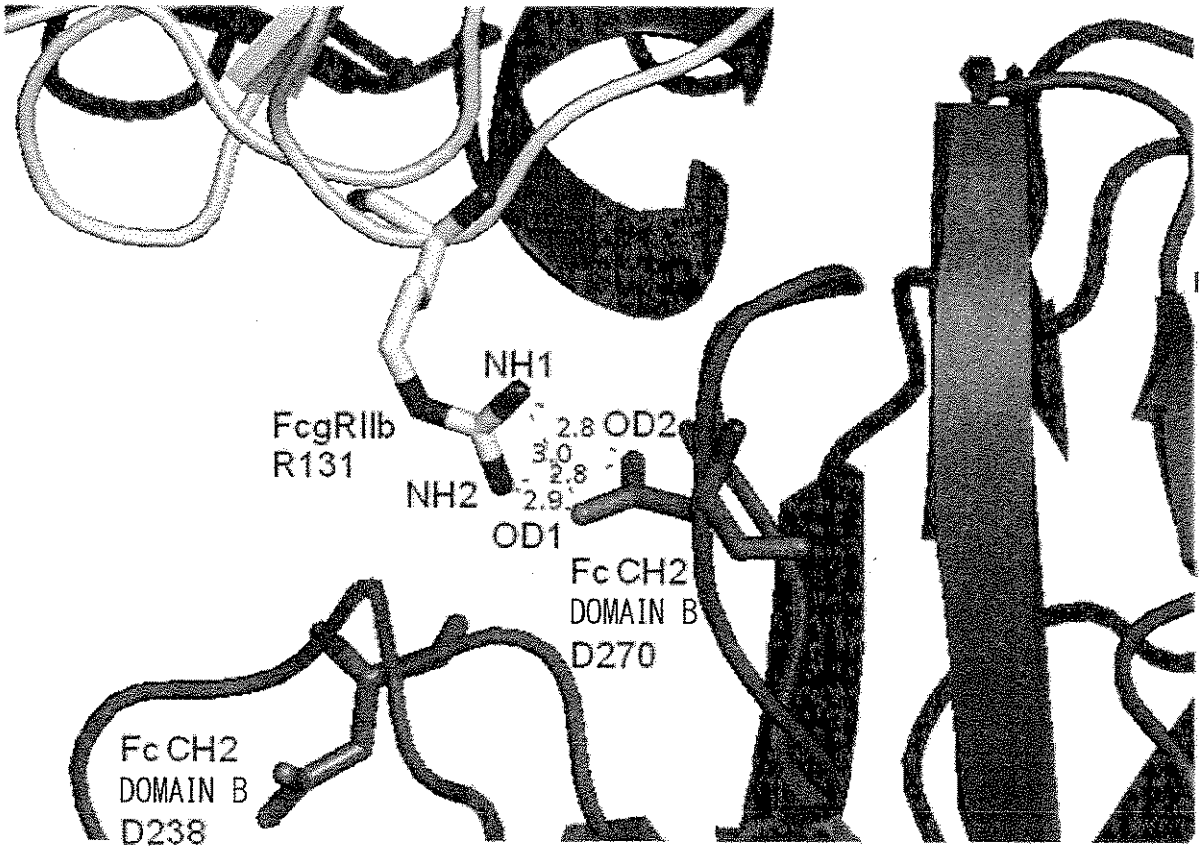

NH1
FcgRIIb
R131
2.8
OD2
3.0
2.8
NH2
2.9
OD1
Fc CH2
DOMAIN B
D270
Fc CH2
DOMAIN B
D238

FIG. 30

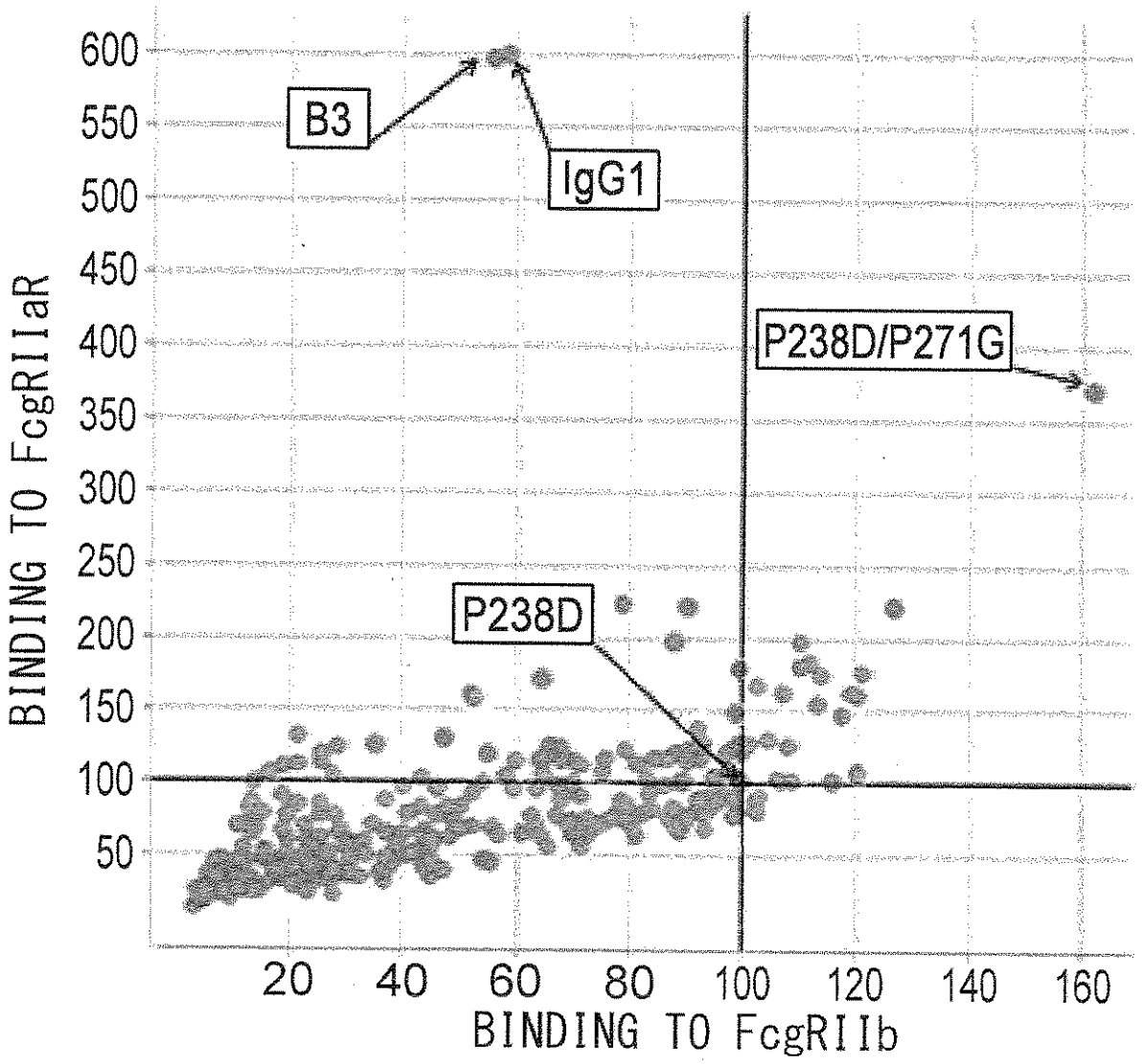

600
550
500
450
400
350
300
250
200
150
100
50
BINDING TO FcgRIIaR
B3
IgG1
P238D/P271G
P238D
20
40
60
80
100
120
140
160
BINDING TO FcgRIIb

FIG. 31

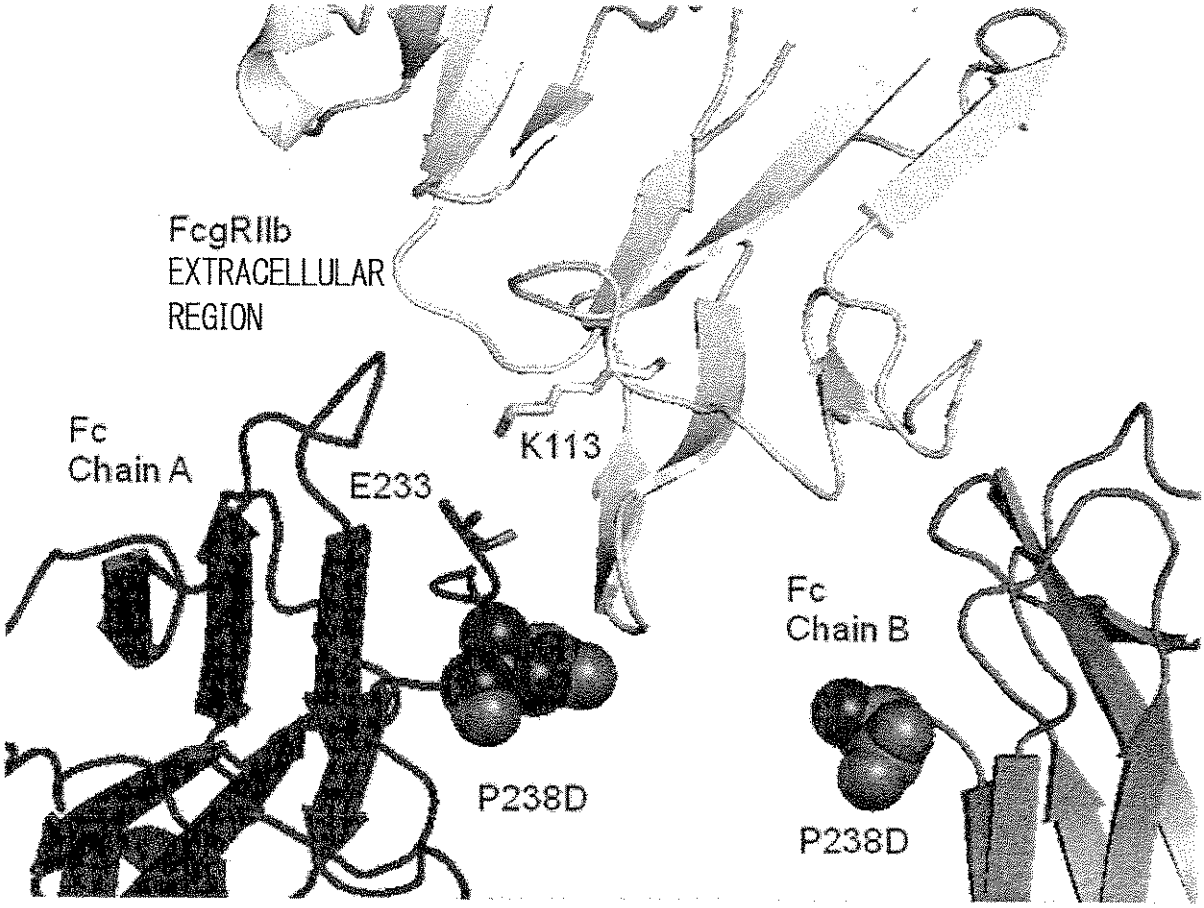

FcgRIIb
EXTRACELLULAR
REGION
Fc
Chain A
E233
K113
P238D
Fc
Chain B
P238D

FIG. 32

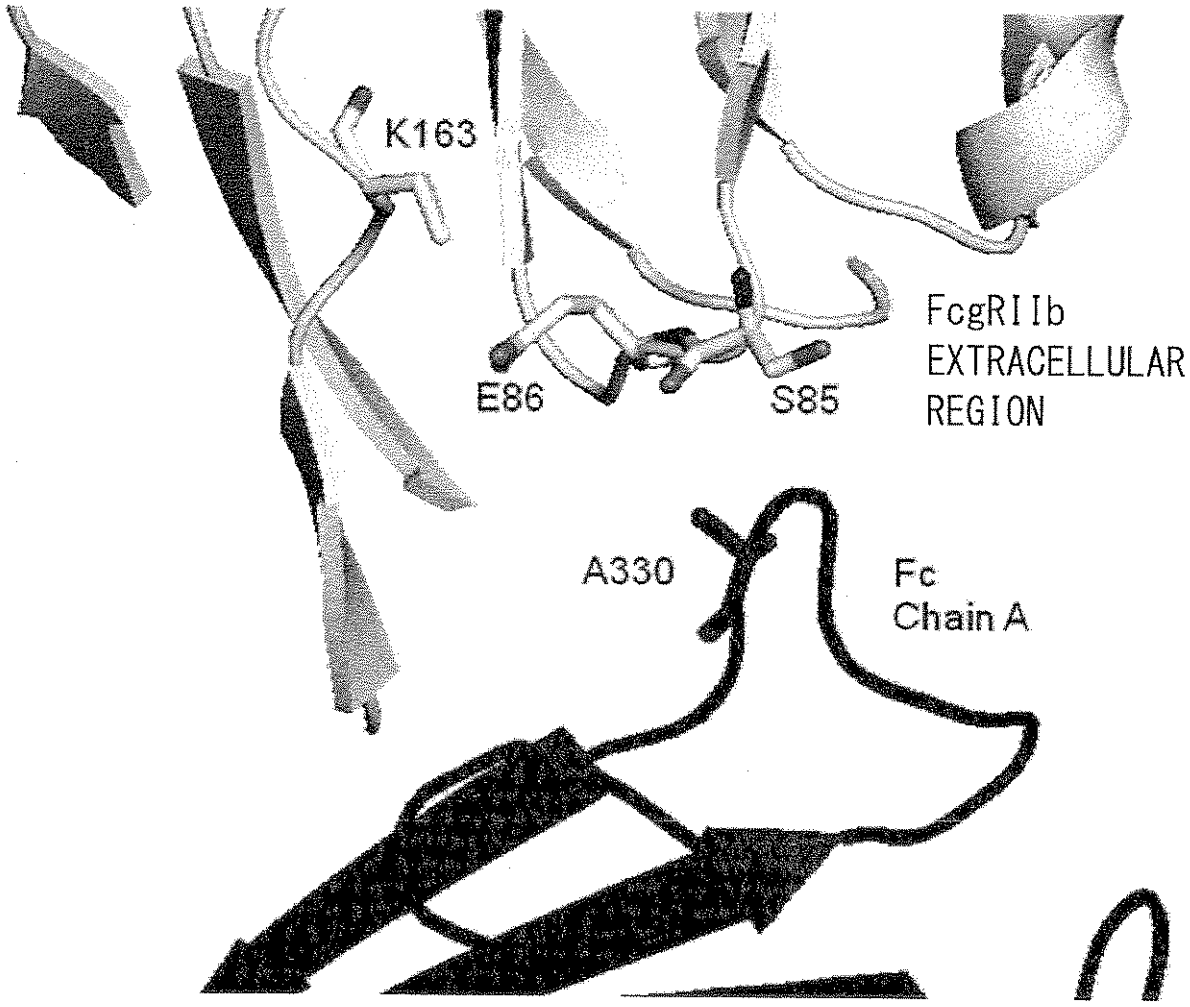
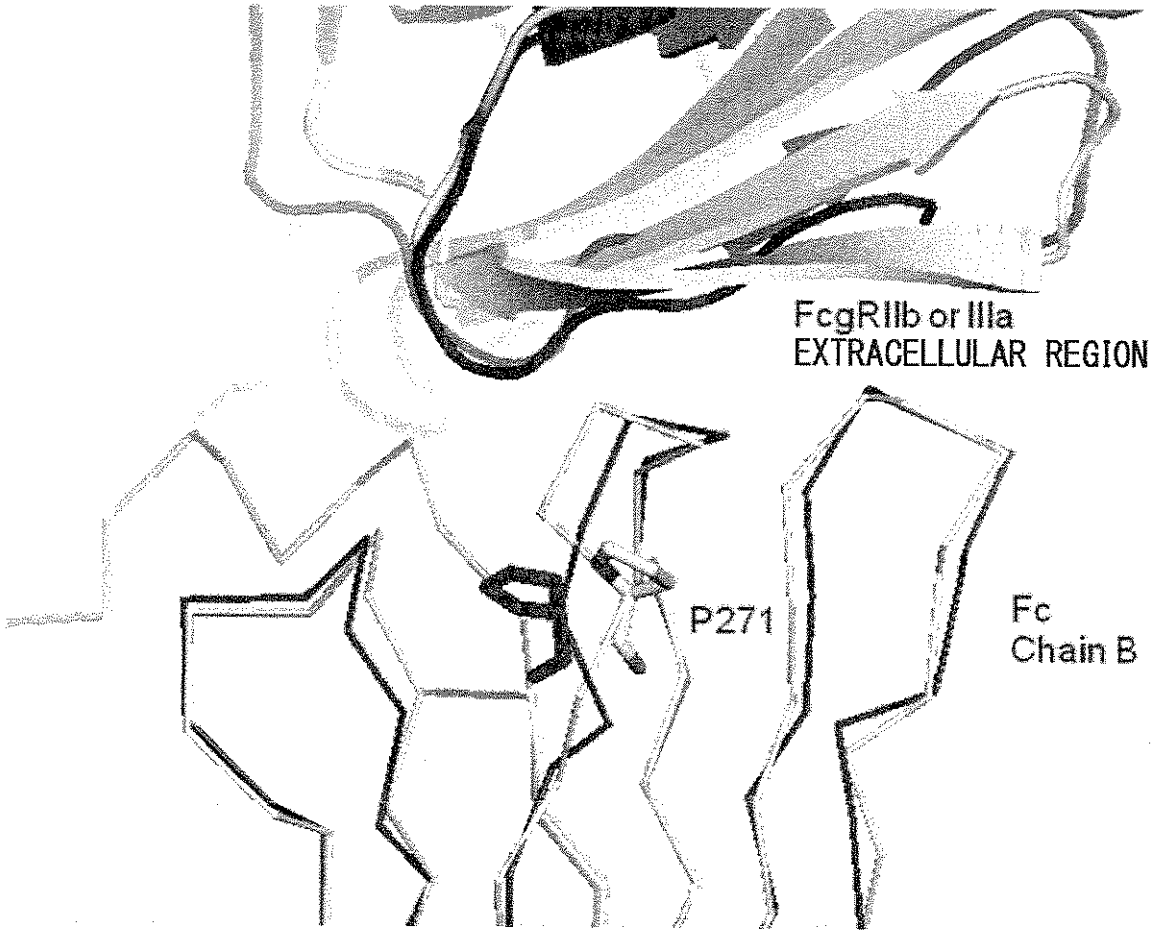


FIG. 33



BLACK CRYSTAL STRUCTURE OF Fc(P238D)/FcγRIIb EXTRACELLULAR REGION COMPLEX
GRAY CRYSTAL STRUCTURE OF Fc(WT)/FcγRIIIa EXTRACELLULAR REGION COMPLEX (PDB code: 3SGJ)

FIG. 34

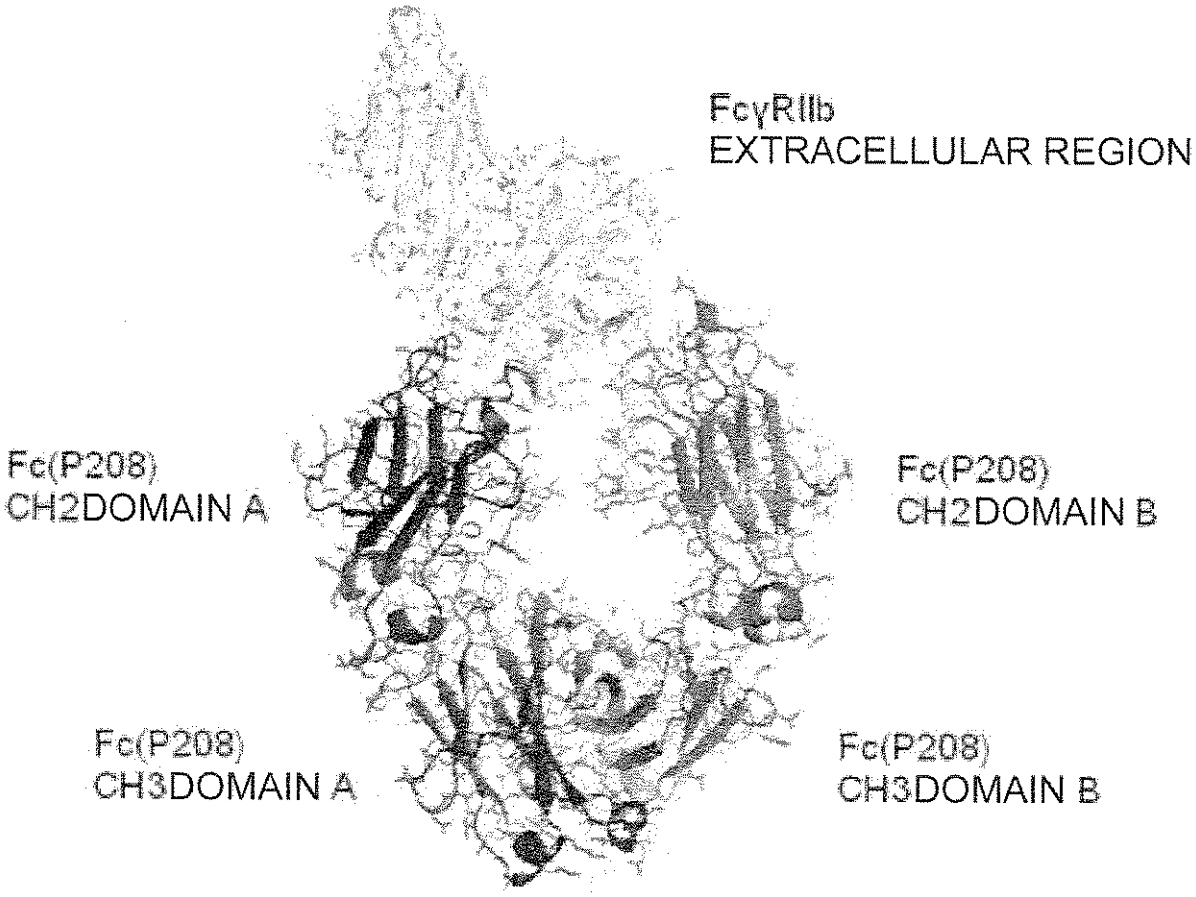
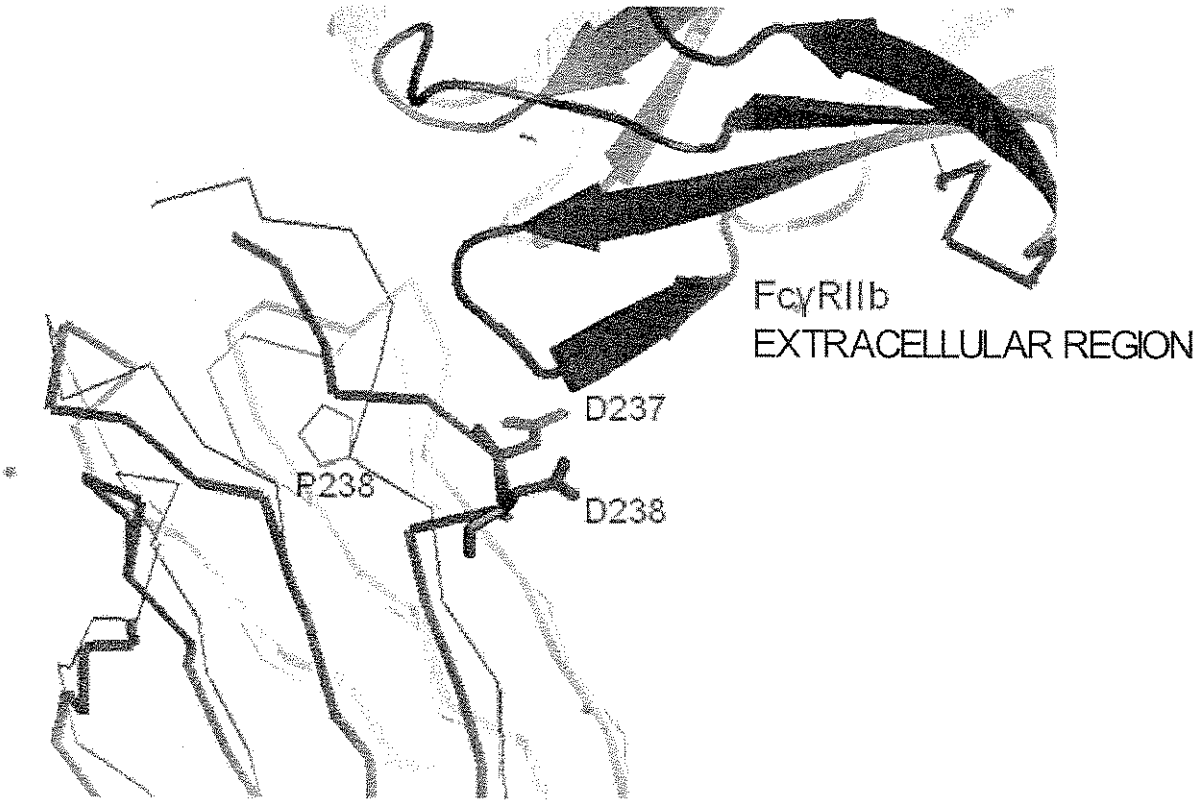

FcγRIIb
EXTRACELLULAR REGION
Fc(P208)
CH2DOMAIN A
Fc(P208)
CH2DOMAIN B
Fc(P208)
CH3DOMAIN A
Fc(P208)
CH3DOMAIN B

FIG. 35



THIN LINE: Fc(Wt) CH2DOMAIN A
HEAVY LINE: Fc(P208) CH2DOMAIN A

FIG. 36

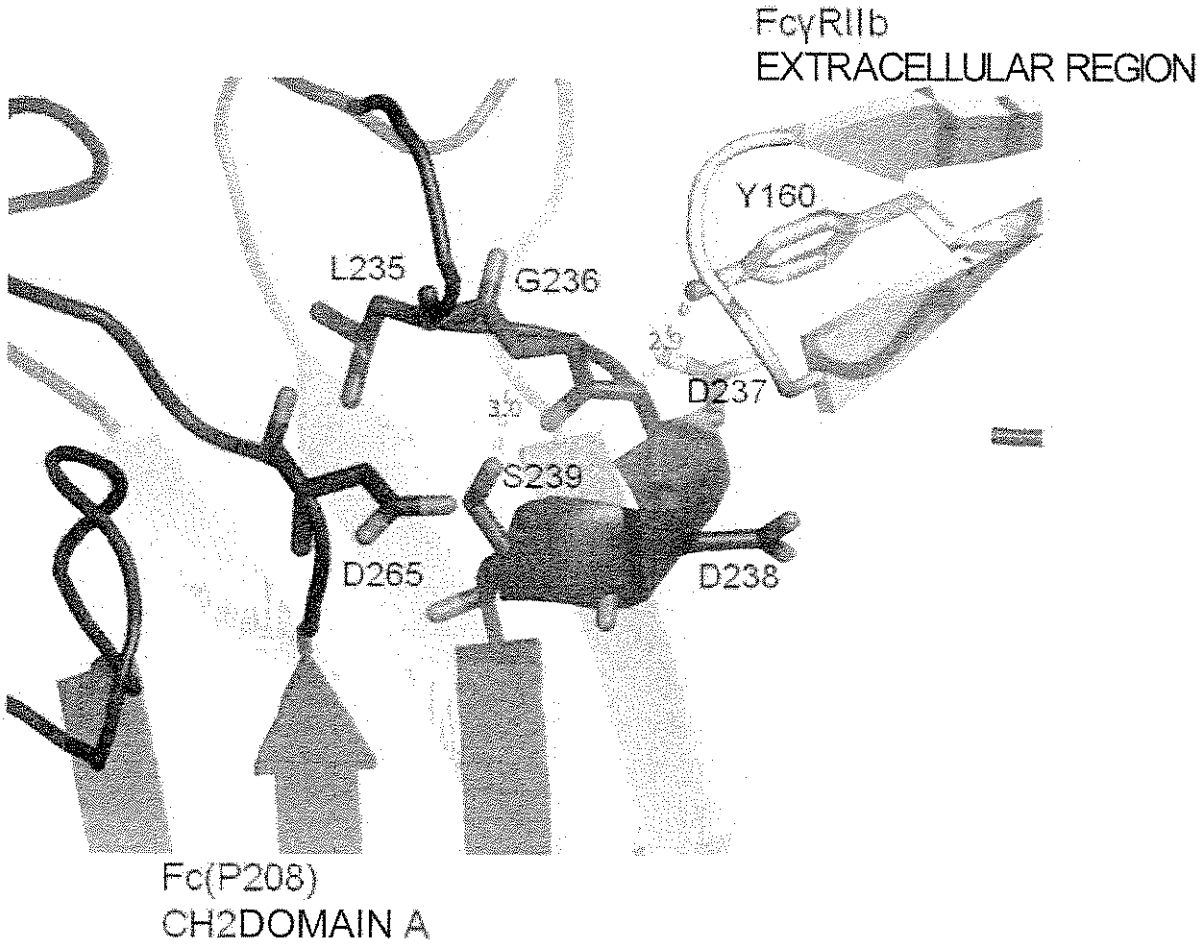

FcγRIIb
EXTRACELLULAR REGION
Y160
L235
G236
D237
S239
D265
D238
Fc(P208)
CH2DOMAIN A

FIG. 37

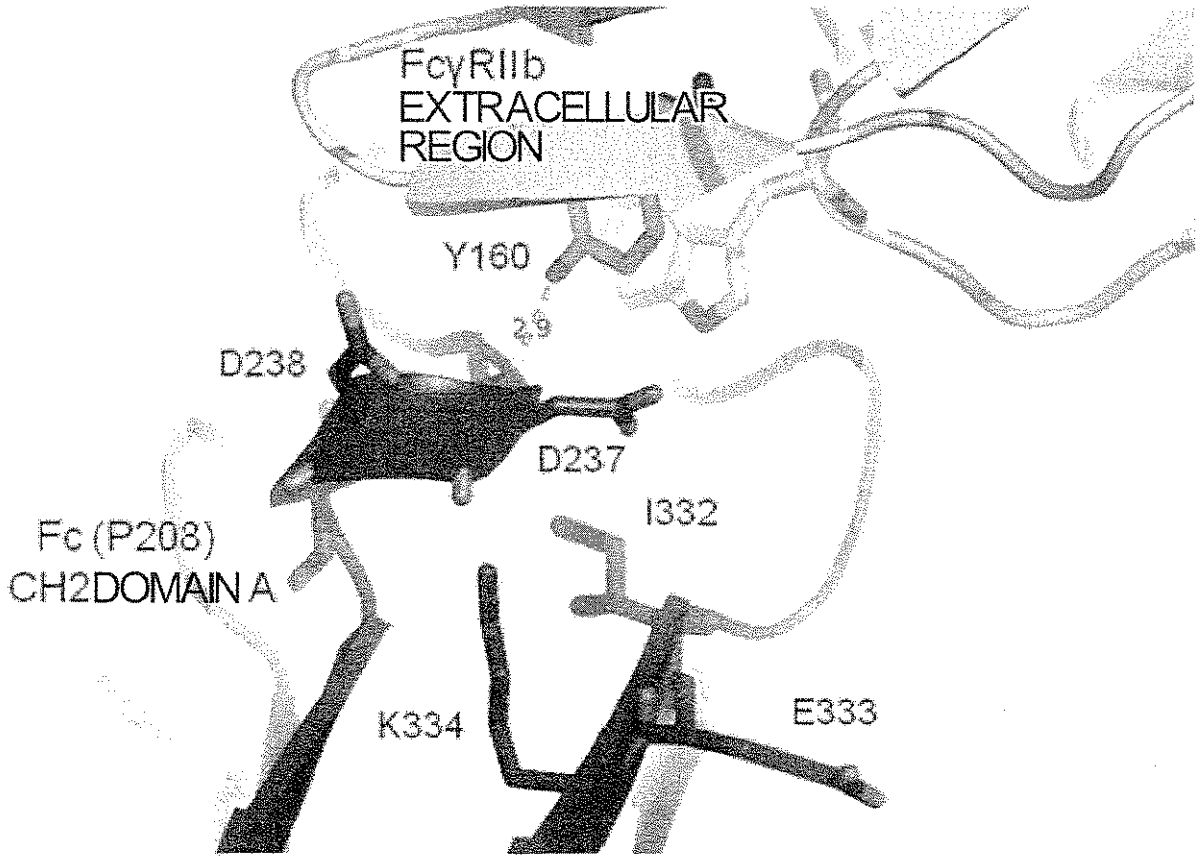
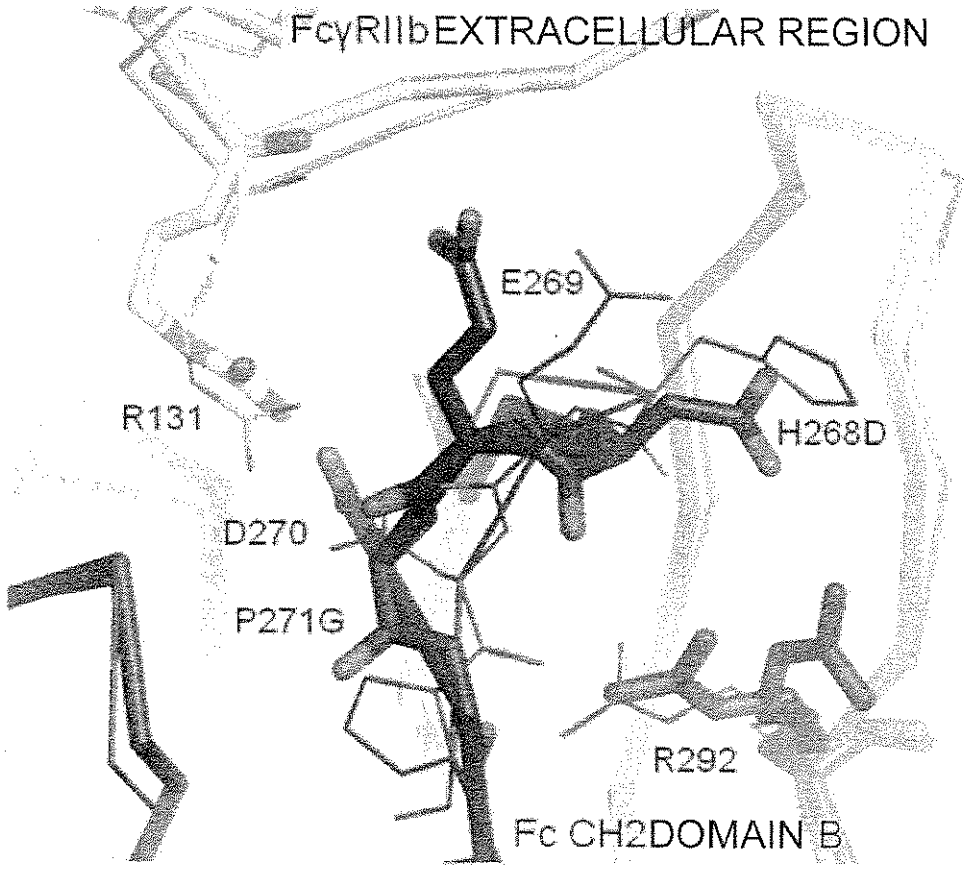
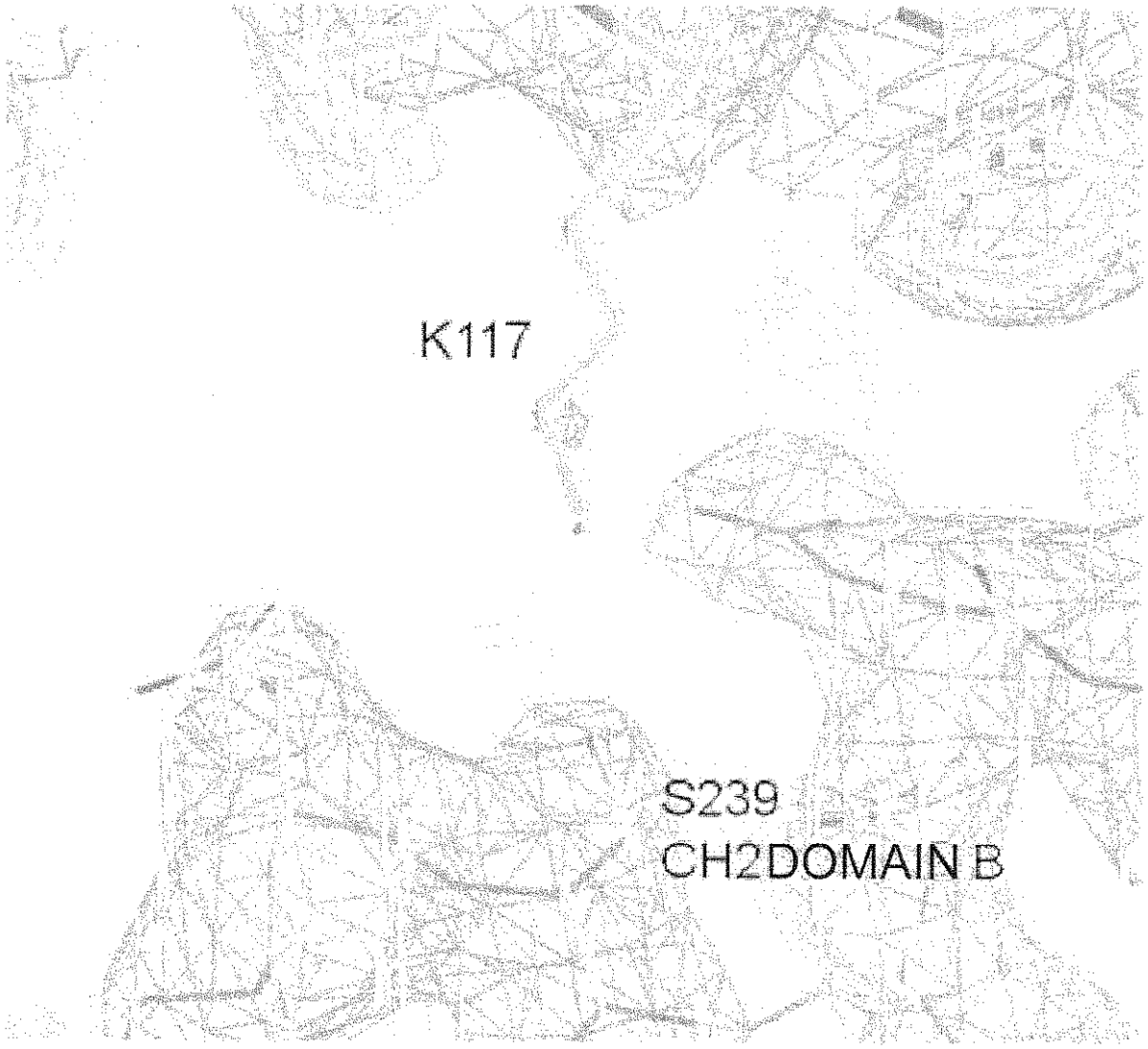

FcγRIIb
EXTRACELLULAR
REGION
Y160
D238
D237
I332
Fc (P208)
CH2DOMAIN A
K334
E333

FIG. 38



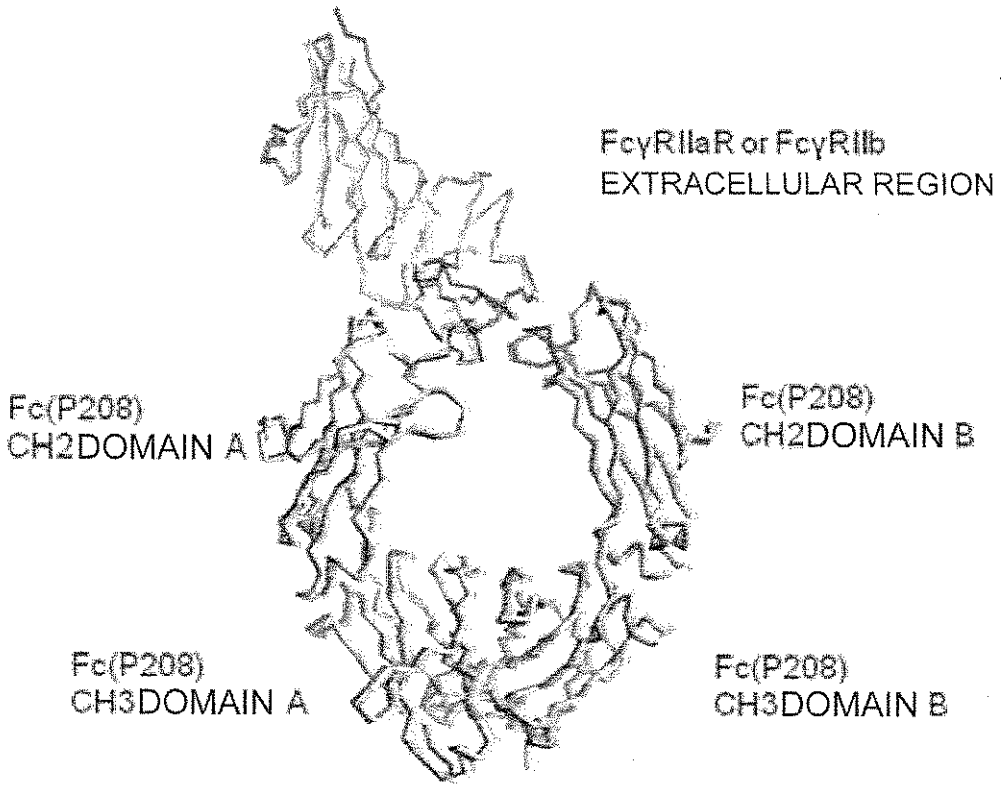
THIN LINE: X-RAY CRYSTAL STRUCTURE OF Fc(P238D)/FcγRIIb EXTRACELLULAR REGION COMPLEX
HEAVY LINE: X-RAY CRYSTAL STRUCTURE OF Fc(P208)/FcγRIIb EXTRACELLULAR REGION COMPLEX

FIG. 39



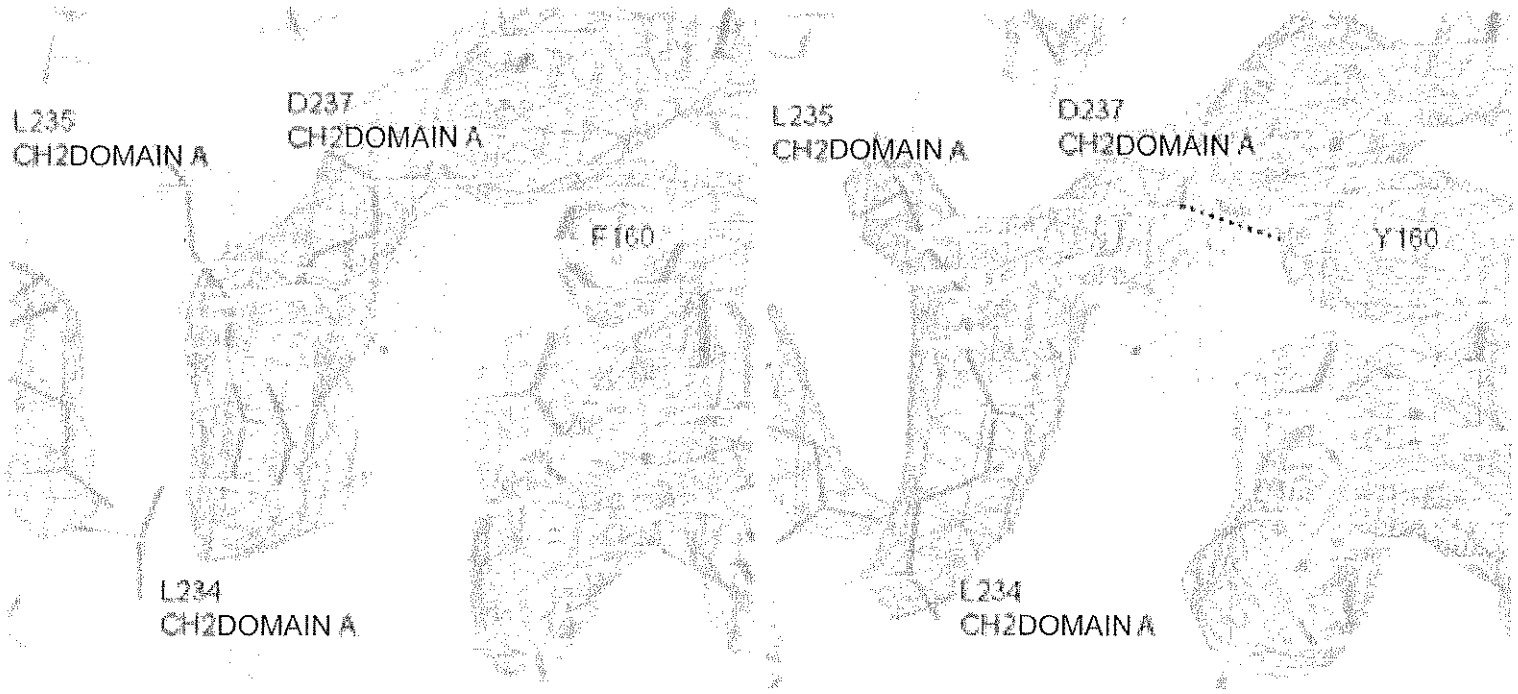
THIN LINE : Fc(P208)
HEAVY LINE : FcγRIIb EXTRACELLULAR REGION

FIG. 40



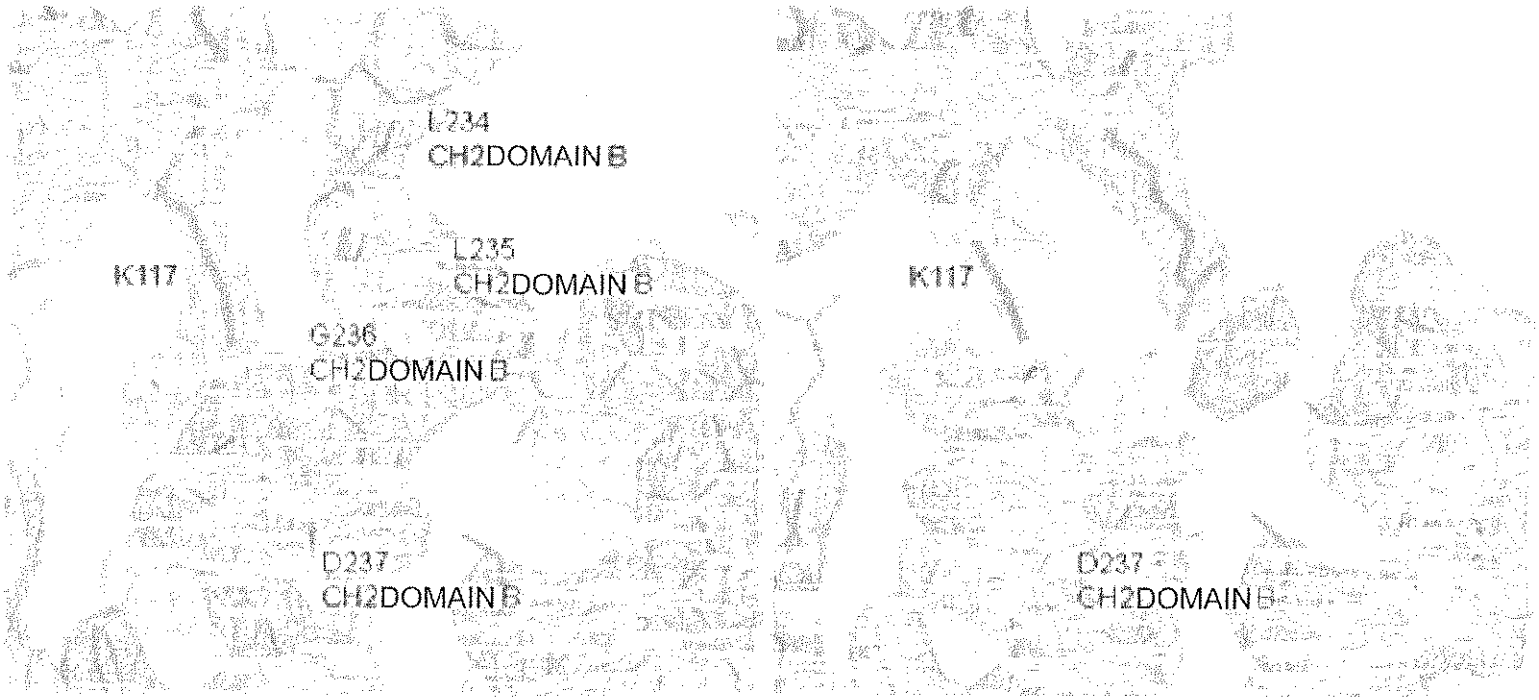
BLACK: X-RAY CRYSTAL STRUCTURE OF Fc(P208)/FcγRIIaR EXTRACELLULAR REGION COMPLEX
GRAY: X-RAY CRYSTAL STRUCTURE OF Fc(P208)/FcγRIIb EXTRACELLULAR REGION COMPLEX

FIG. 41



THIN LINE: Fc(P208)
HEAVY LINE: FcγRIIaR or FcγRIIb EXTRACELLULAR REGION
LEFT: X-RAY CRYSTAL STRUCTURE OF Fc(P208)/FcγRIIaR EXTRACELLULAR REGION COMPLEX
RIGHT: X-RAY CRYSTAL STRUCTURE OF Fc(P208)/FcγRIIb EXTRACELLULAR REGION COMPLEX

FIG. 42



THIN LINE: Fc(P208)

HEAVY LINE: FcγRIIaR or FcγRIIb EXTRACELLULAR REGION

LEFT: X-RAY CRYSTAL STRUCTURE OF Fc(P208)/FcγRIIaR EXTRACELLULAR REGION COMPLEX

RIGHT: X-RAY CRYSTAL STRUCTURE OF Fc(P208)/FcγRIIb EXTRACELLULAR REGION COMPLEX

FIG. 43


G1d 118 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
G4d 118 ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPS
G1d 208 NTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
G4d 208 NTKVDKRVESK---YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN
G1d 298 STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
G4d 298 STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
G1d 388 ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP (SEQ ID NO : 1 6 1)
G4d 388 ENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSL (SEQ ID NO : 1 6 2)

FIG. 44

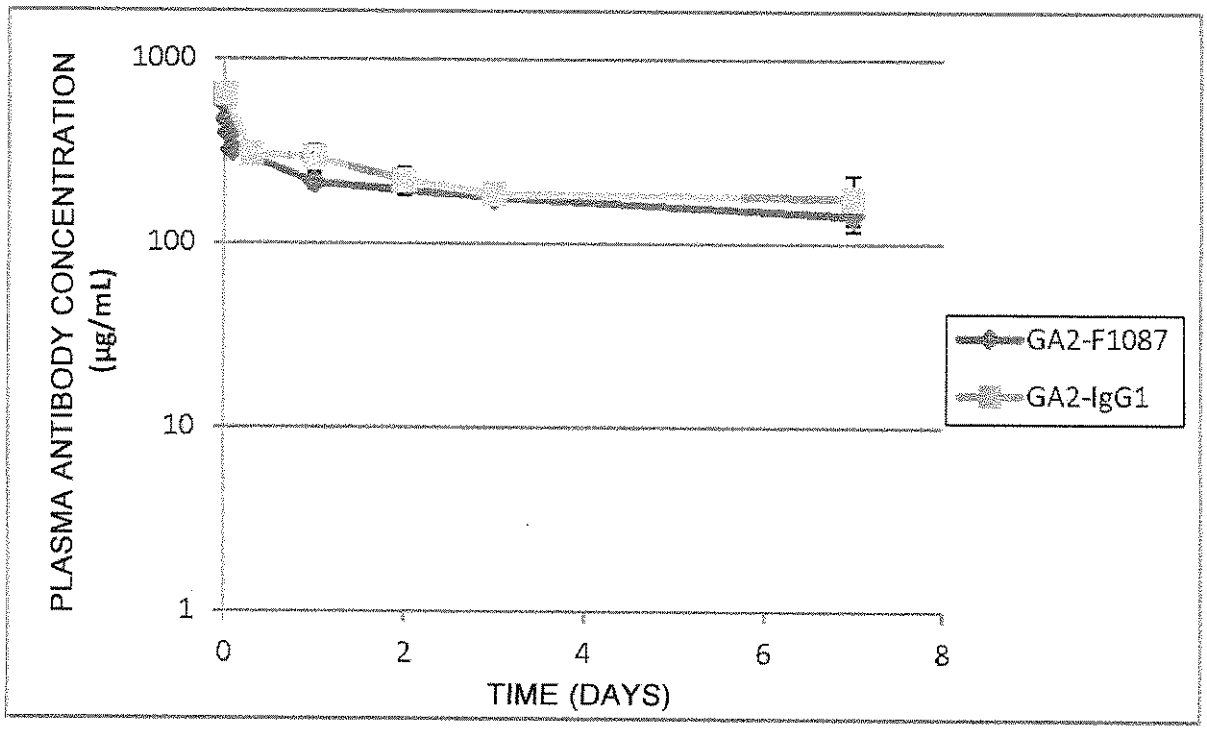

PLASMA ANTIBODY CONCENTRATION (µg/mL)
1000
100
10
1
0
2
4
6
8
TIME (DAYS)
GA2-F1087
GA2-IgG1

FIG. 45

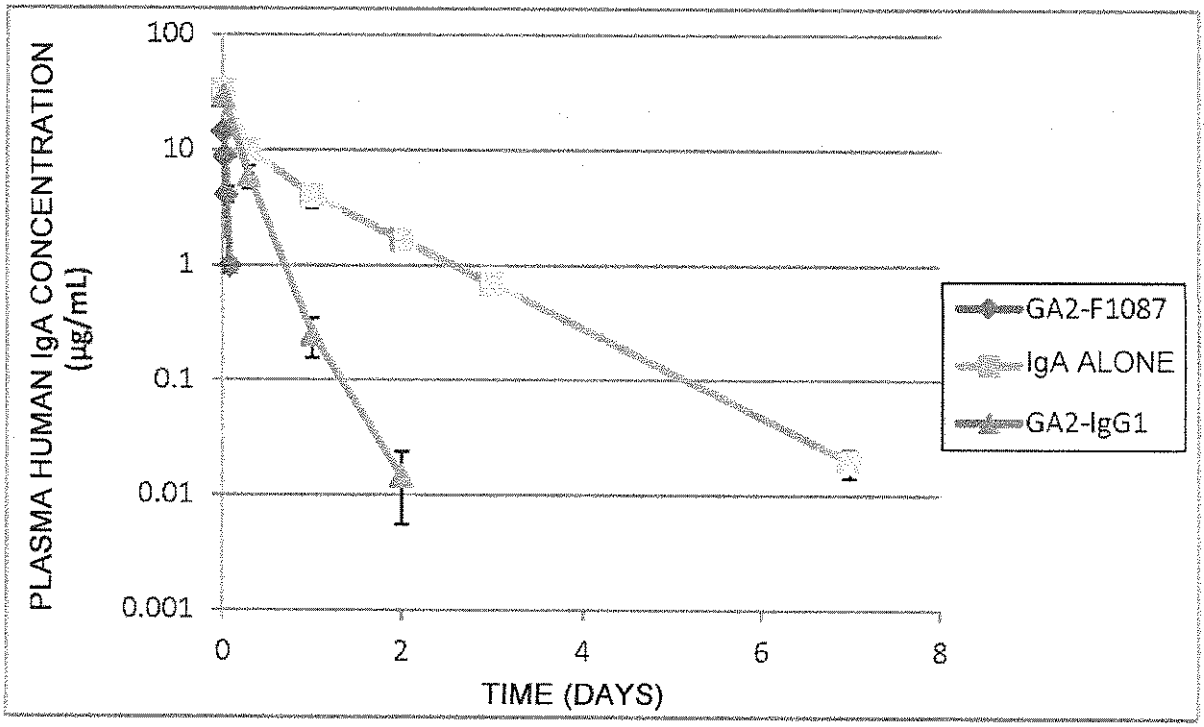

PLASMA HUMAN IgA CONCENTRATION (µg/mL)
100
10
1
0.1
0.01
0.001
0
2
4
6
8
TIME (DAYS)
GA2-F1087
IgA ALONE
GA2-IgG1

FIG. 46

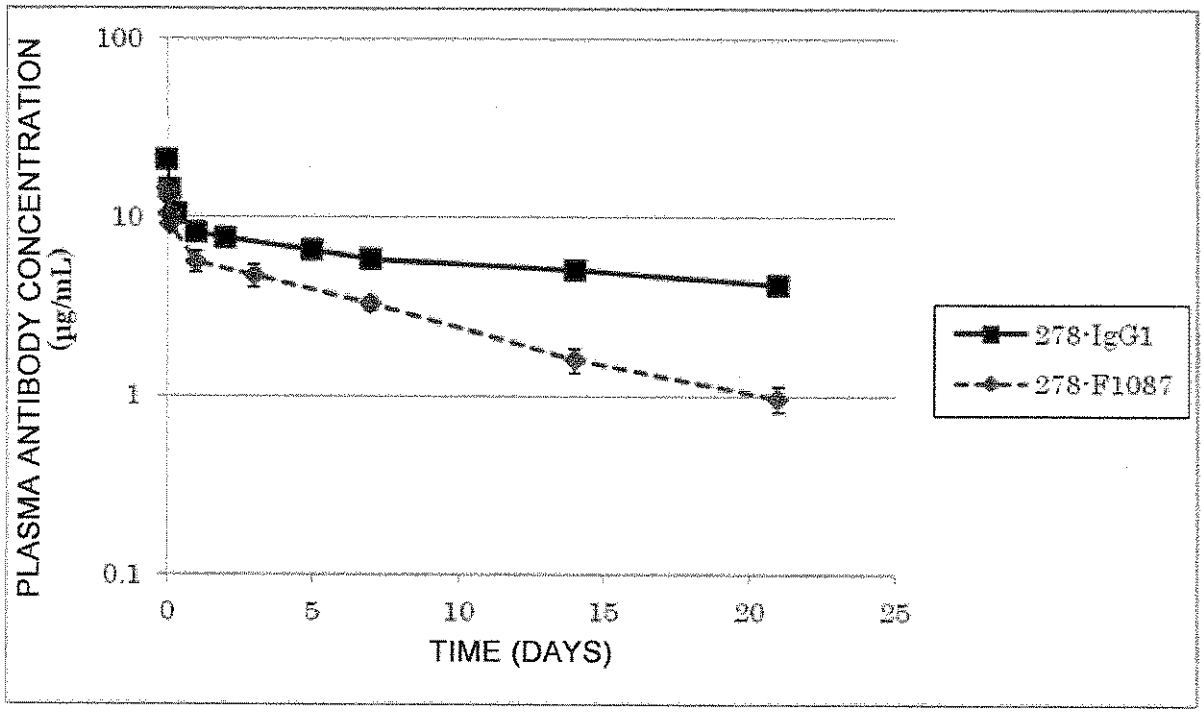

PLASMA ANTIBODY CONCENTRATION (μg/mL)
100
10
1
0.1
0
5
10
15
20
25
TIME (DAYS)
278-IgG1
278-F1087

FIG. 47

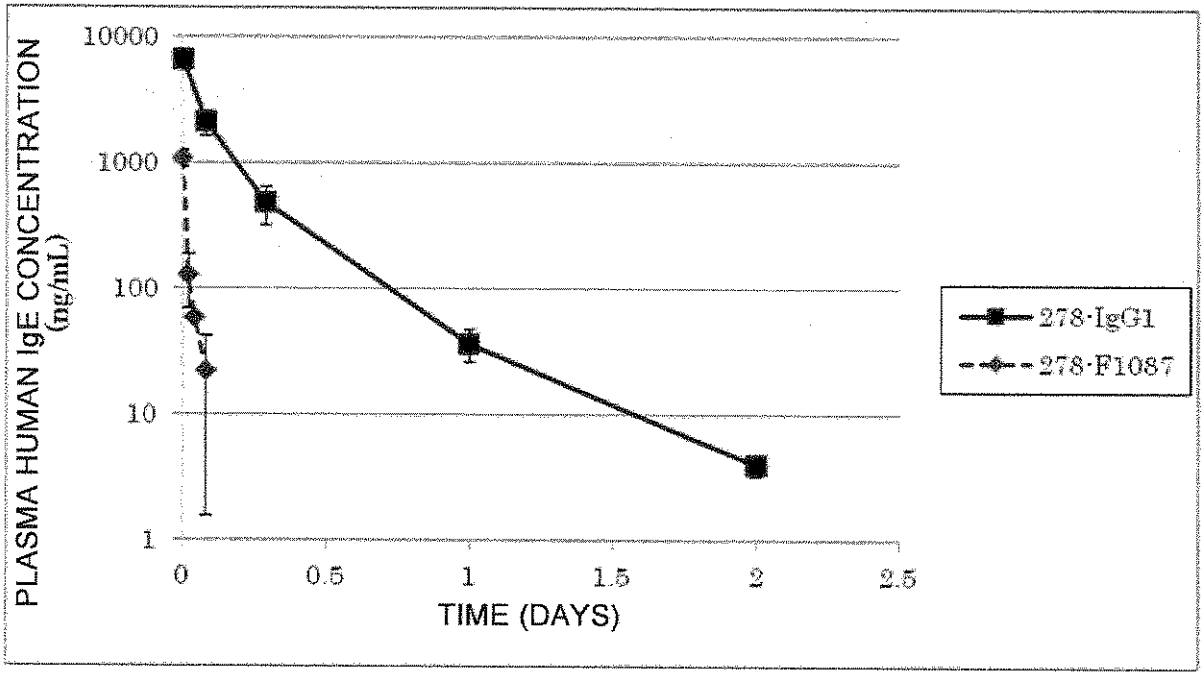
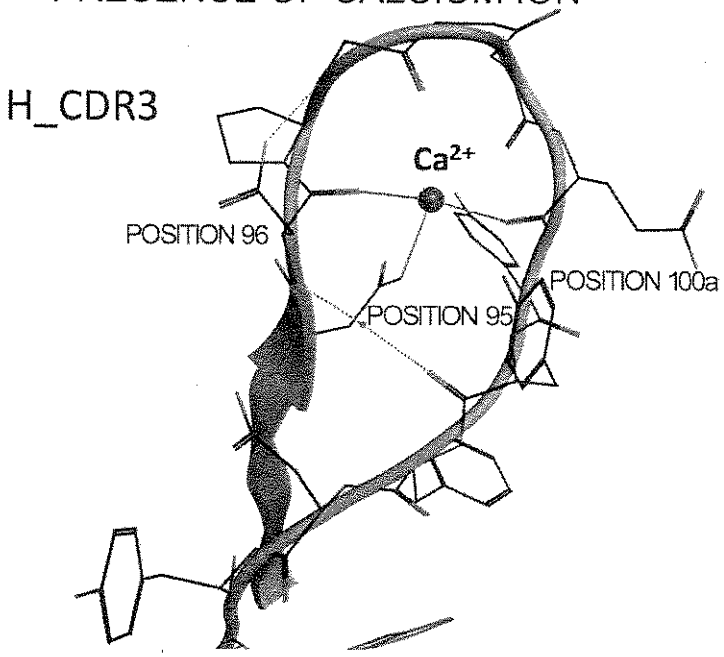

PLASMA HUMAN IgE CONCENTRATION (ng/mL)
10000
1000
100
10
1
0
0.5
1
1.5
2
2.5
TIME (DAYS)
278-IgG1
278-F1087

FIG. 48

(i) CRYSTAL STRUCTURE IN THE PRESENCE OF CALCIUM ION



(ii) CRYSTAL STRUCTURE IN THE ABSENCE OF CALCIUM ION

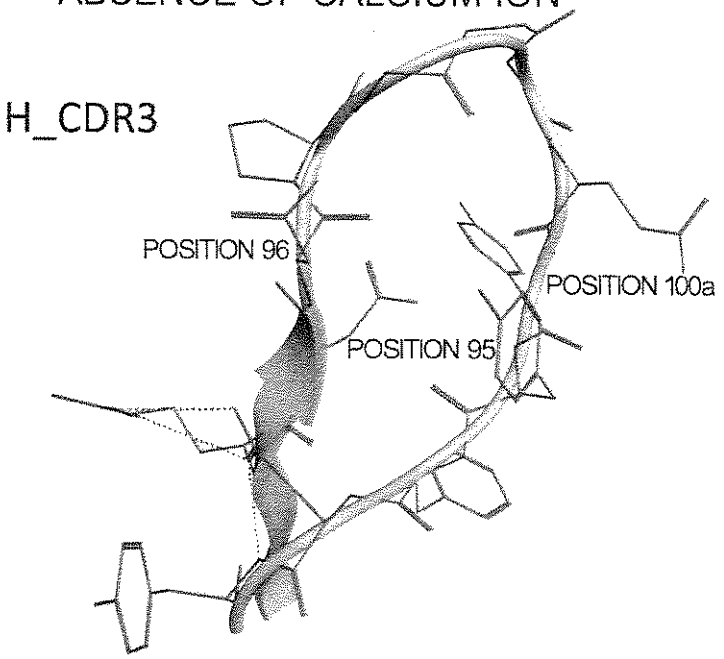


FIG. 49

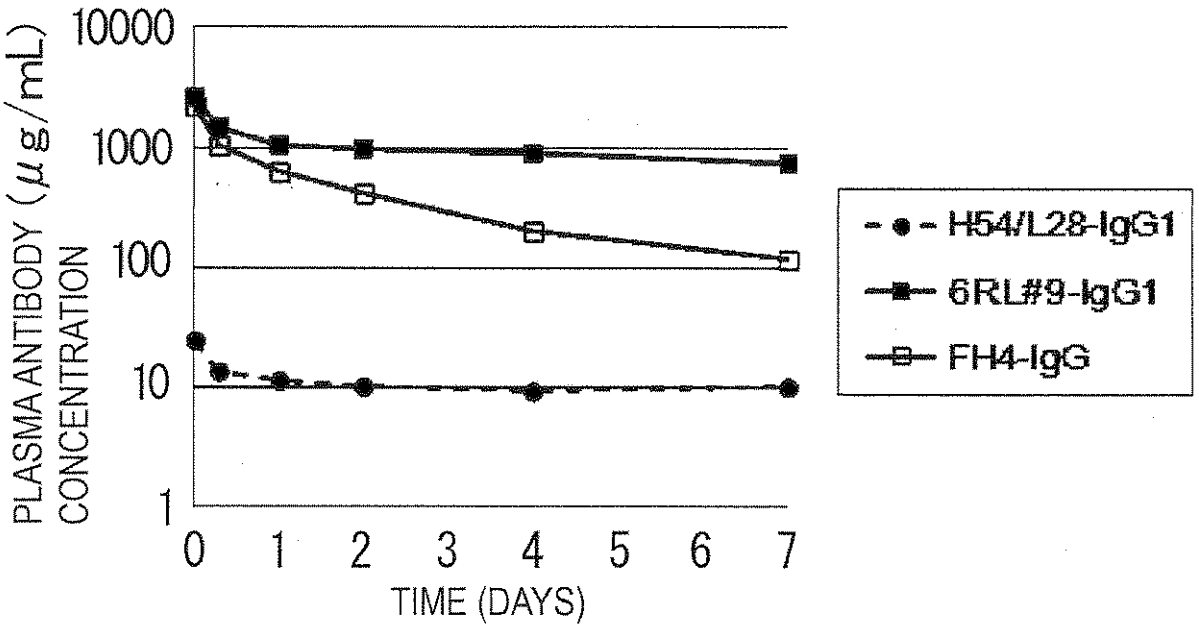

PLASMA ANTIBODY CONCENTRATION (μg/mL)
10000
1000
100
10
1
0
1
2
3
4
5
6
7
TIME (DAYS)
H54/L28-IgG1
6RL#9-IgG1
FH4-IgG

FIG. 50

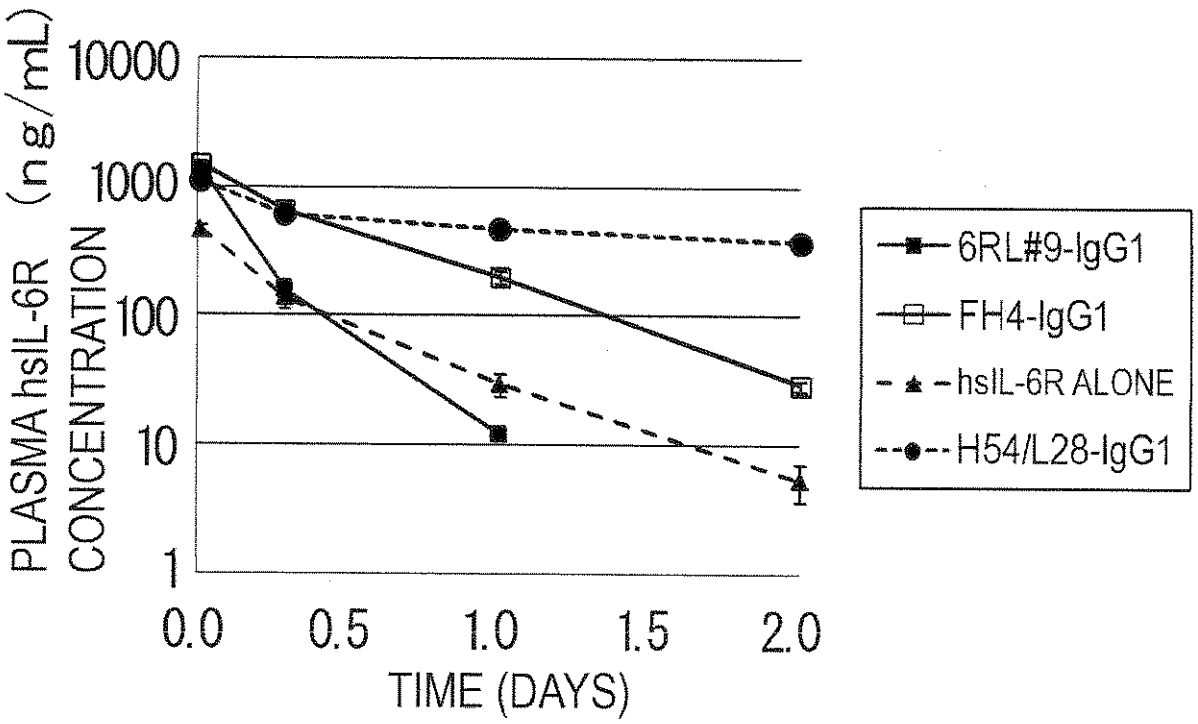

PLASMA hsIL-6R (ng/mL) CONCENTRATION
10000
1000
100
10
1
0.0
0.5
1.0
1.5
2.0
TIME (DAYS)
6RL#9-IgG1
FH4-IgG1
hsIL-6R ALONE
H54/L28-IgG1

FIG. 51

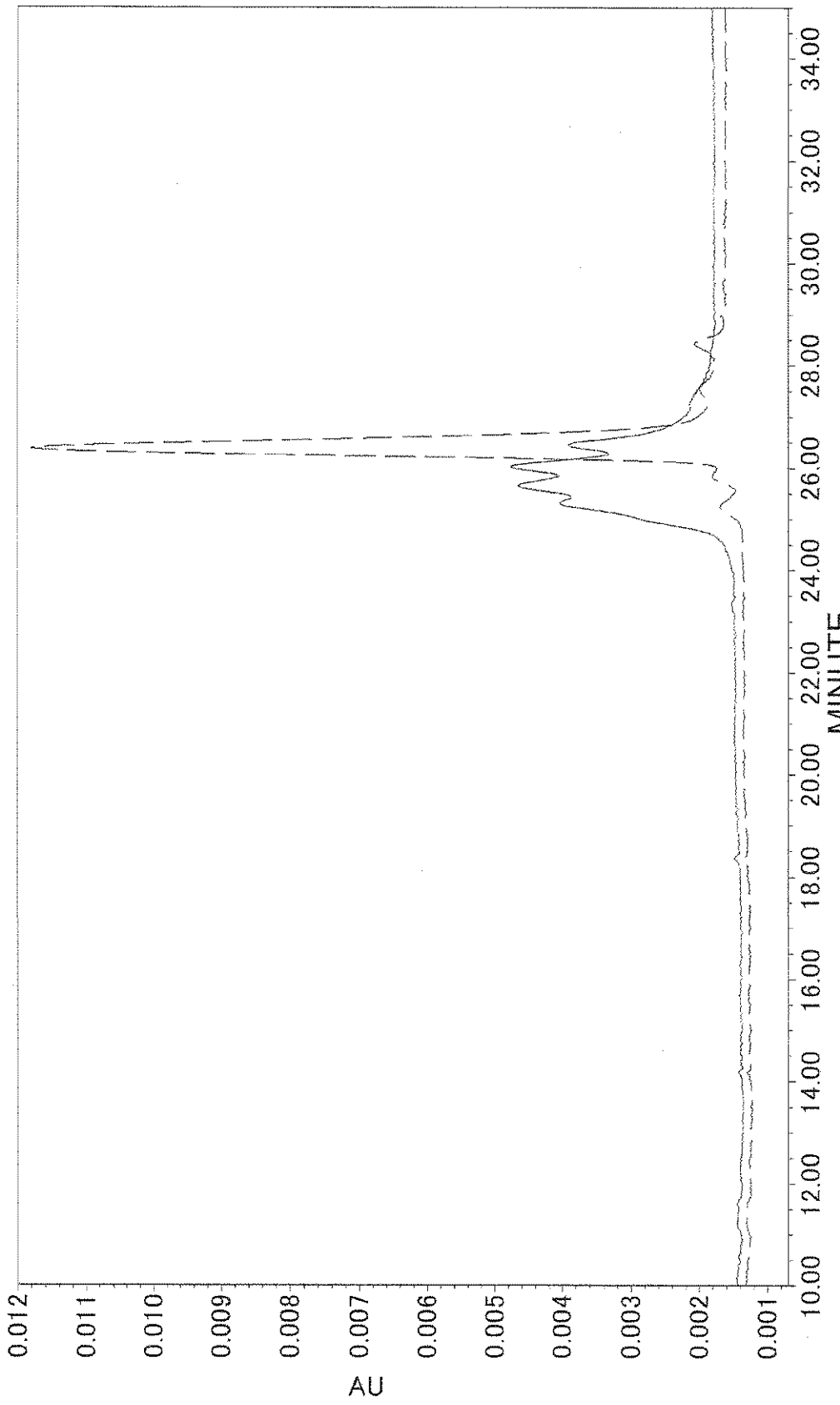

0.012
0.011
0.010
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
AU
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
34.00
MINUTE

FIG. 52

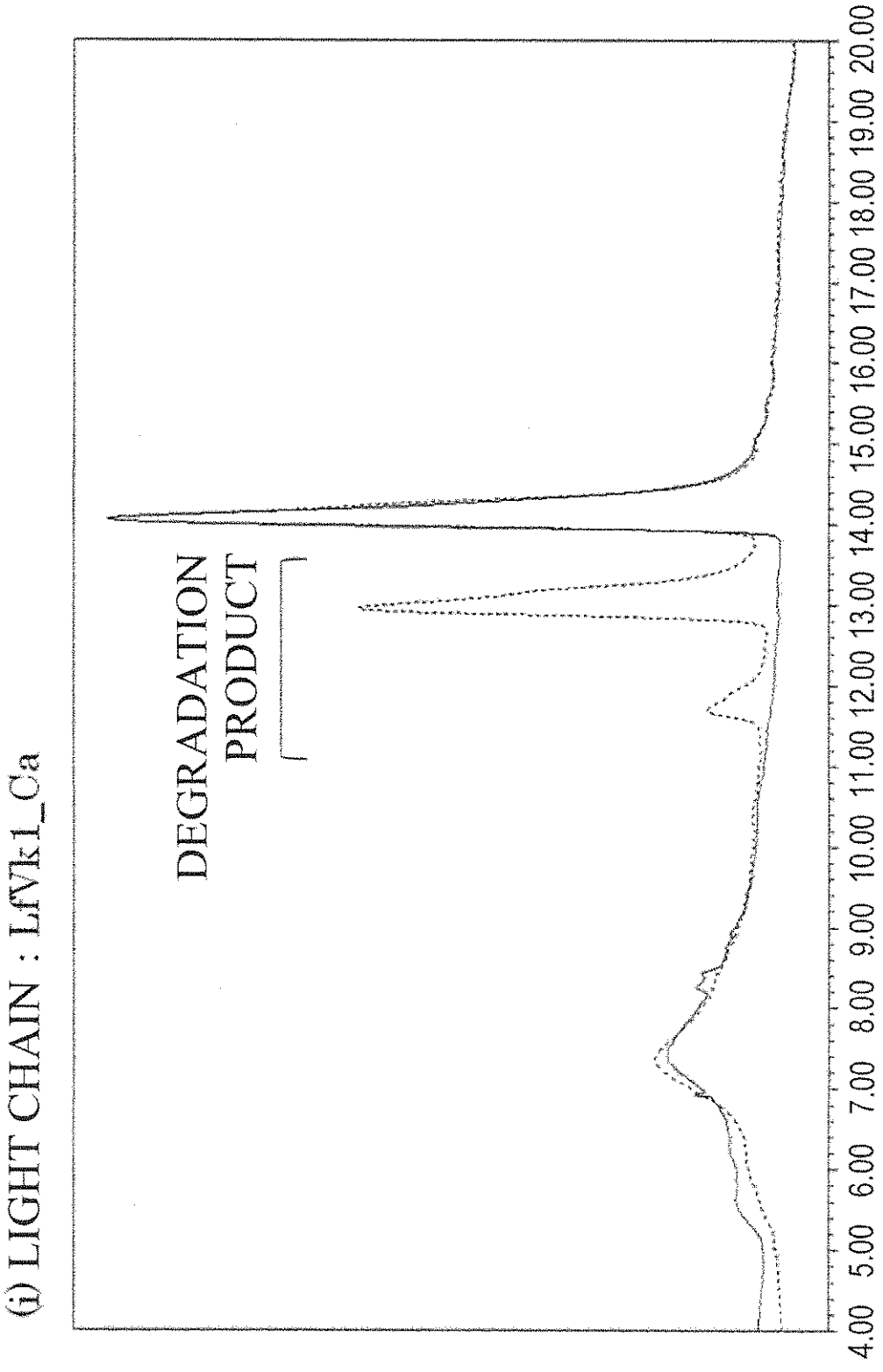

(i) LIGHT CHAIN : LfVk1_Ca
DEGRADATION PRODUCT
4.00 5.00 6.00 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00
MINUTE

FIG. 53A

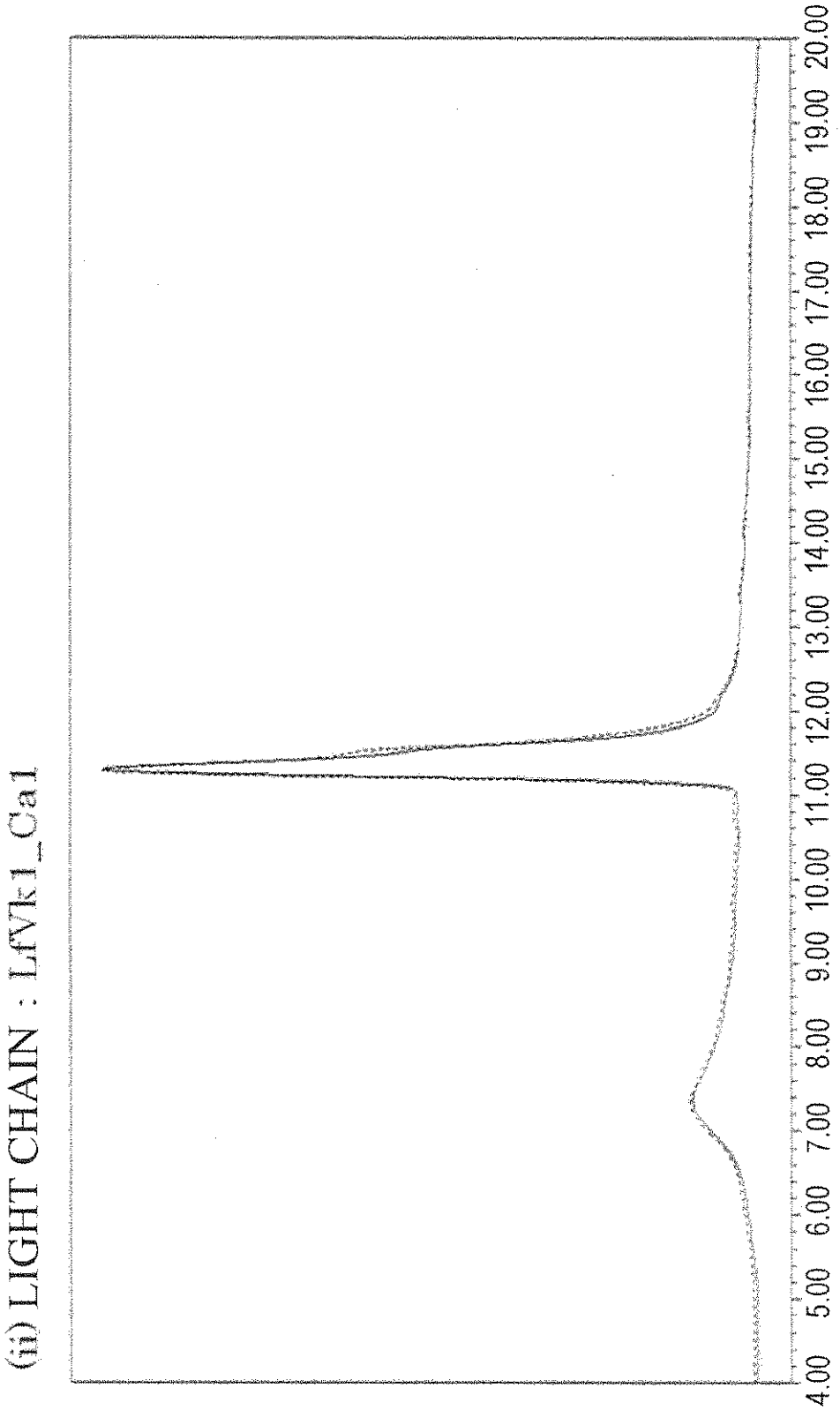

(ii) LIGHT CHAIN : LfVk1_Ca1
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
13.00
14.00
15.00
16.00
17.00
18.00
19.00
20.00
MINUTE

FIG. 53B

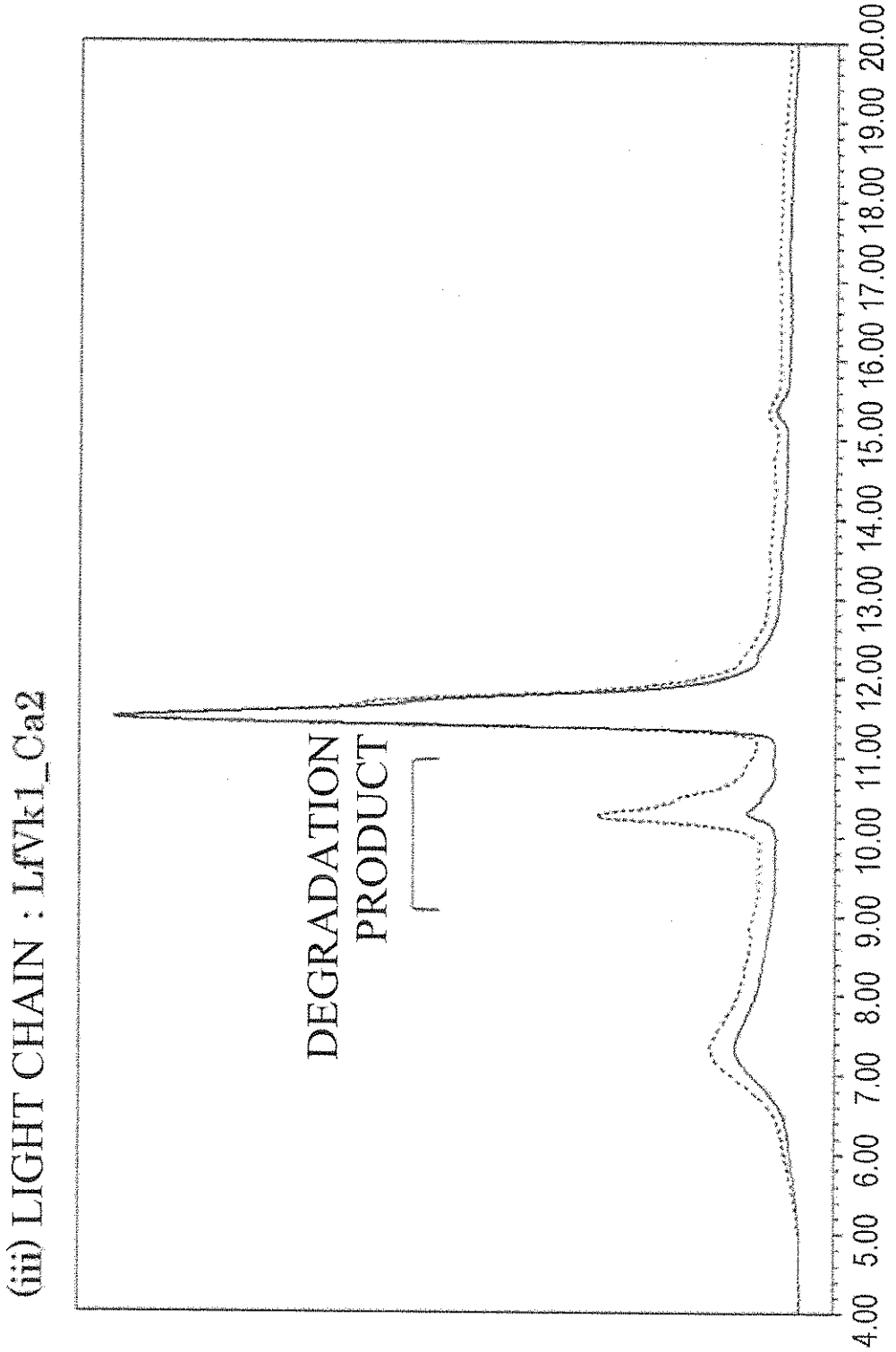

(iii) LIGHT CHAIN : LfVk1_Ca2
DEGRADATION PRODUCT
4.00 5.00 6.00 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00
MINUTE

FIG. 53C

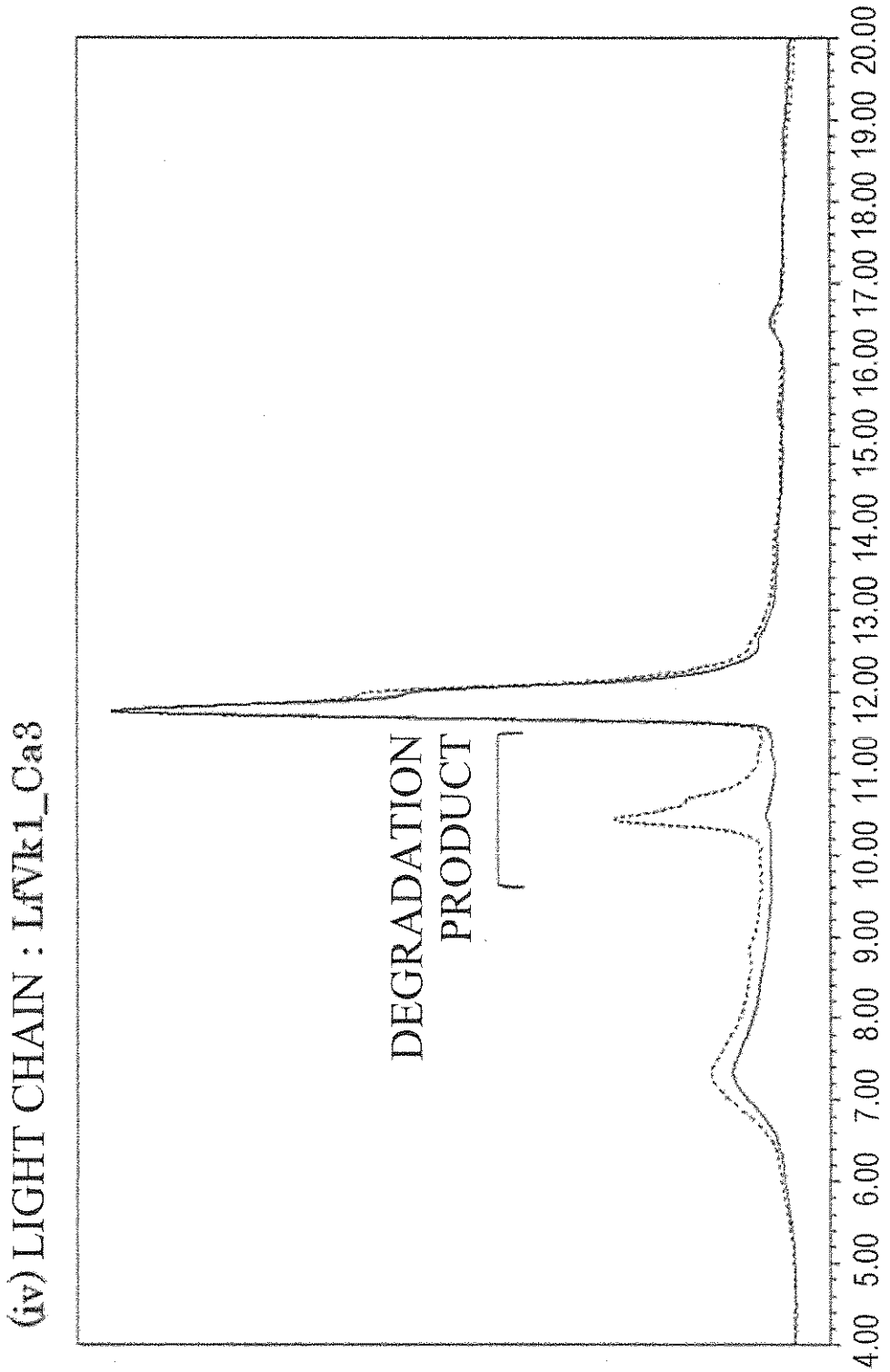

(iv) LIGHT CHAIN : LfVk1_Ca3
DEGRADATION PRODUCT
4.00 5.00 6.00 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00
MINUTE

FIG. 53D

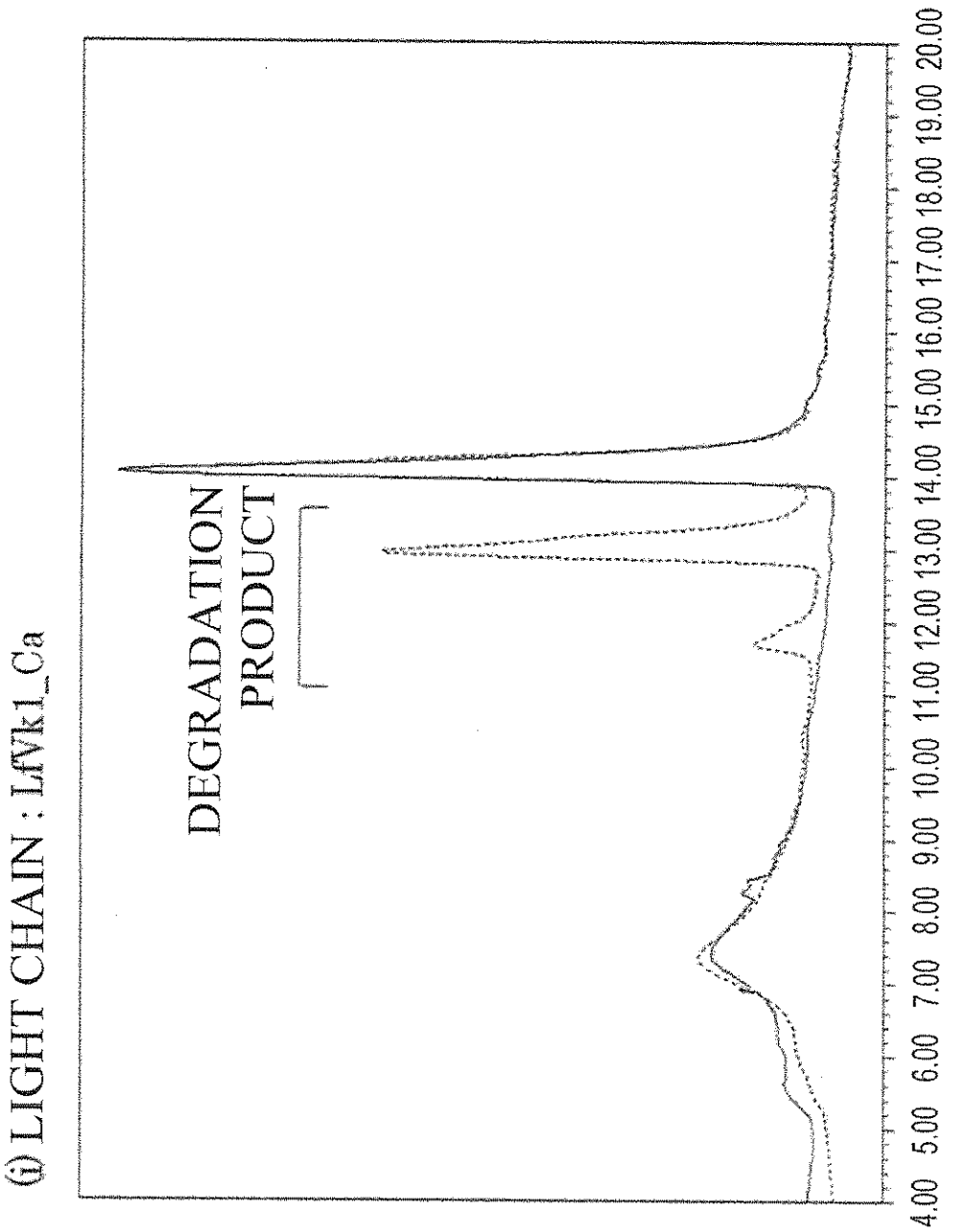

(i) LIGHT CHAIN : LfVk1_Ca
DEGRADATION PRODUCT
4.00 5.00 6.00 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00
MINUTE

FIG. 54A

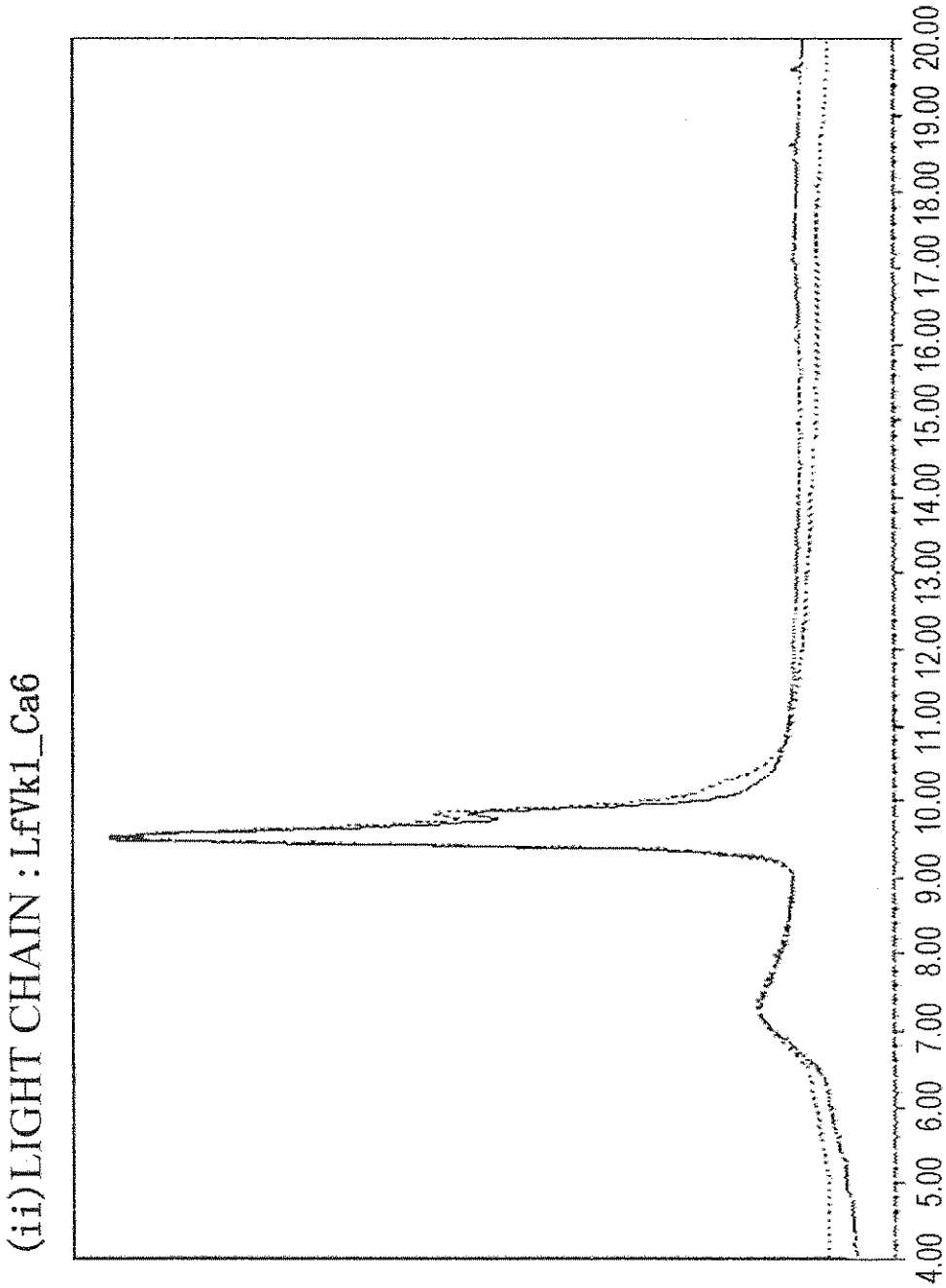

(ii) LIGHT CHAIN : LfVk1_Ca6
4.00 5.00 6.00 7.00 8.00 9.00 10.00 11.00 12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00
MINUTE

FIG. 54B

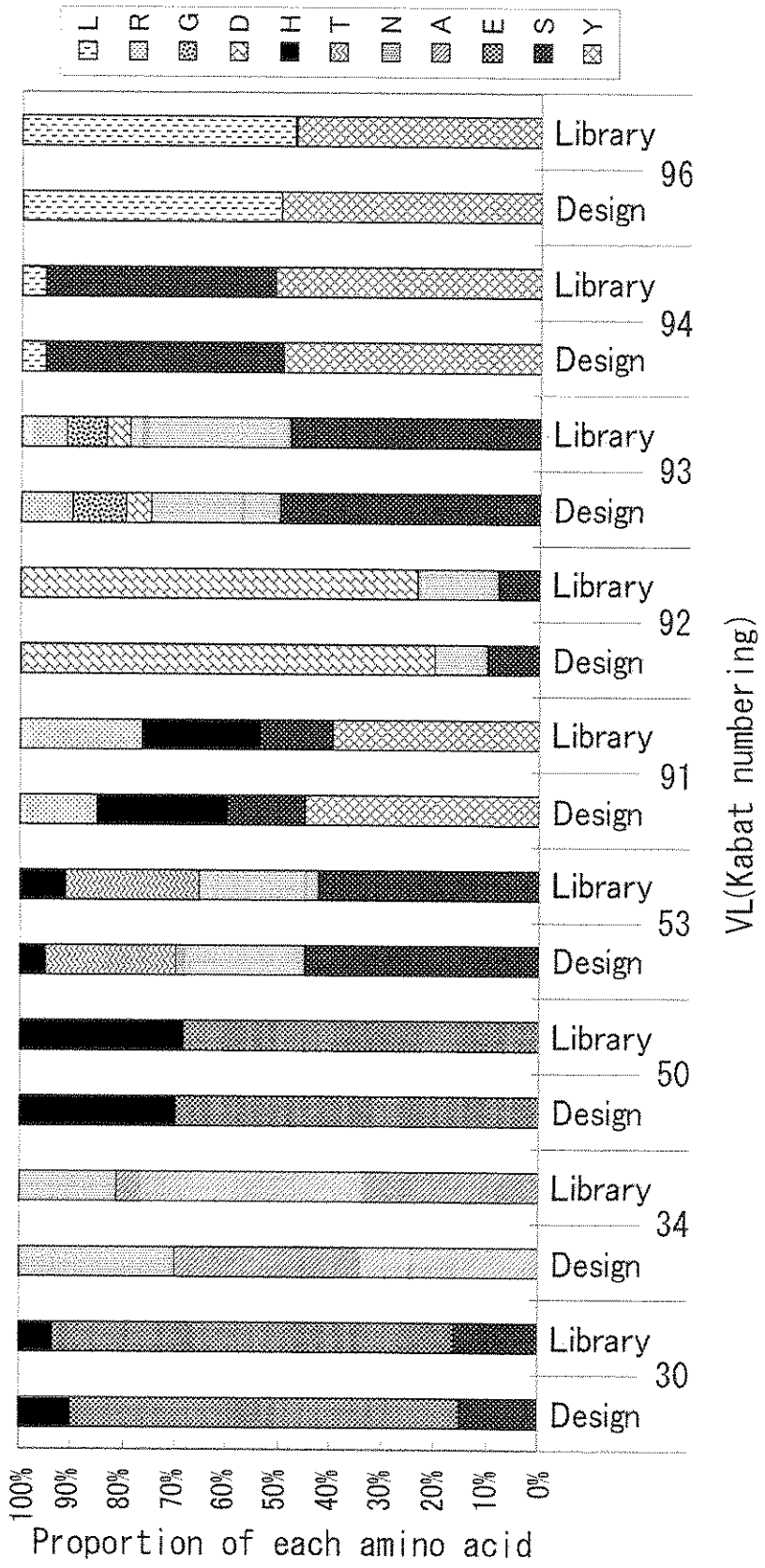

L
R
G
D
H
T
N
A
E
S
Y
Library
96
Design
Library
94
Design
Library
93
Design
Library
92
Design
Library
91
Design
Library
53
Design
Library
50
Design
Library
34
Design
Library
30
Design
VL(Kabat numbering)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Proportion of each amino acid

FIG. 55

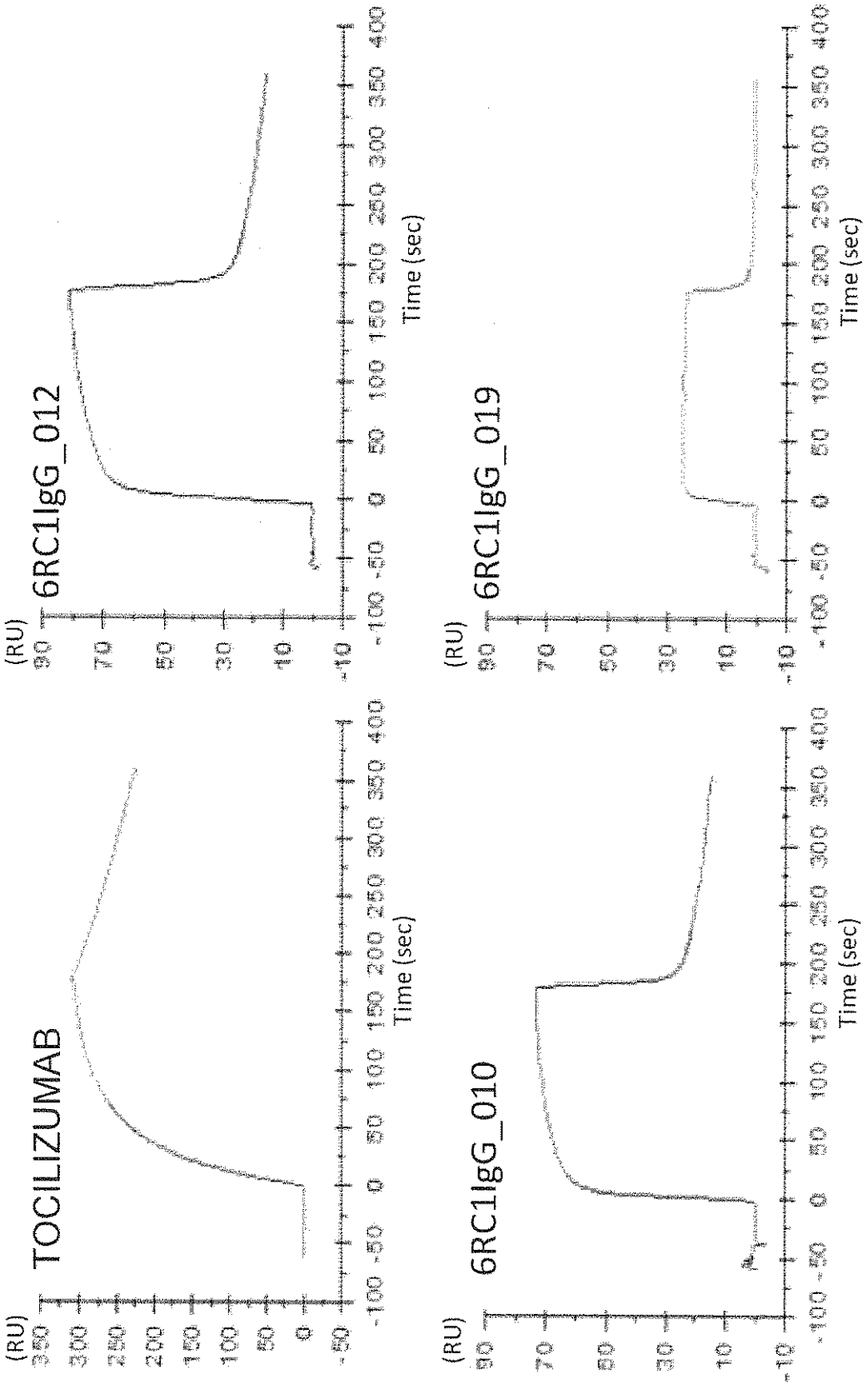

TOCILIZUMAB
6RC1IgG_012
6RC1IgG_010
6RC1IgG_019
(RU)
Time (sec)

FIG. 56

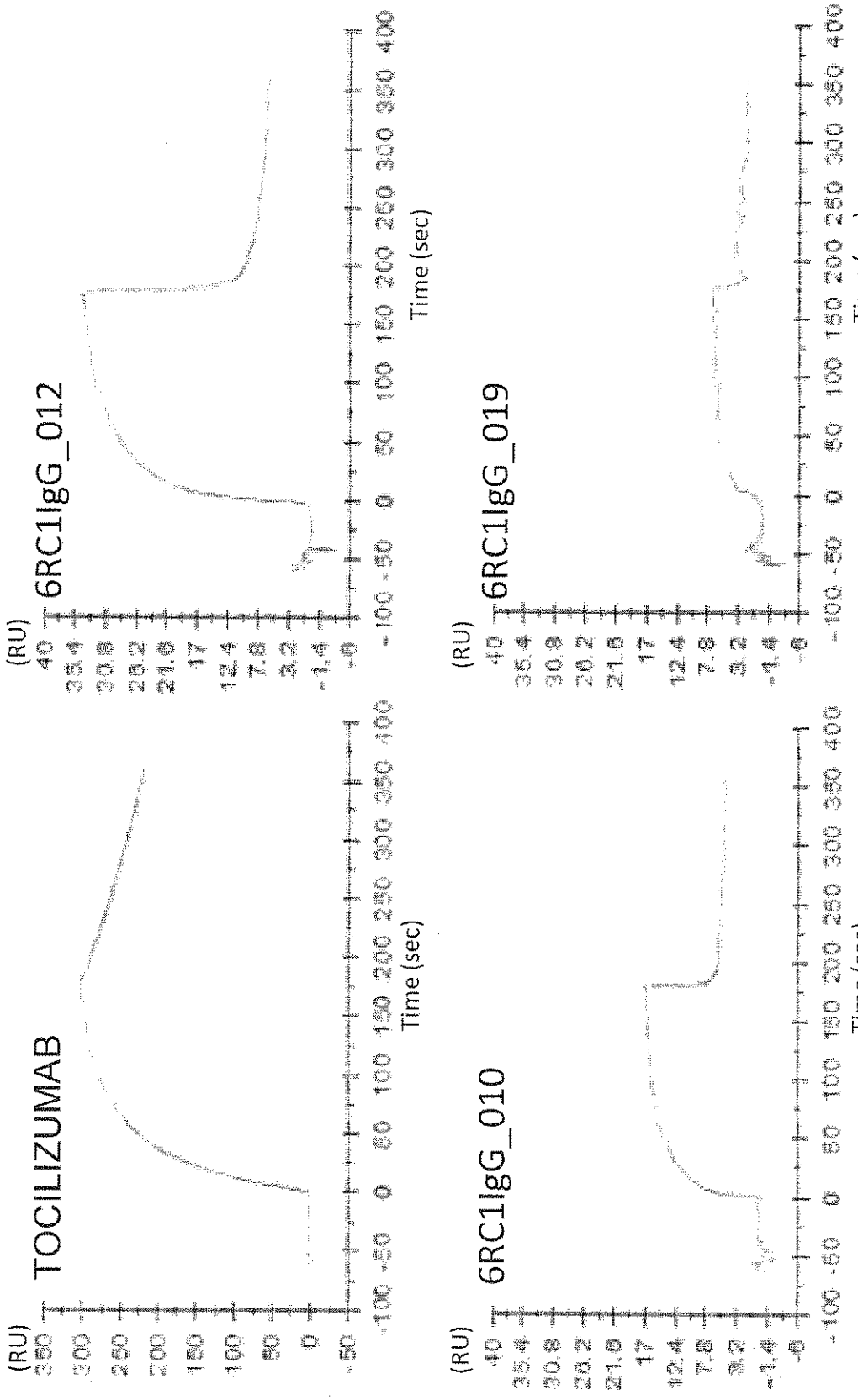

TOCILIZUMAB
6RC1IgG_012
6RC1IgG_010
6RC1IgG_019
(RU)
Time (sec)

FIG. 57

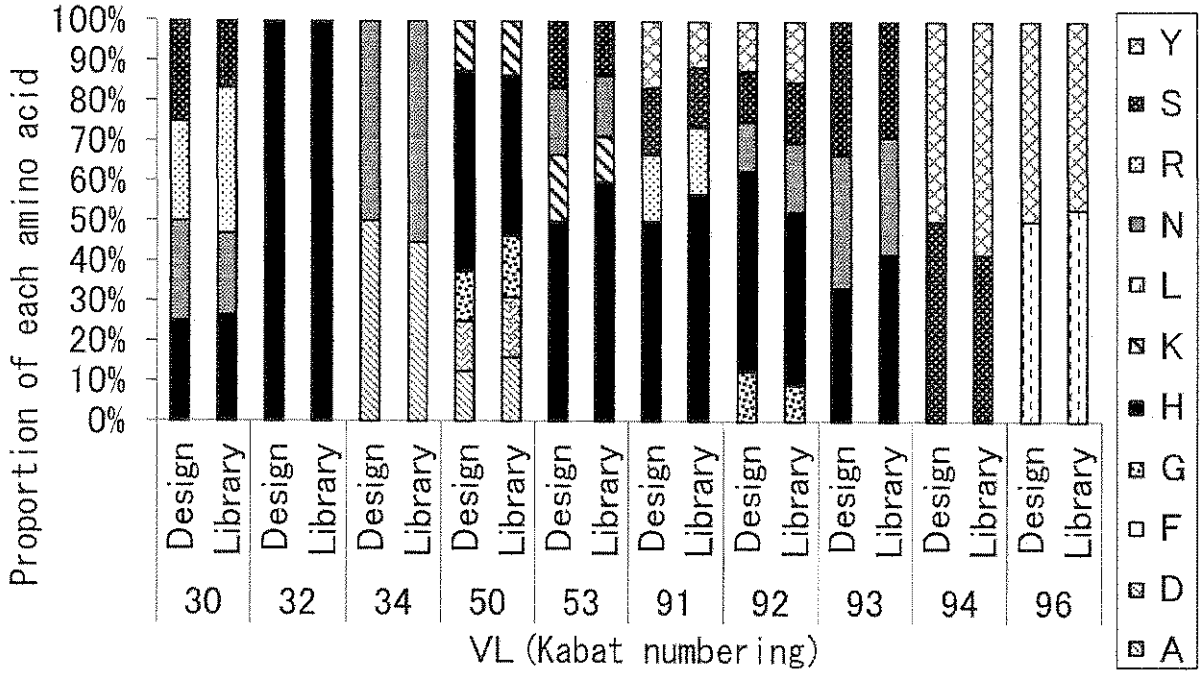

Proportion of each amino acid
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Design
Library
30
32
34
50
53
91
92
93
94
96
VL (Kabat numbering)
Y
S
R
N
L
K
H
G
F
D
A

FIG. 58

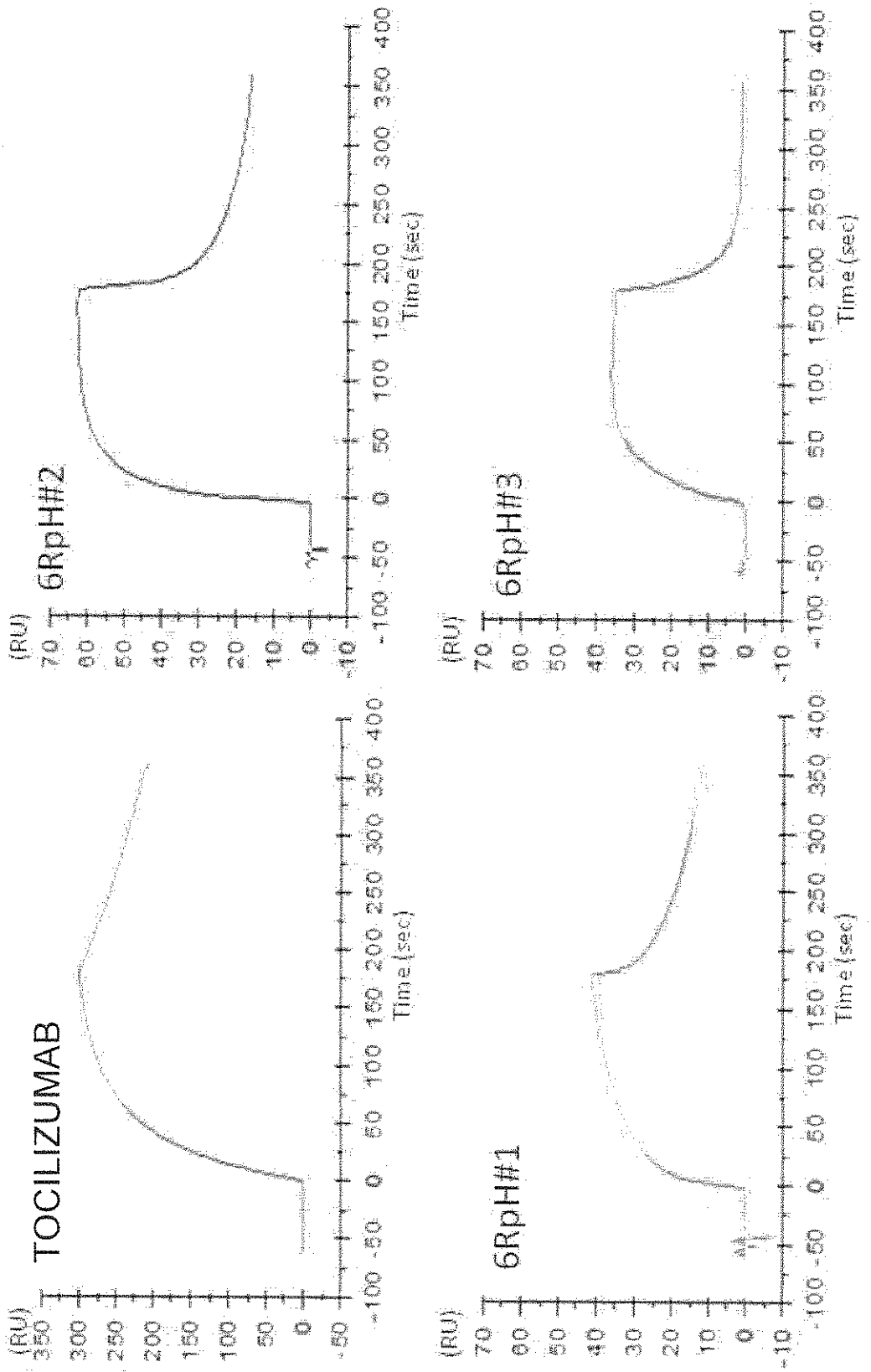


FIG. 59

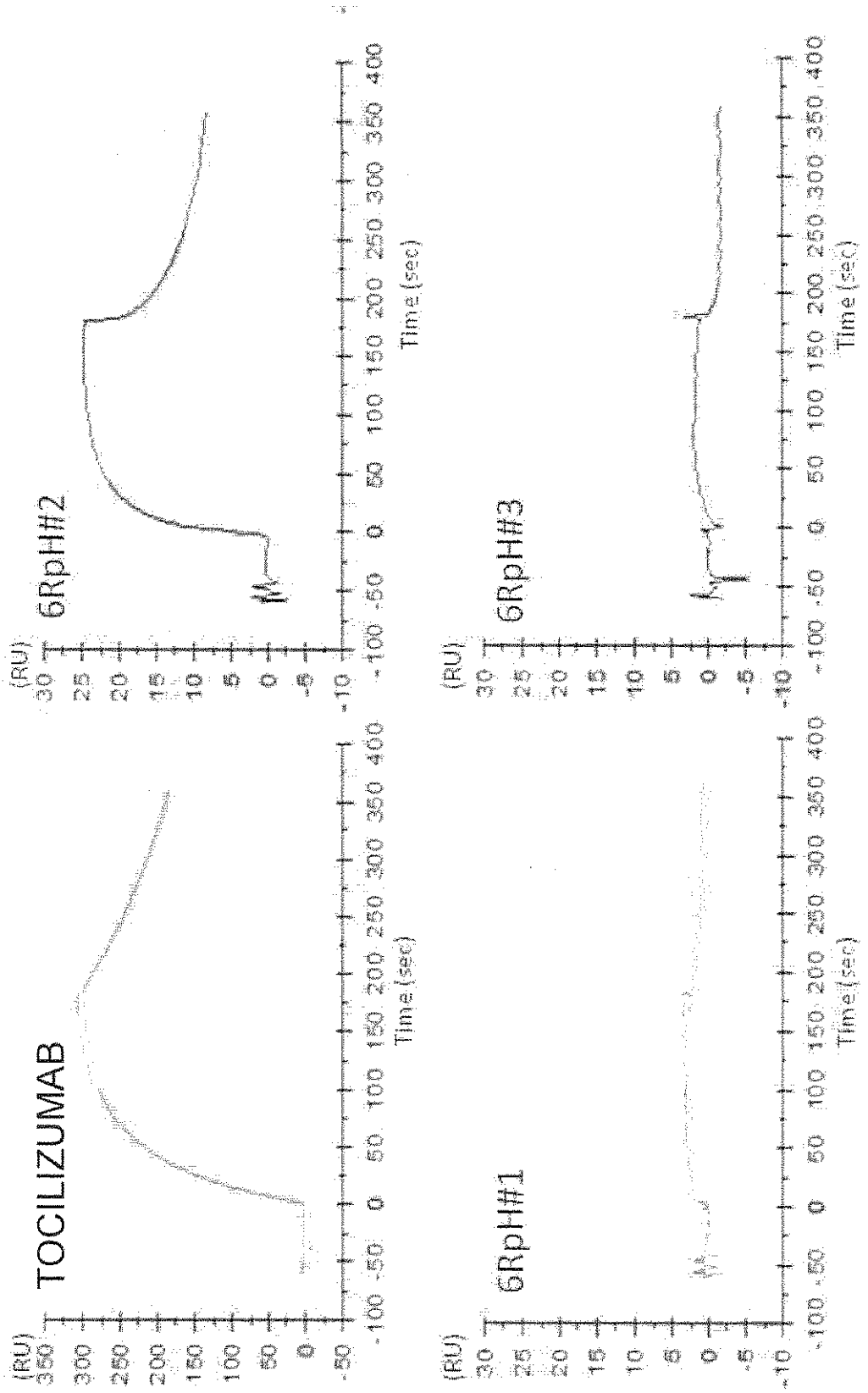


FIG. 60

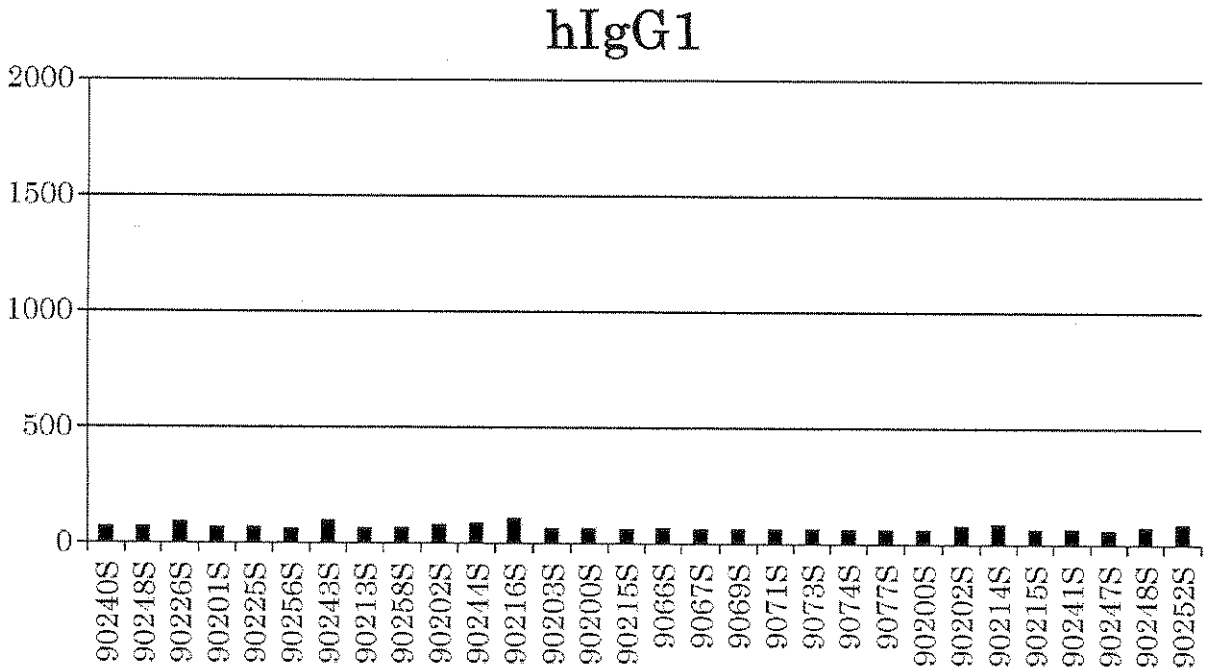

hIgG1
2000
1500
1000
500
0
90240S
90248S
90226S
90201S
90225S
90256S
90243S
90213S
90258S
90202S
90244S
90216S
90203S
90200S
90215S
9066S
9067S
9069S
9071S
9073S
9074S
9077S
90200S
90202S
90214S
90215S
90241S
90247S
90248S
90252S

FIG. 61A

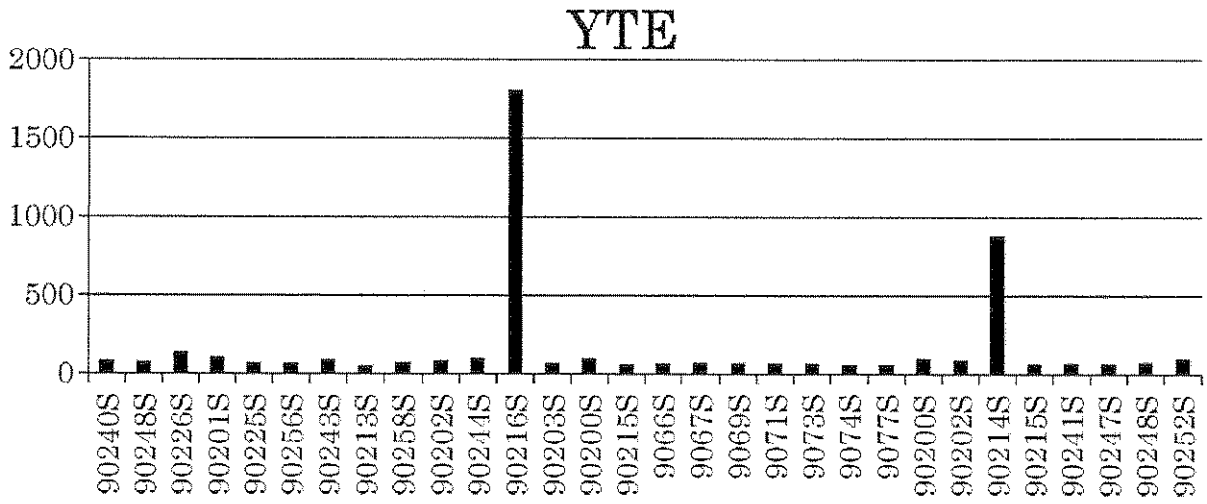

YTE
2000
1500
1000
500
0
90240S
90248S
90226S
90201S
90225S
90256S
90243S
90213S
90258S
90202S
90244S
90216S
90203S
90200S
90215S
9066S
9067S
9069S
9071S
9073S
9074S
9077S
90200S
90202S
90214S
90215S
90241S
90247S
90248S
90252S

FIG. 61B

F1166

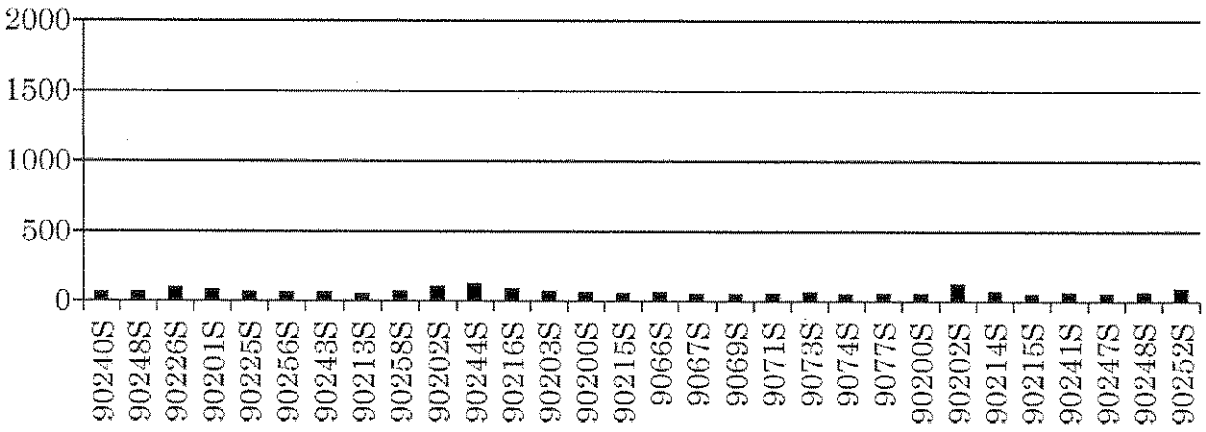


FIG. 61C

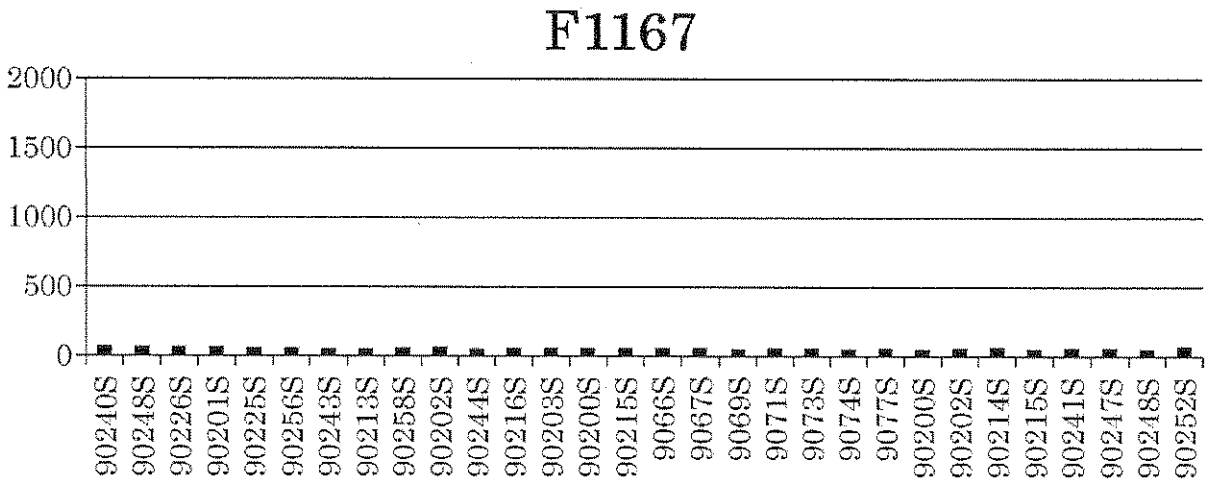

F1167
2000
1500
1000
500
0
90240S
90248S
90226S
90201S
90225S
90256S
90243S
90213S
90258S
90202S
90244S
90216S
90203S
90200S
90215S
9066S
9067S
9069S
9071S
9073S
9074S
9077S
90200S
90202S
90214S
90215S
90241S
90247S
90248S
90252S

FIG. 61D

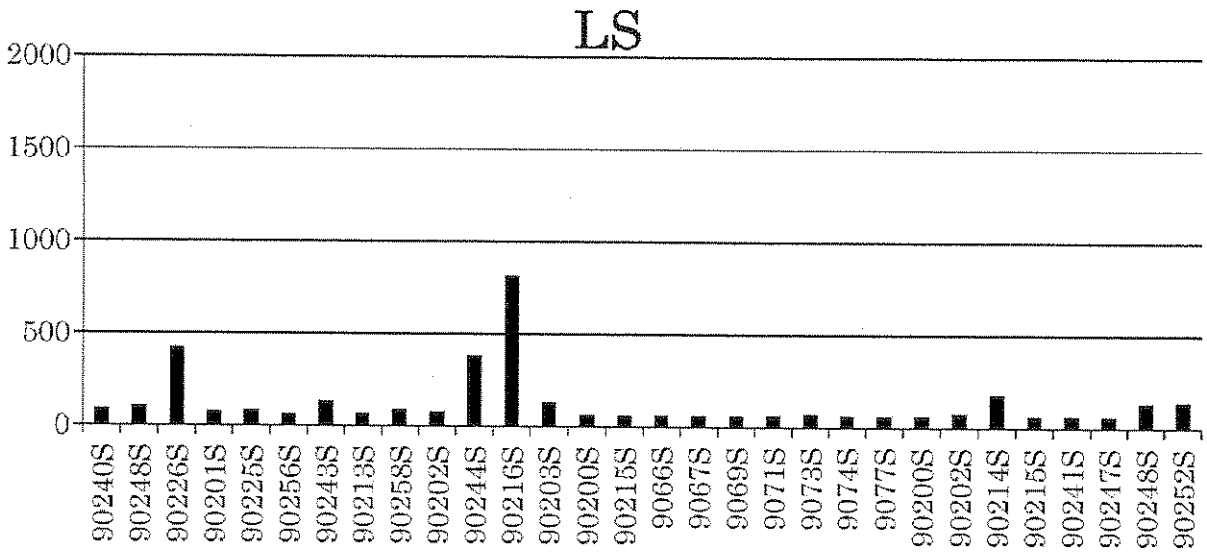

LS
2000
1500
1000
500
0
90240S
90248S
90226S
90201S
90225S
90256S
90243S
90213S
90258S
90202S
90244S
90216S
90203S
90200S
90215S
9066S
9067S
9069S
9071S
9073S
9074S
9077S
90200S
90202S
90214S
90215S
90241S
90247S
90248S
90252S

FIG. 61E

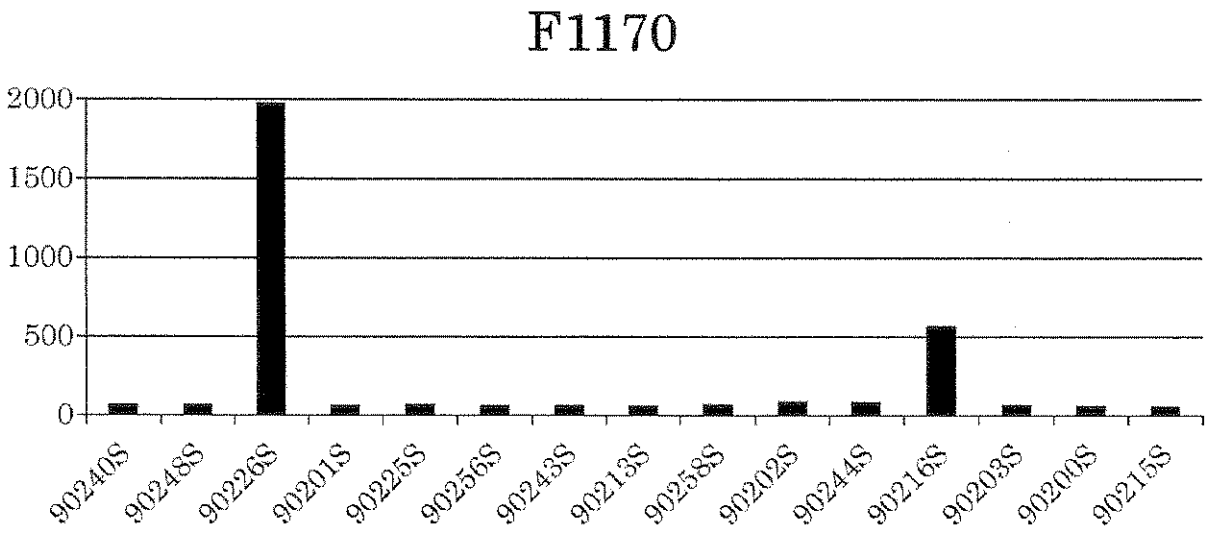

F1170
2000
1500
1000
500
0
90240S
90248S
90226S
90201S
90225S
90256S
90243S
90213S
90258S
90202S
90244S
90216S
90203S
90200S
90215S

FIG. 61F

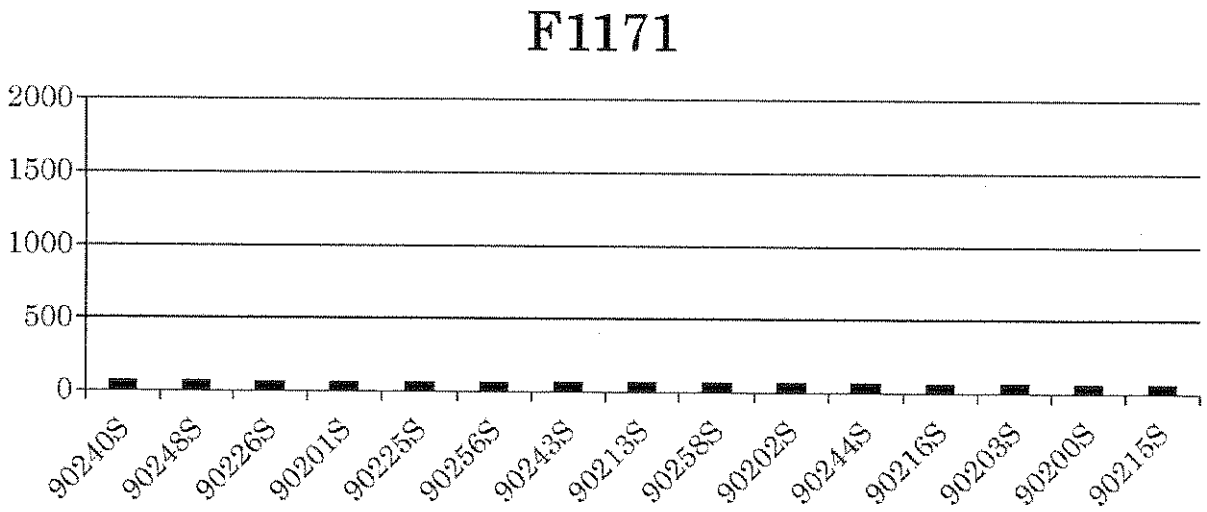

F1171
2000
1500
1000
500
0
90240S
90248S
90226S
90201S
90225S
90256S
90243S
90213S
90258S
90202S
90244S
90216S
90203S
90200S
90215S

FIG. 61G

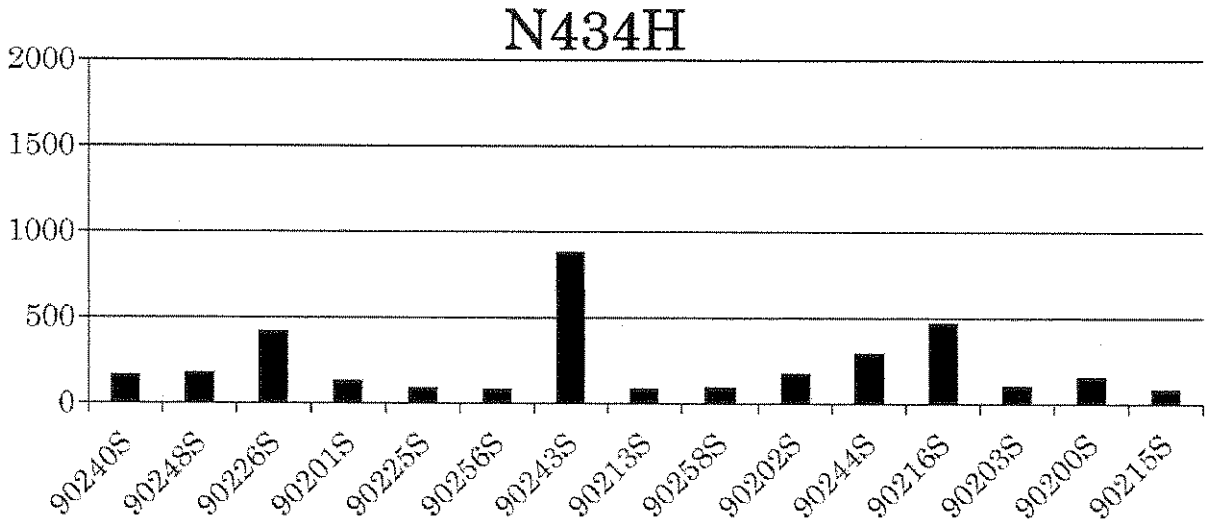

N434H
2000
1500
1000
500
0
90240S
90248S
90226S
90201S
90225S
90256S
90243S
90213S
90258S
90202S
90244S
90216S
90203S
90200S
90215S

FIG. 61H

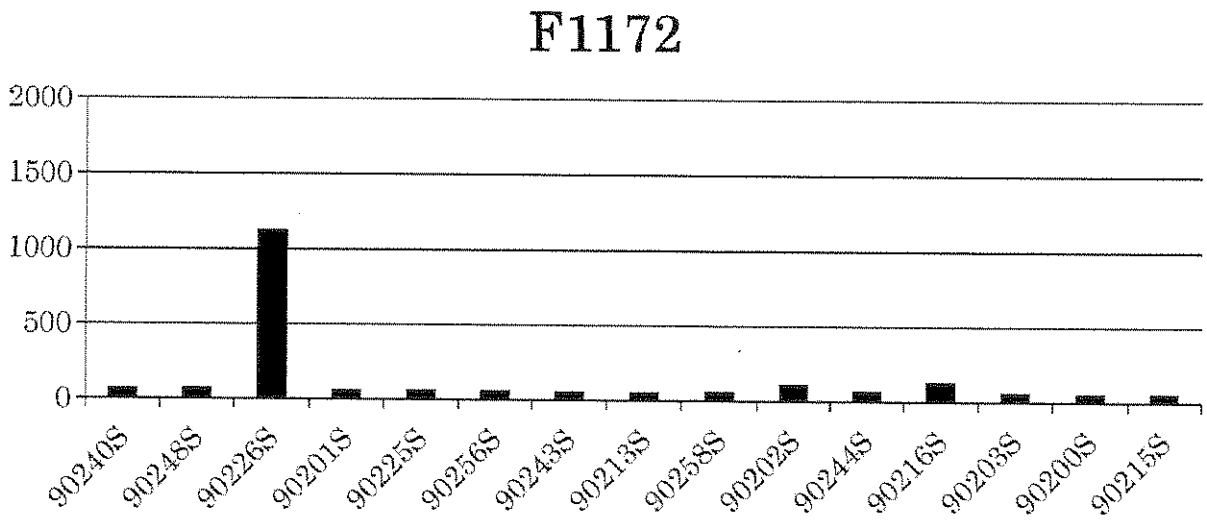

F1172
2000
1500
1000
500
0
90240S
90248S
90226S
90201S
90225S
90256S
90243S
90213S
90258S
90202S
90244S
90216S
90203S
90200S
90215S

FIG. 61I

F1173

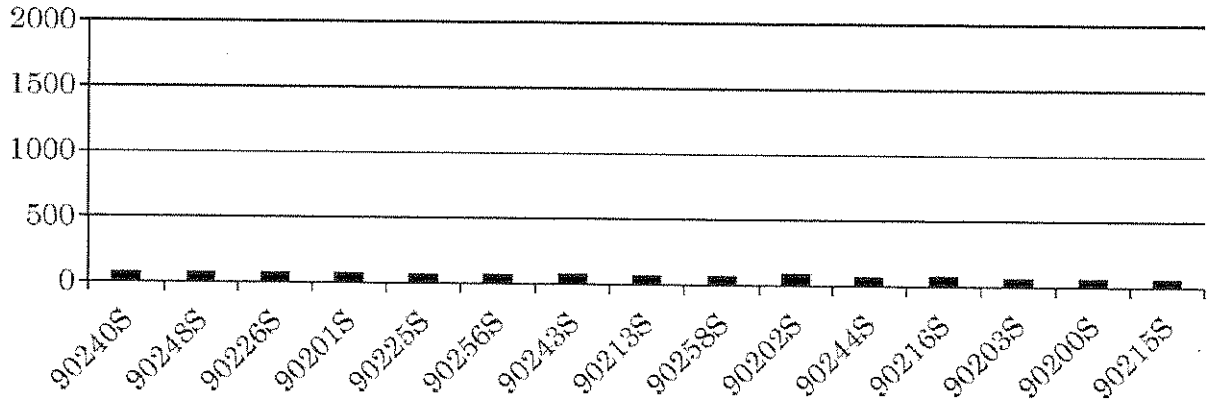


FIG. 61J

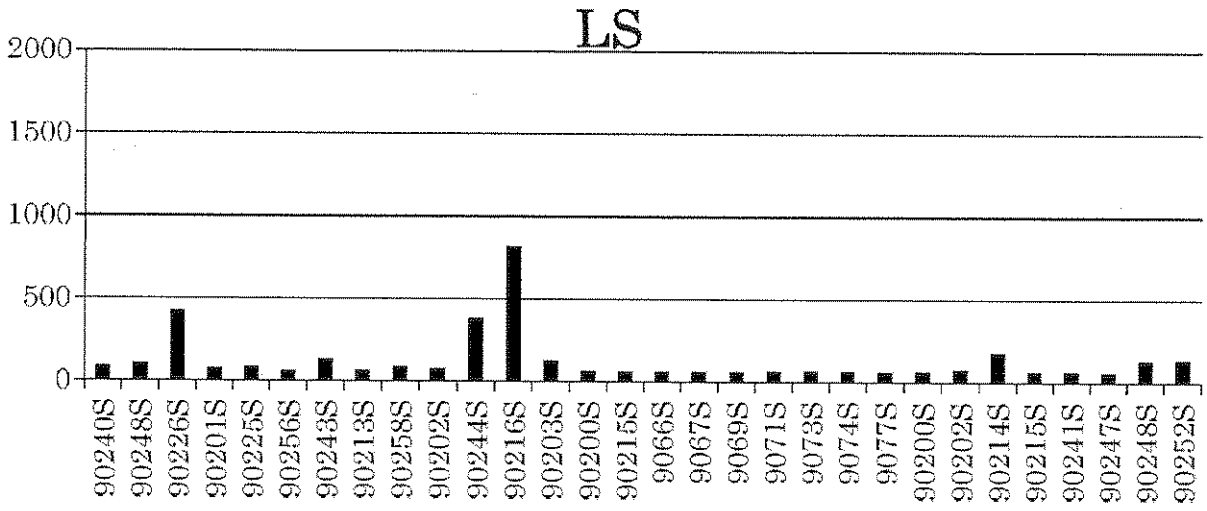

LS
2000
1500
1000
500
0
90240S
90248S
90226S
90201S
90225S
90256S
90243S
90213S
90258S
90202S
90244S
90216S
90203S
90200S
90215S
9066S
9067S
9069S
9071S
9073S
9074S
9077S
90200S
90202S
90214S
90215S
90241S
90247S
90248S
90252S

FIG. 62A

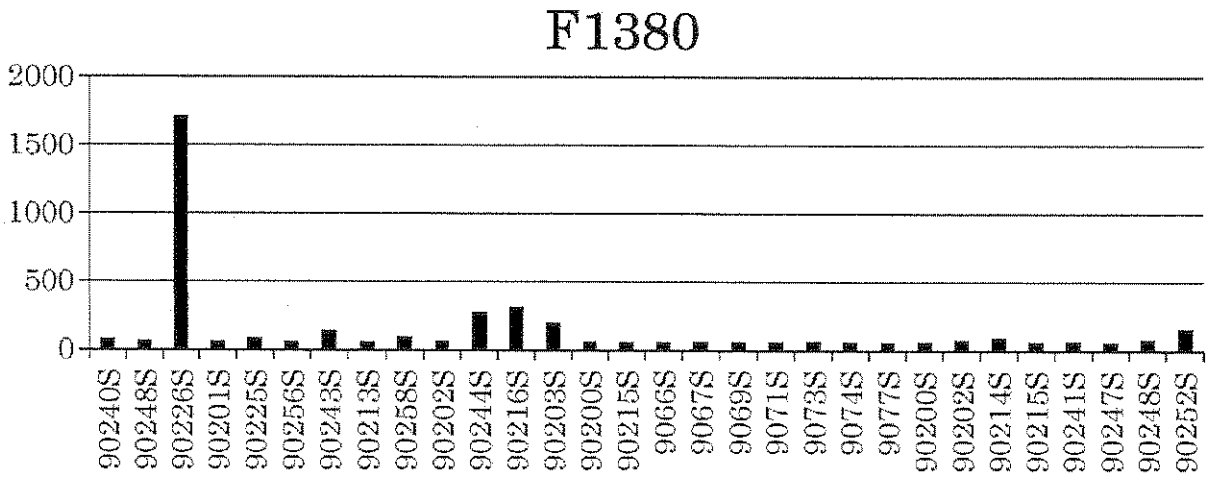

F1380
2000
1500
1000
500
0
90240S
90248S
90226S
90201S
90225S
90256S
90243S
90213S
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90202S
90244S
90216S
90203S
90200S
90215S
9096S
9097S
9069S
9071S
9073S
9074S
9077S
90200S
90203S
90214S
90215S
90241S
90247S
90248S
90252S

FIG. 62B

F1384

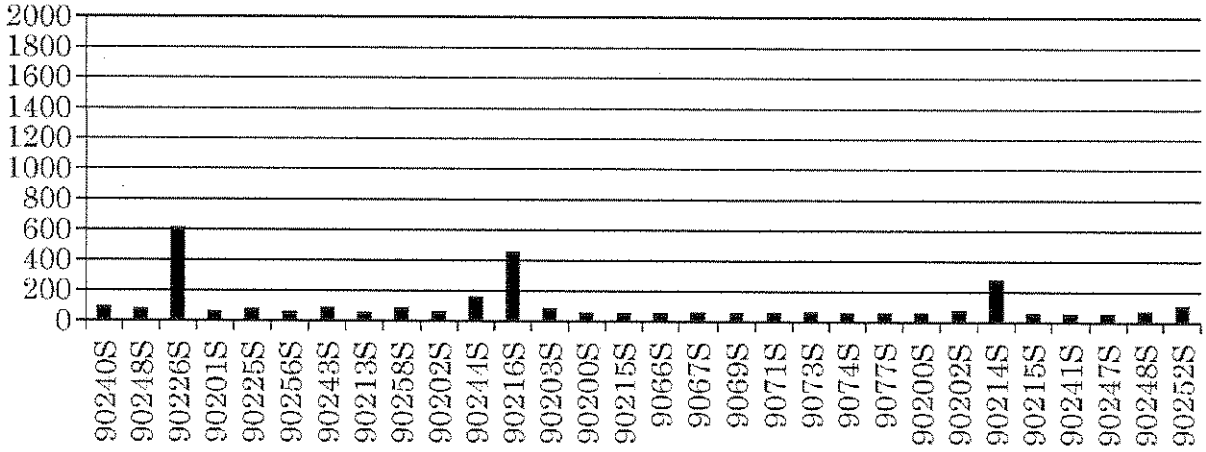


FIG. 62C

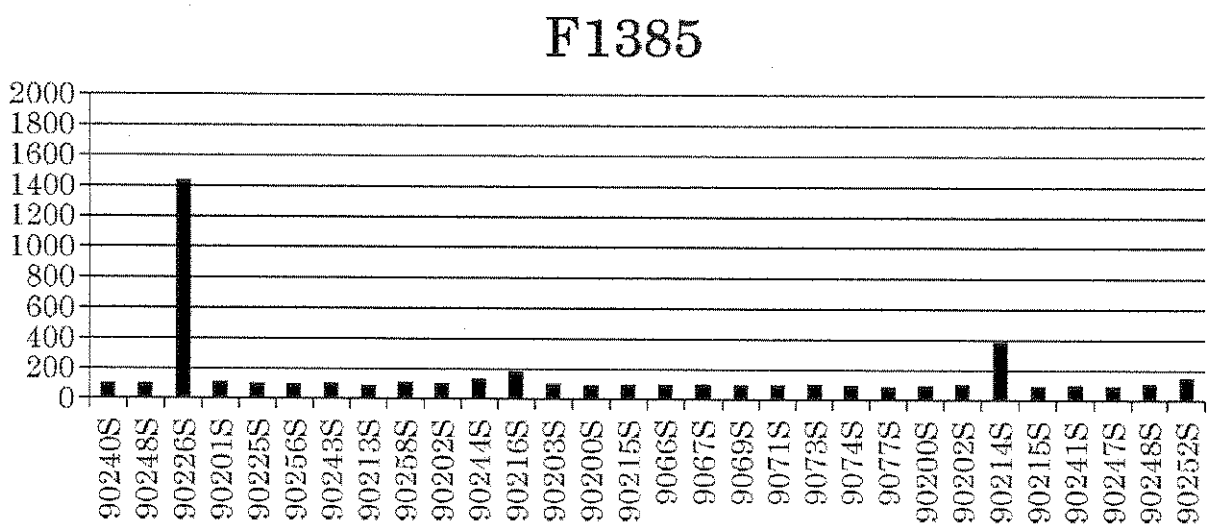

F1385
2000
1800
1600
1400
1200
1000
800
600
400
200
0
90240S
90248S
90226S
90201S
90225S
90256S
90243S
90213S
90258S
90202S
90244S
90216S
90203S
90200S
90215S
9066S
9067S
9069S
9071S
9073S
9074S
9077S
90200S
90202S
90214S
90215S
90241S
90247S
90248S
90252S

FIG. 62D

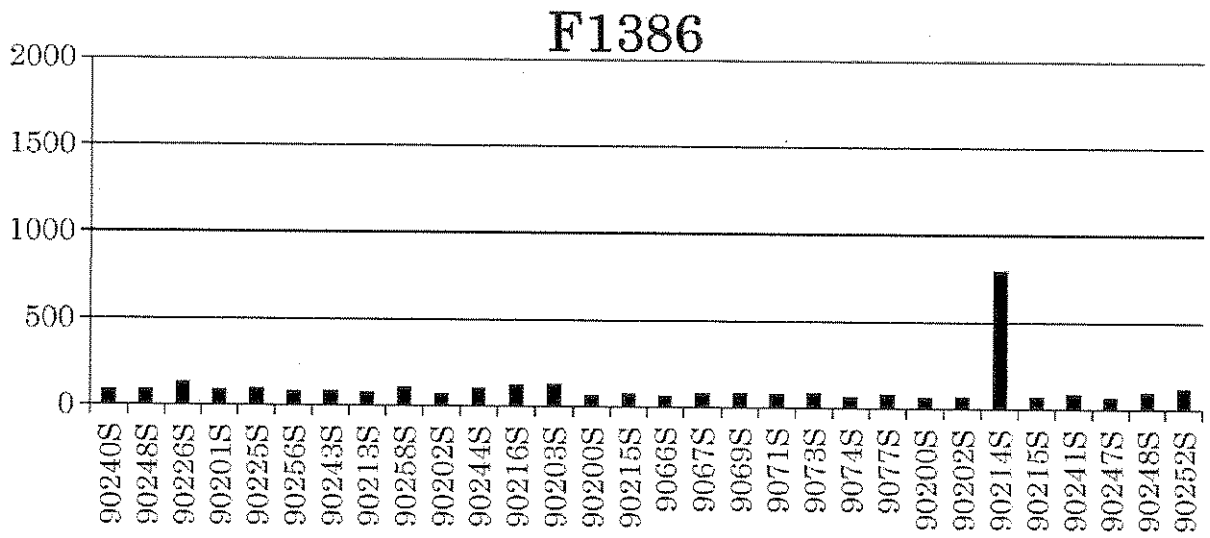

F1386
2000
1500
1000
500
0
90240S
90248S
90226S
90201S
90225S
90256S
90243S
90213S
90258S
90202S
90244S
90216S
90203S
90200S
90215S
9066S
9067S
9069S
9071S
9073S
9074S
9077S
90200S
90202S
90214S
90215S
90241S
90247S
90248S
90252S

FIG. 62E

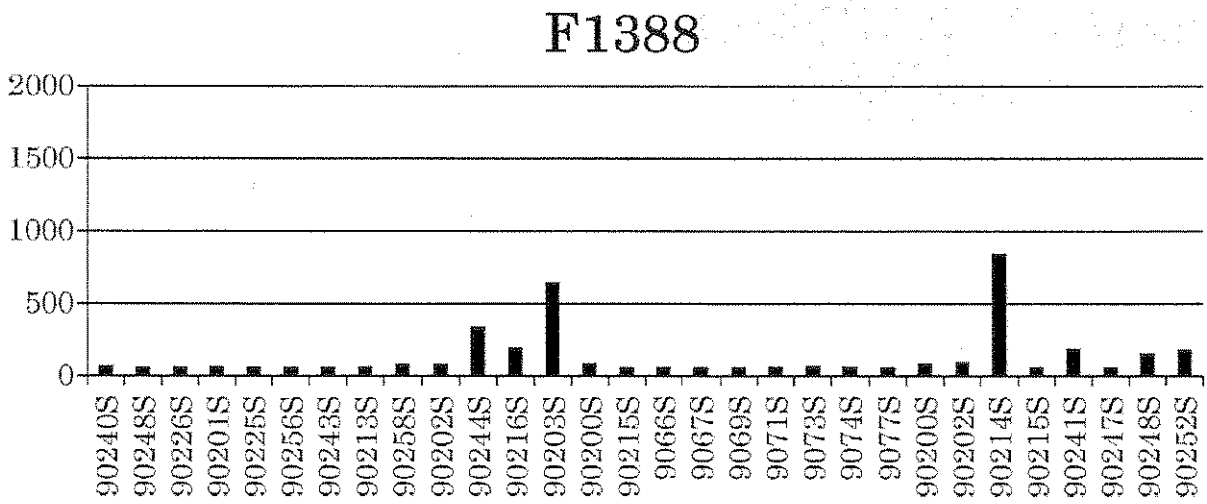

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500
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90248S
90226S
90201S
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90256S
90243S
90213S
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90216S
90203S
90200S
90215S
9066S
9067S
9069S
9071S
9073S
9074S
9077S
90200S
90202S
90214S
90215S
90241S
90247S
90248S
90252S

FIG. 62F

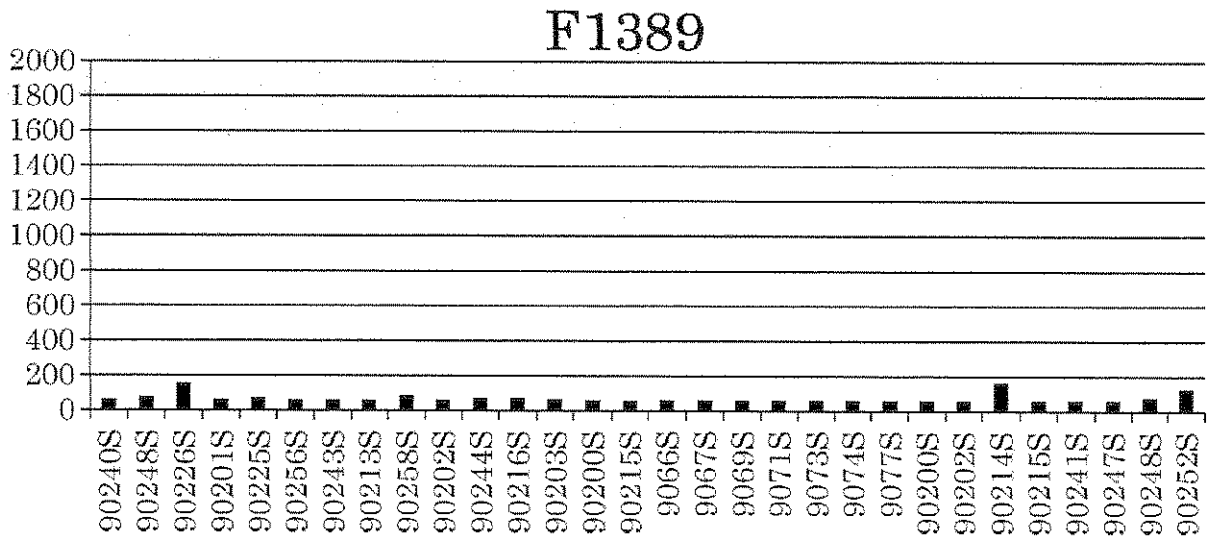

F1389
2000
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1600
1400
1200
1000
800
600
400
200
0
90240S
90248S
90226S
90201S
90225S
90256S
90243S
90213S
90258S
90202S
90244S
90216S
90203S
90200S
90215S
9066S
9067S
9069S
9071S
9073S
9074S
9077S
90200S
90202S
90214S
90215S
90241S
90247S
90248S
90252S

FIG. 62G