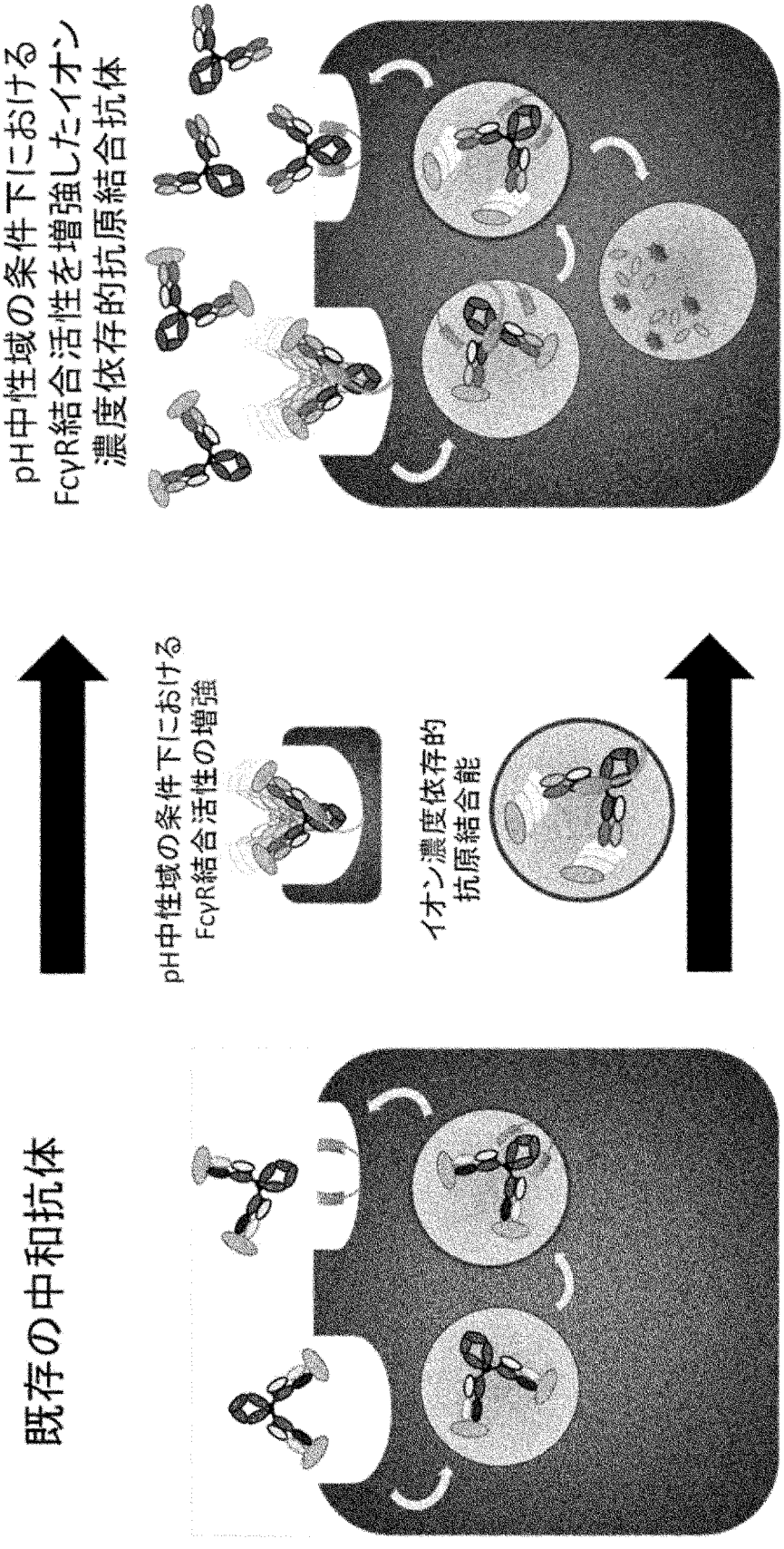
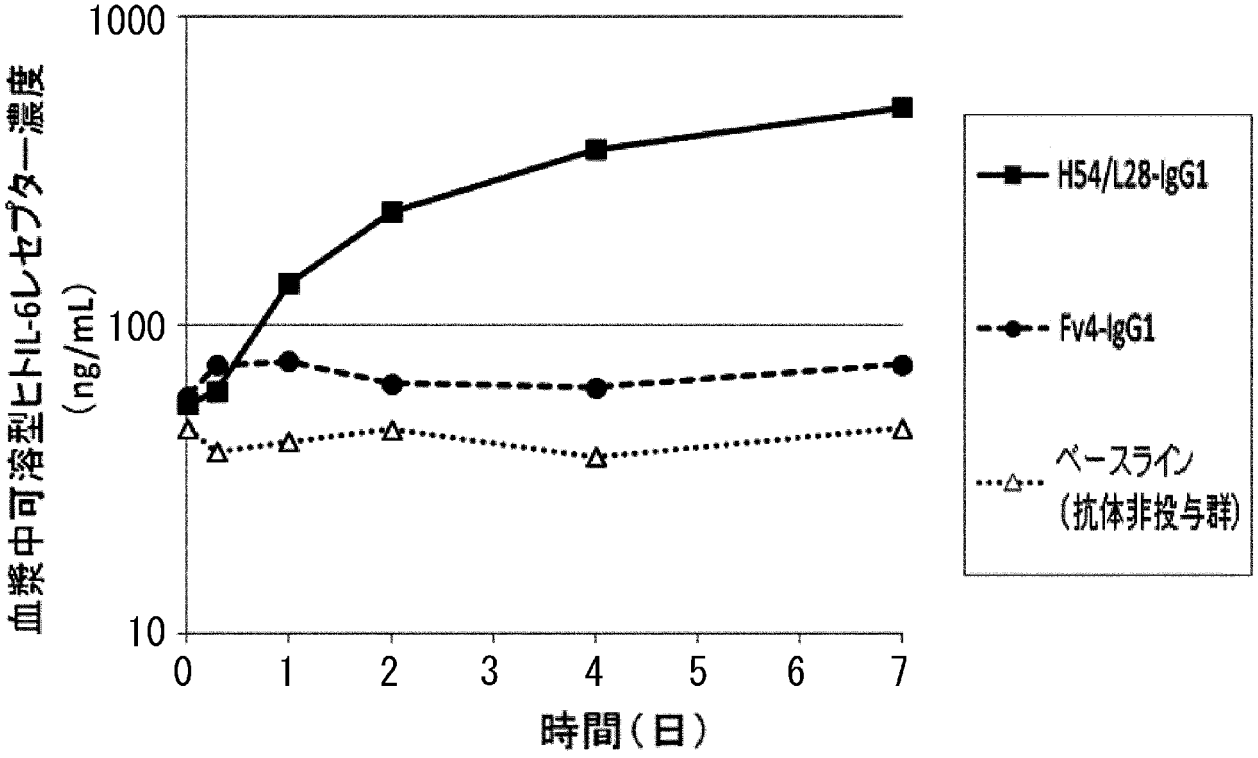
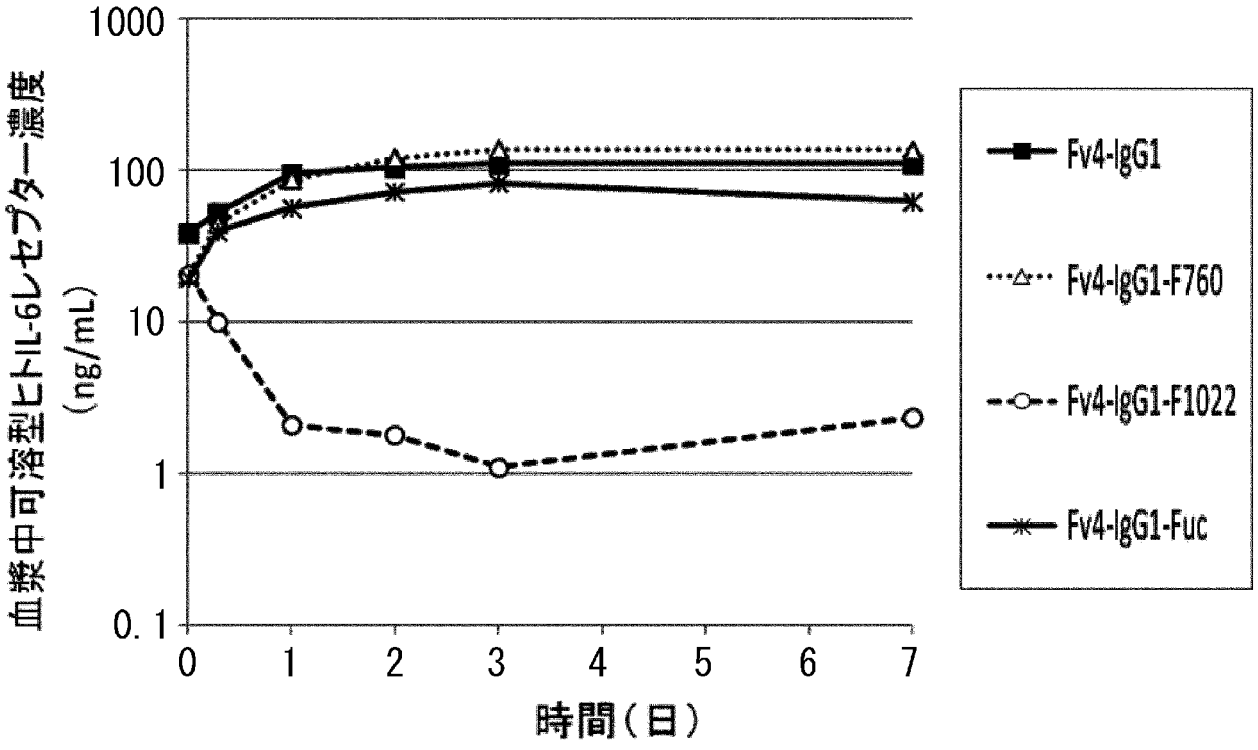
[図1]


既存の中和抗体
pH中性域の条件下における
FcγR結合活性の増強
イオン濃度依存的
抗原結合能
pH中性域の条件下における
FcγR結合活性を増強したイオン
濃度依存的抗原結合抗体

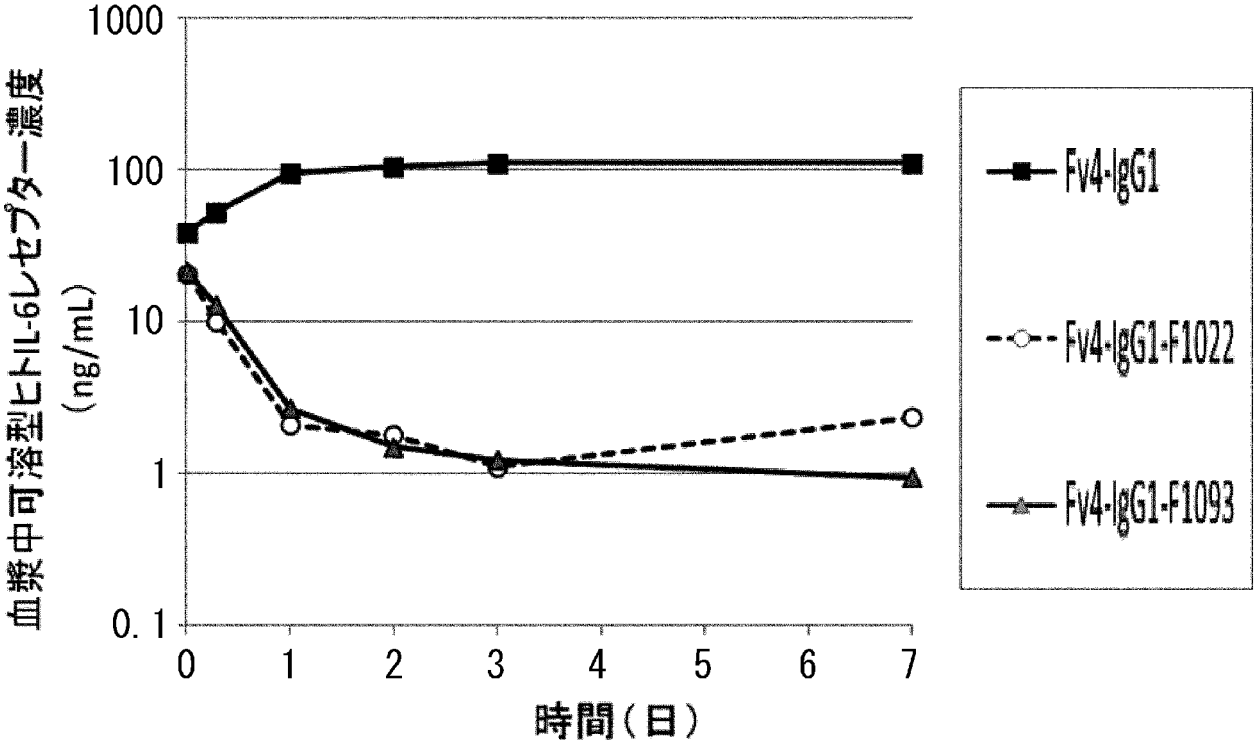
[図2]


1000
100
10
0
1
2
3
4
5
6
7
血漿中可溶型ヒトIL-6レセプター濃度
(ng/mL)
時間(日)
H54/L28-IgG1
Fv4-IgG1
ベースライン
(抗体非投与群)

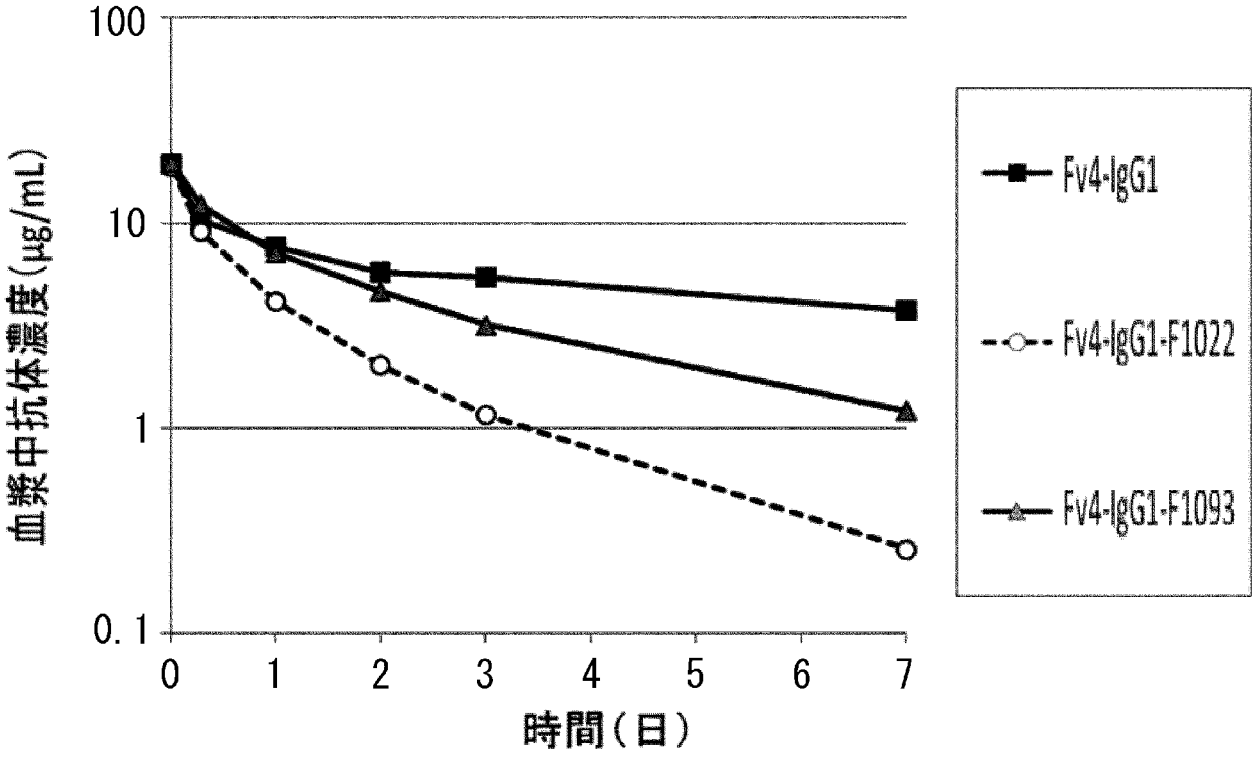
[図3]


1000
100
10
1
0.1
0
1
2
3
4
5
6
7
血漿中可溶型ヒトIL-6レセプター濃度
(ng/mL)
時間(日)
Fv4-IgG1
Fv4-IgG1-F760
Fv4-IgG1-F1022
Fv4-IgG1-Fuc

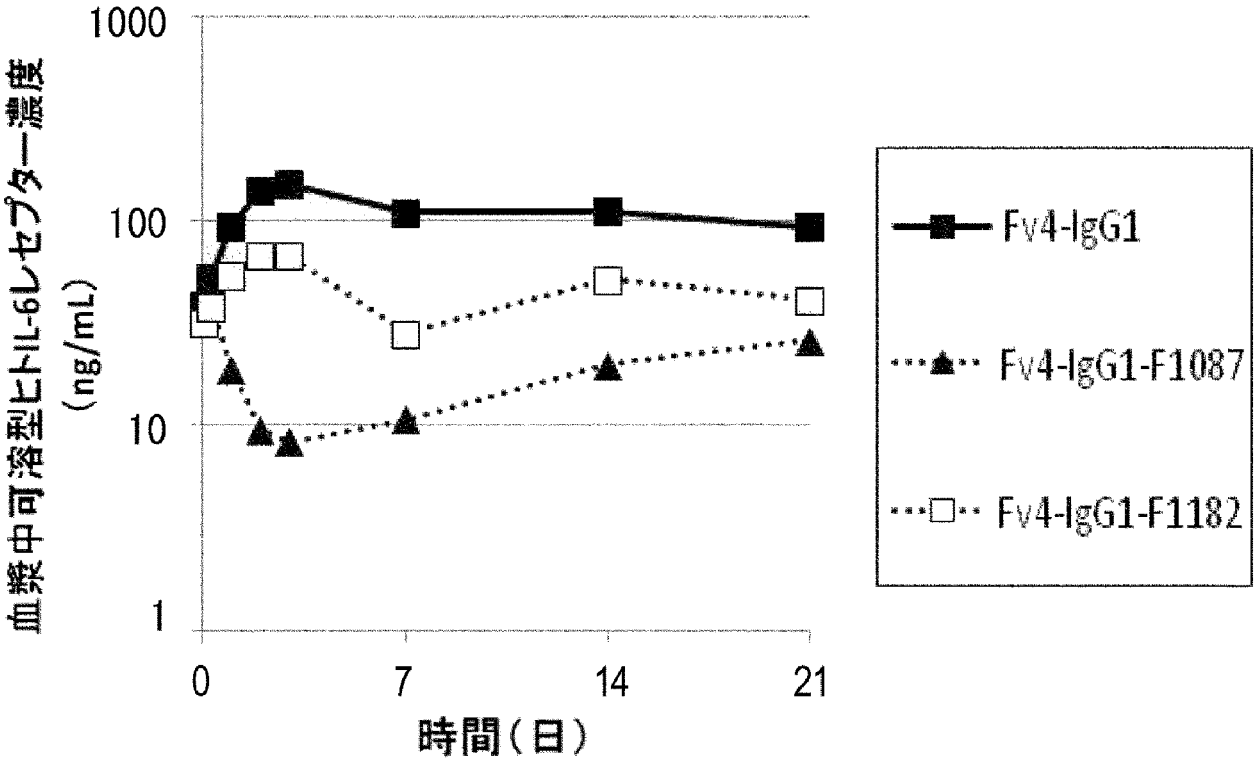
[図4]


1000
100
10
1
0.1
血漿中可溶型ヒトIL-6レセプター濃度 (ng/mL)
0
1
2
3
4
5
6
7
時間(日)
Fv4-IgG1
Fv4-IgG1-F1022
Fv4-IgG1-F1093

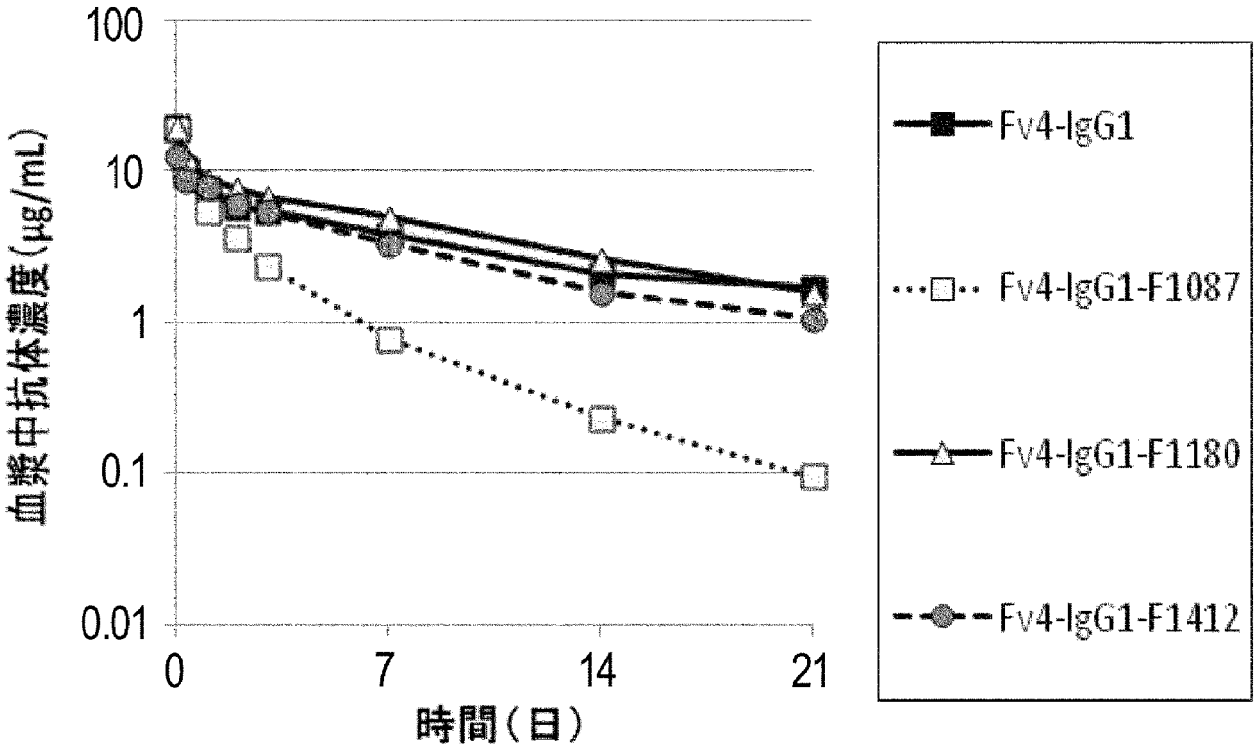
[図5]


100
10
1
0.1
血漿中抗体濃度(μg/mL)
0
1
2
3
4
5
6
7
時間(日)
Fv4-IgG1
Fv4-IgG1-F1022
Fv4-IgG1-F1093

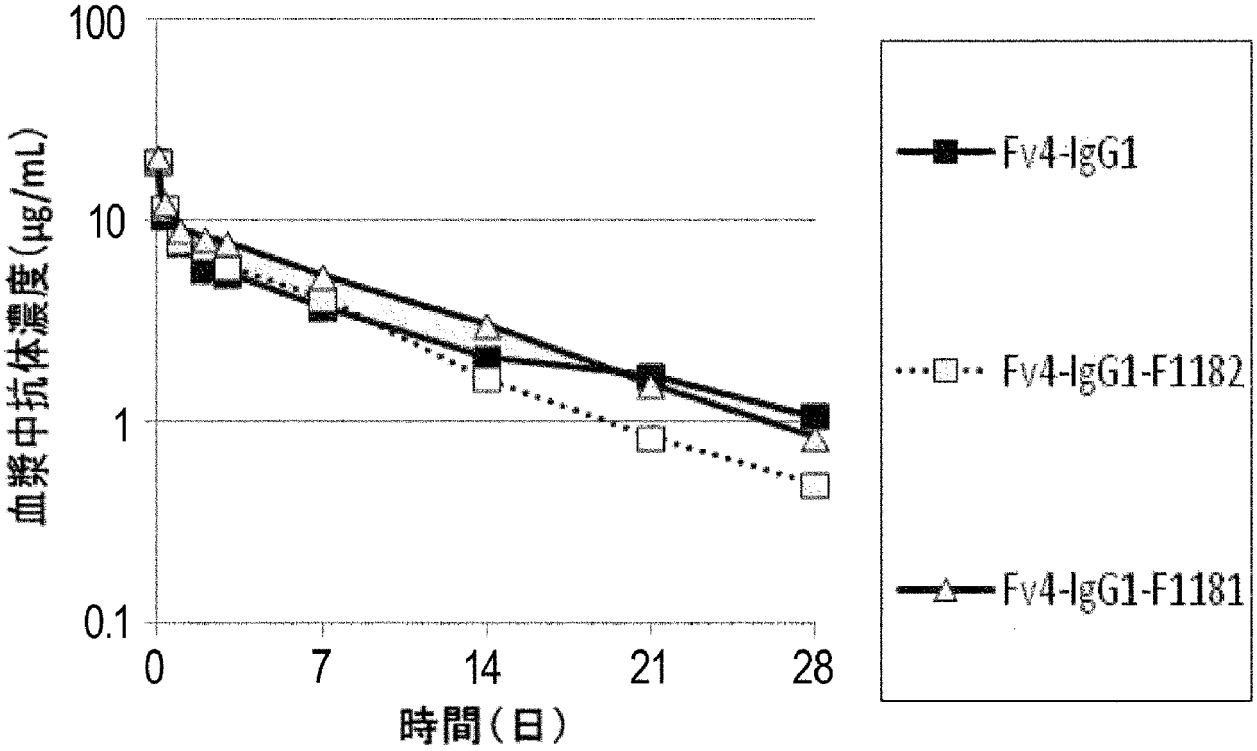
[図6]


1000
100
10
1
血漿中可溶型ヒトIL-6レセプター濃度 (ng/mL)
0
7
14
21
時間(日)
Fv4-IgG1
Fv4-IgG1-F1087
Fv4-IgG1-F1182

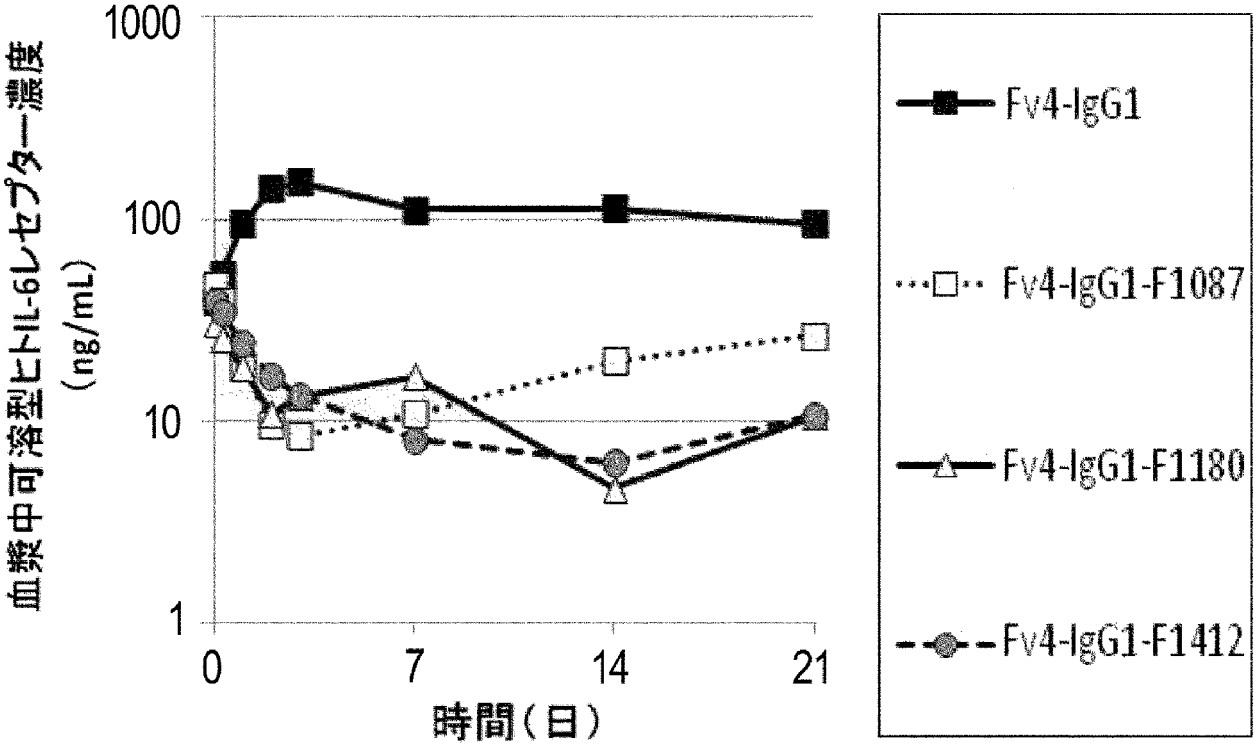
[図7]


100
10
1
0.1
0.01
血漿中抗体濃度(μg/mL)
0
7
14
21
時間(日)
Fv4-IgG1
Fv4-IgG1-F1087
Fv4-IgG1-F1180
Fv4-IgG1-F1412

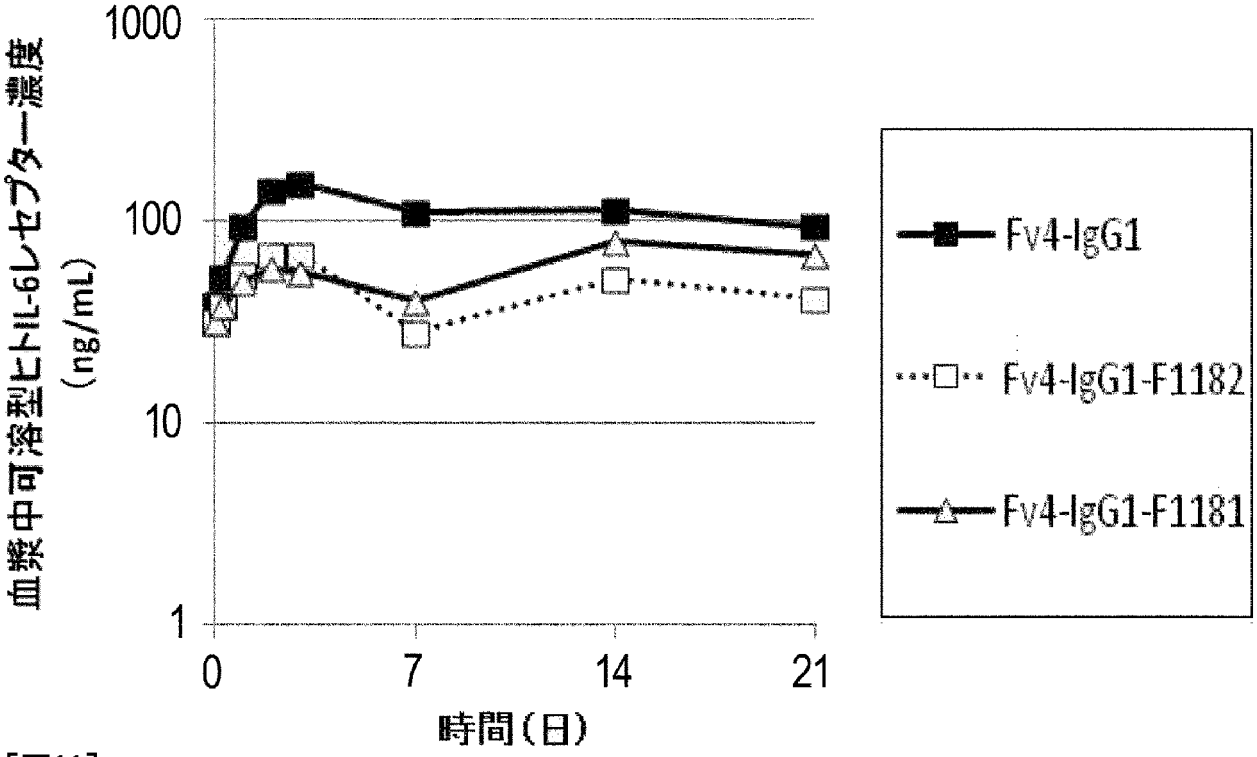
[図8]


100
10
1
0.1
血漿中抗体濃度(μg/mL)
0
7
14
21
28
時間(日)
Fv4-IgG1
Fv4-IgG1-F1182
Fv4-IgG1-F1181

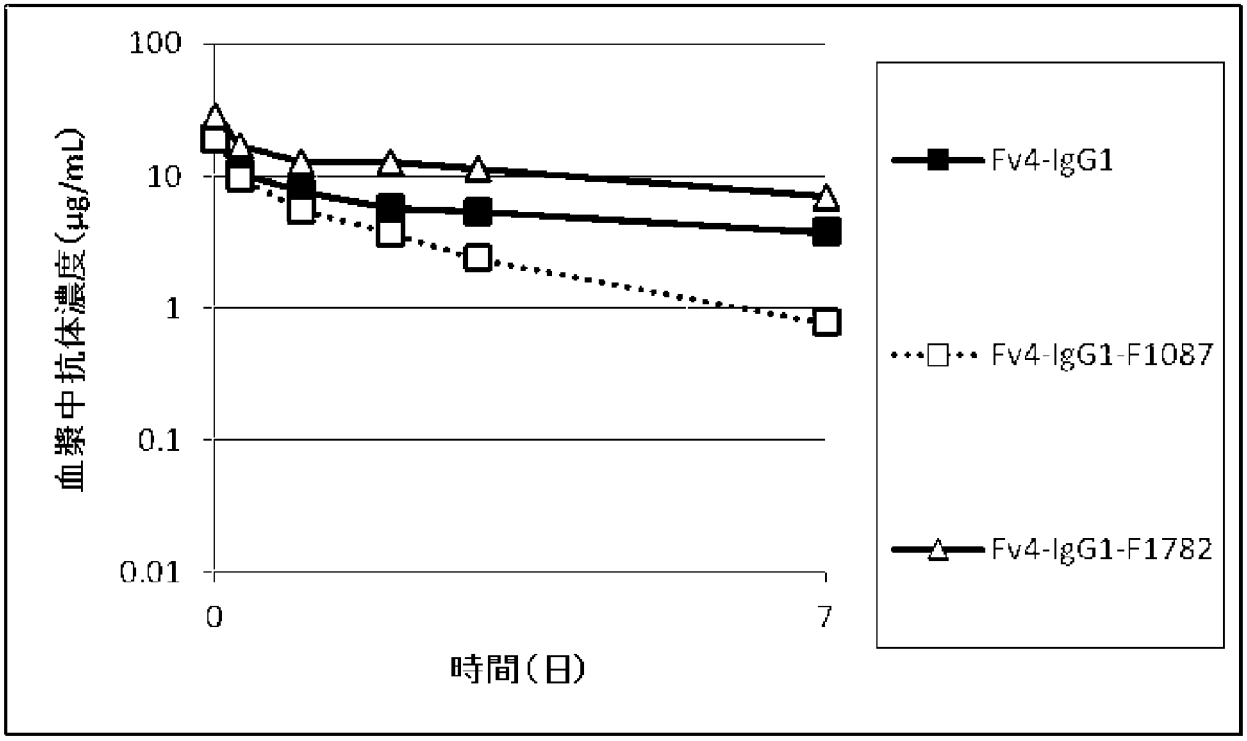
[図9]


1000
100
10
1
血漿中可溶型ヒトIL-6レセプター濃度(ng/mL)
0
7
14
21
時間(日)
Fv4-IgG1
Fv4-IgG1-F1087
Fv4-IgG1-F1180
Fv4-IgG1-F1412

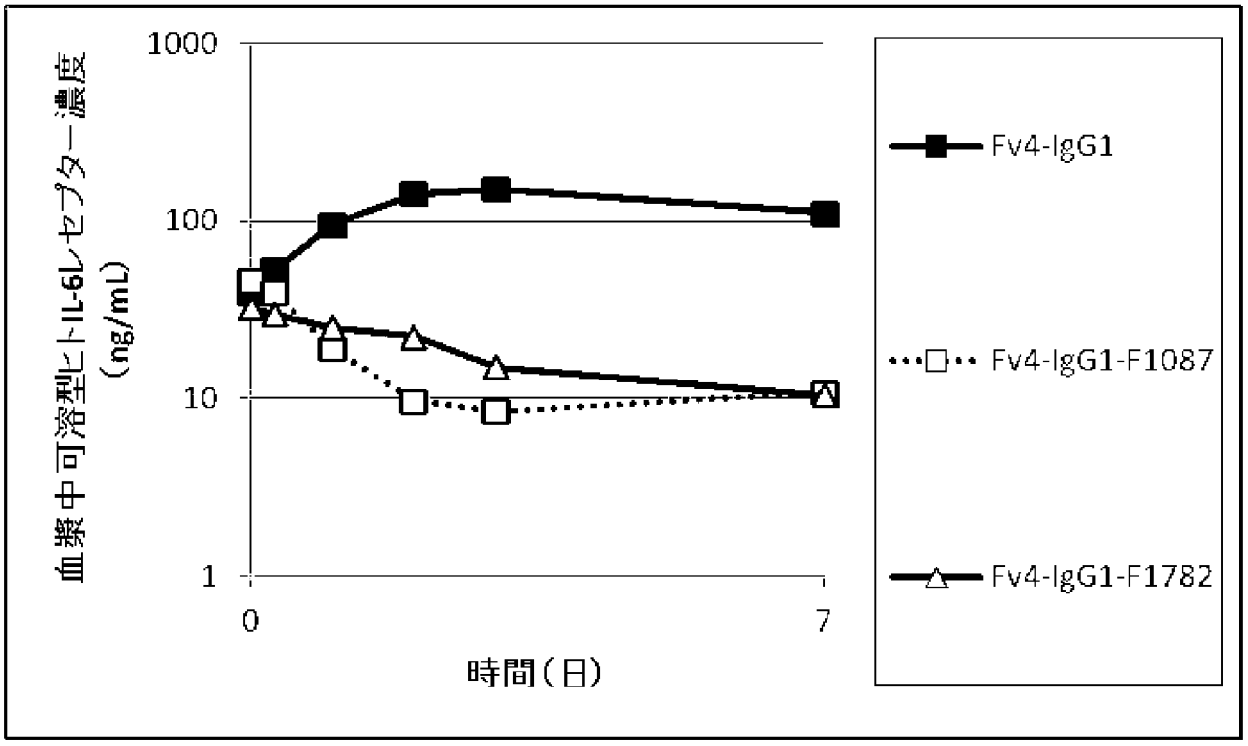
[図10]


血漿中可溶型ヒトIL-6レセプター濃度 (ng/mL)
1000
100
10
1
0
7
14
21
時間(日)
Fv4-IgG1
Fv4-IgG1-F1182
Fv4-IgG1-F1181

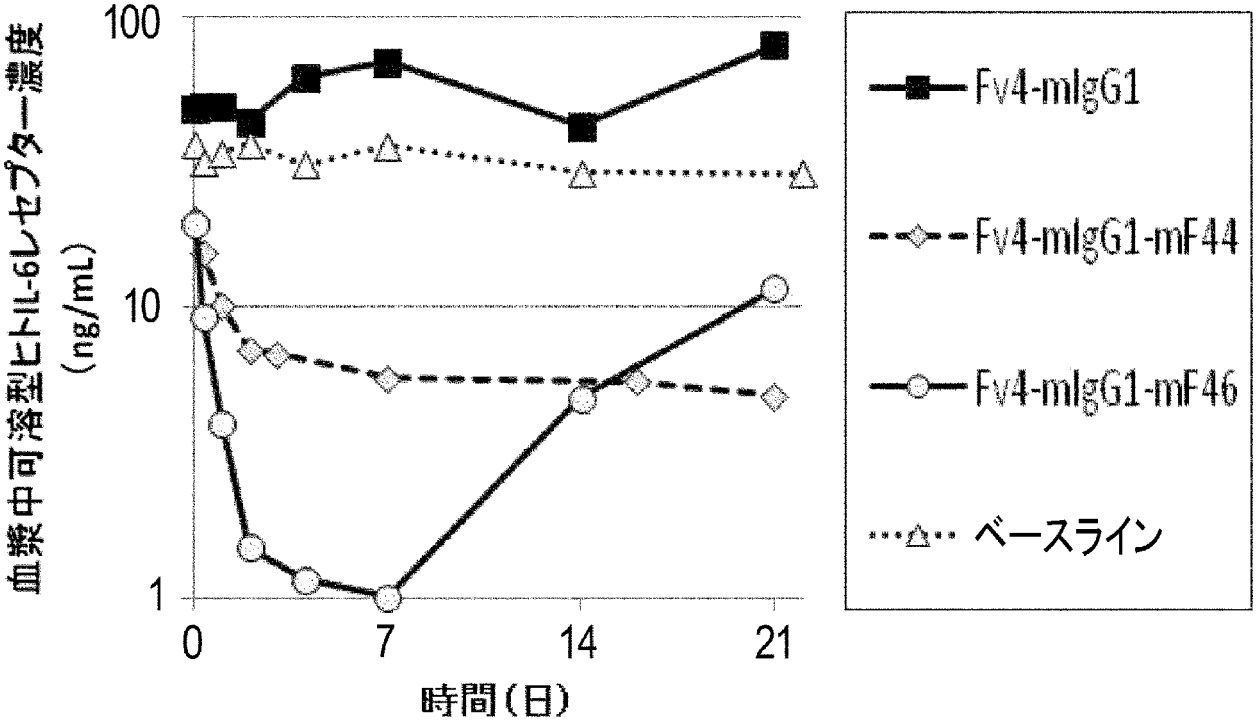
[図11]


血漿中抗体濃度(μg/mL)
100
10
1
0.1
0.01
0
7
時間(日)
Fv4-IgG1
Fv4-IgG1-F1087
Fv4-IgG1-F1782

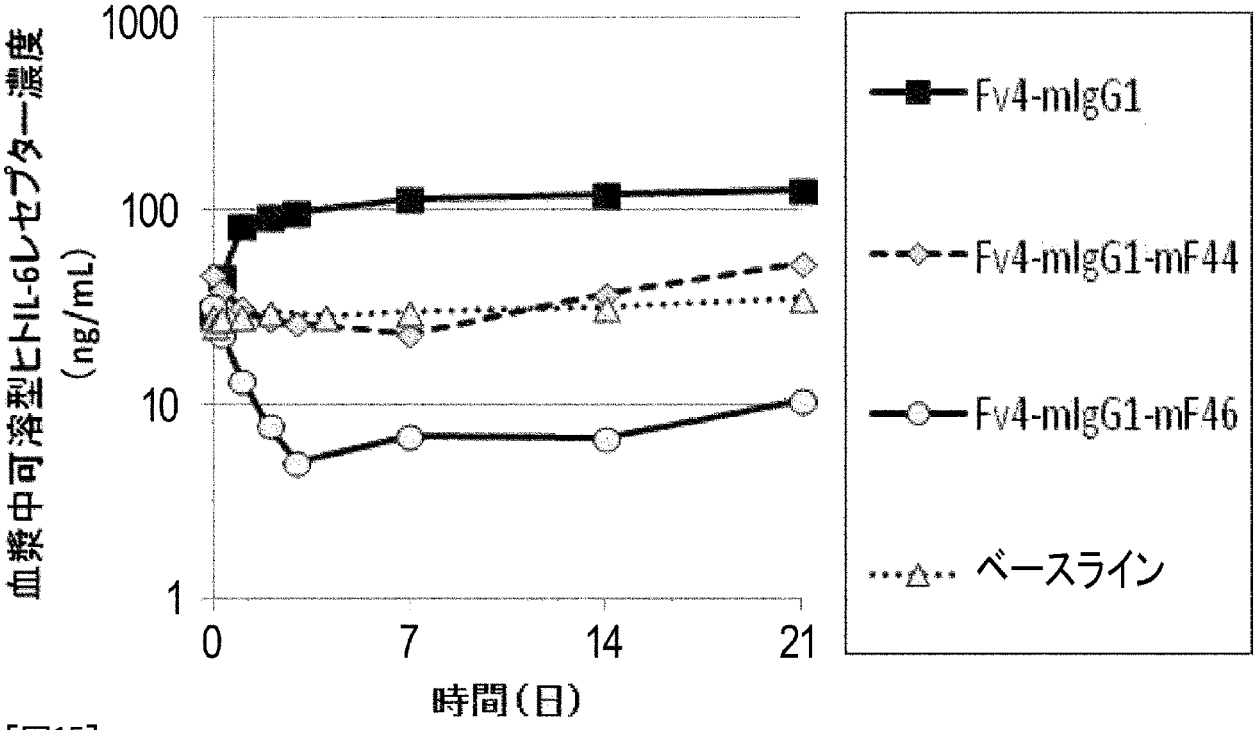
[図12]


1000
100
10
1
0
7
血漿中可溶型ヒトIL-6レセプター濃度
(ng/mL)
時間(日)
Fv4-IgG1
Fv4-IgG1-F1087
Fv4-IgG1-F1782

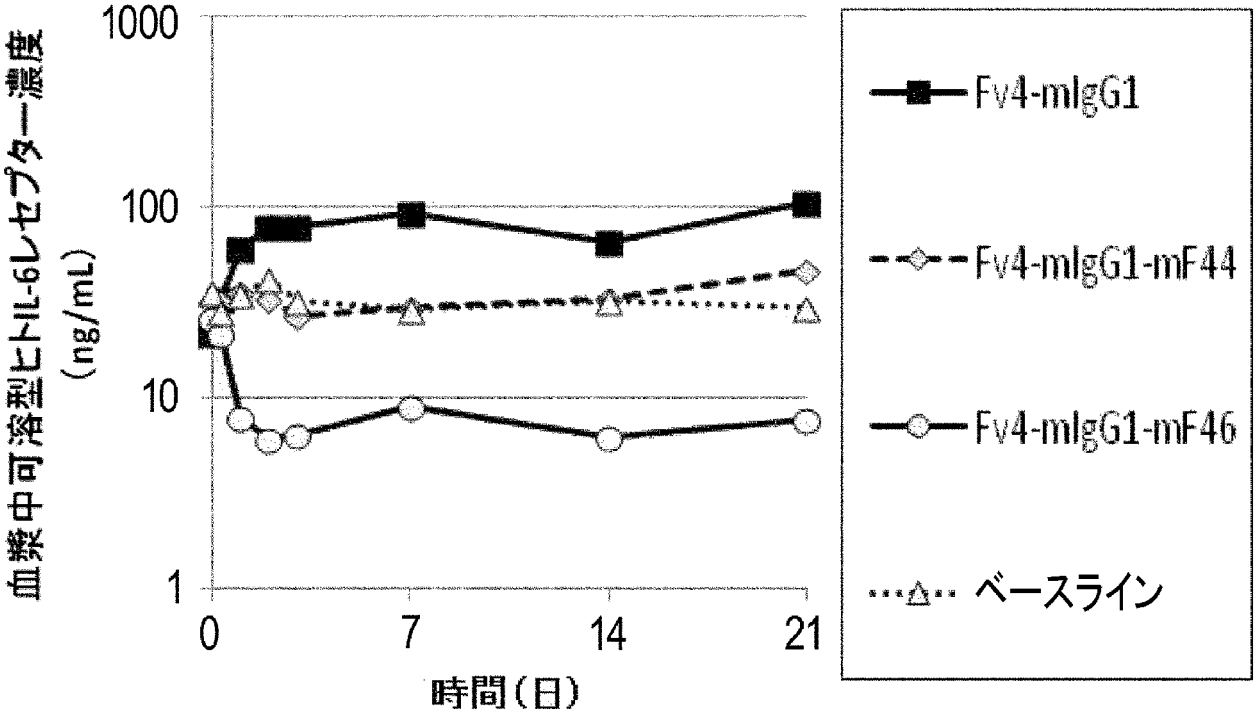
[図13]


100
10
1
0
7
14
21
血漿中可溶型ヒトIL-6レセプター濃度
(ng/mL)
時間(日)
Fv4-mIgG1
Fv4-mIgG1-mF44
Fv4-mIgG1-mF46
ベースライン

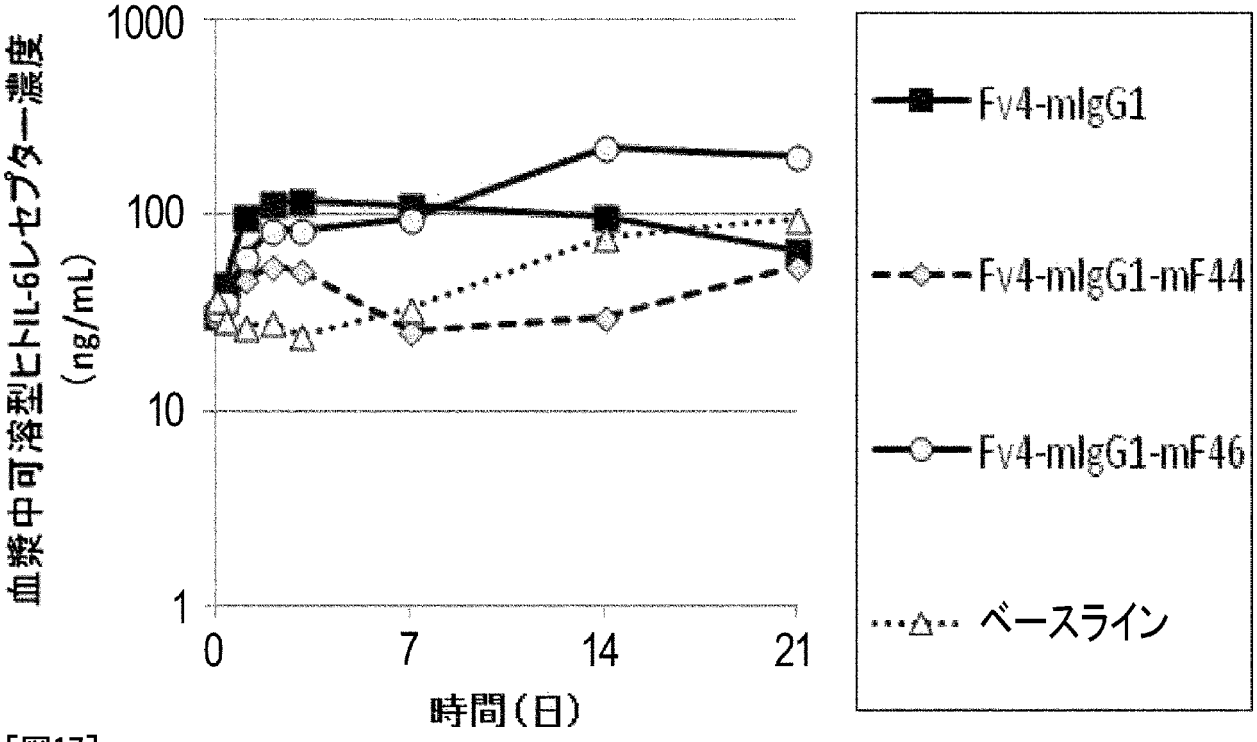
[図14]


血漿中可溶型ヒトIL-6レセプター濃度
(ng/mL)
1000
100
10
1
0
7
14
21
時間(日)
Fv4-mIgG1
Fv4-mIgG1-mF44
Fv4-mIgG1-mF46
ベースライン

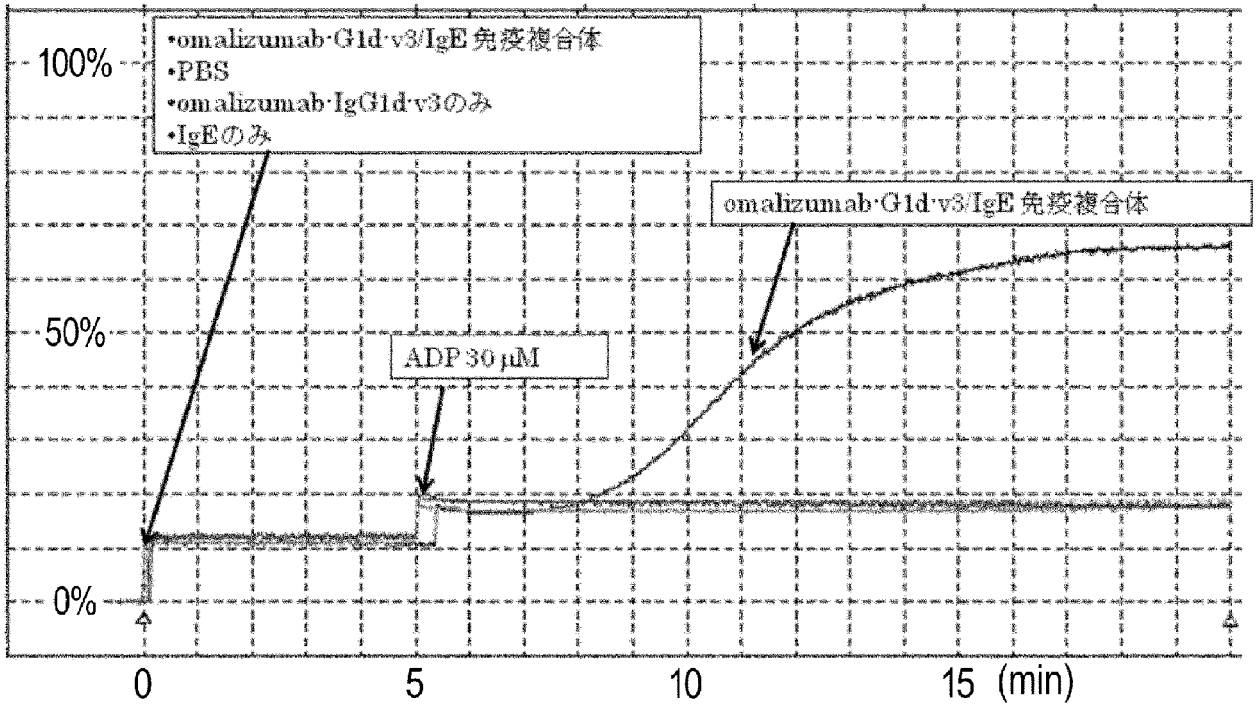
[図15]


血漿中可溶型ヒトIL-6レセプター濃度
(ng/mL)
1000
100
10
1
0
7
14
21
時間(日)
Fv4-mIgG1
Fv4-mIgG1-mF44
Fv4-mIgG1-mF46
ベースライン

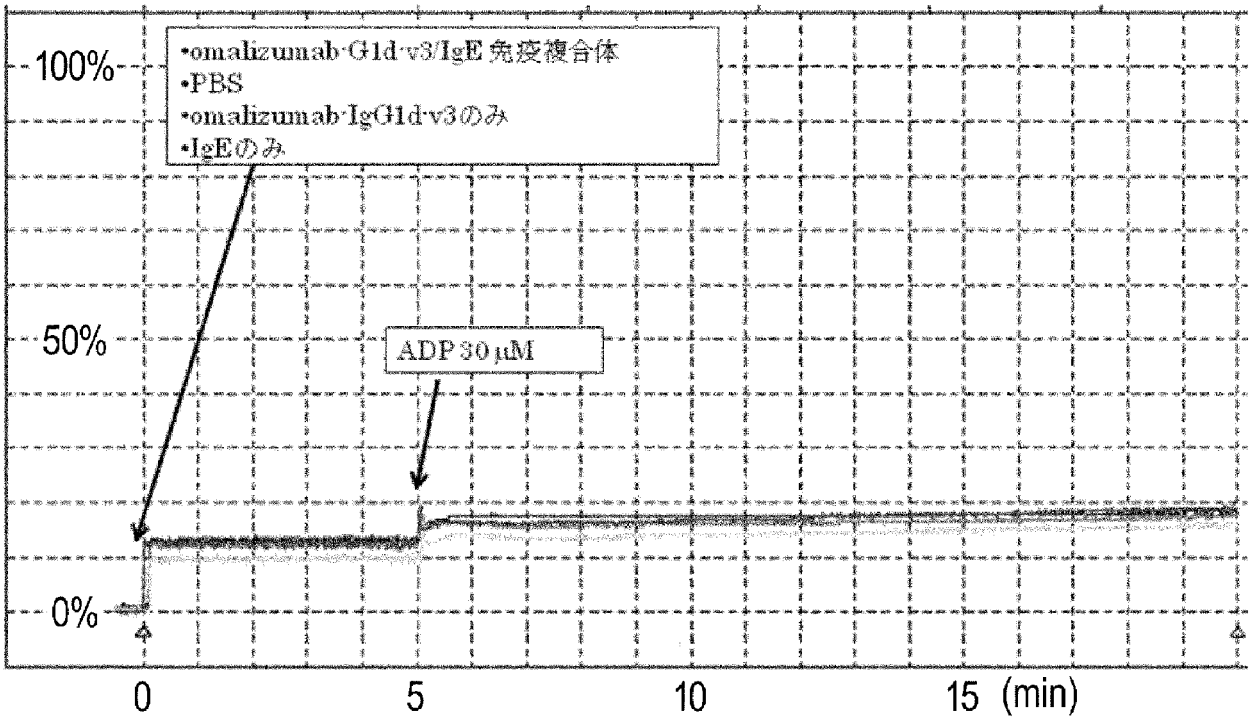
[図16]


血漿中可溶型ヒトIL-6レセプター濃度 (ng/mL)
1000
100
10
1
0
7
14
21
時間(日)
Fv4-mIgG1
Fv4-mIgG1-mF44
Fv4-mIgG1-mF46
ベースライン

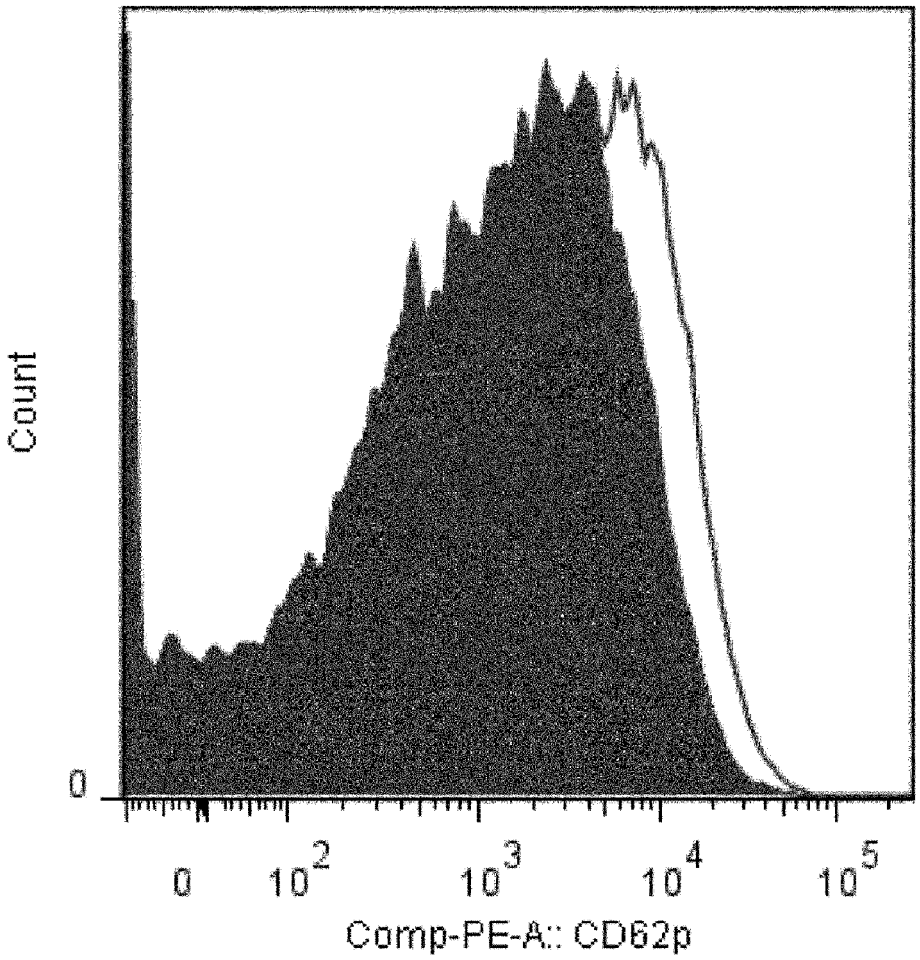
[図17]


•omalizumab·G1d·v3/IgE免疫複合体
•PBS
•omalizumab·IgG1d·v3のみ
•IgEのみ
100%
50%
0%
ADP 30 μM
omalizumab·G1d·v3/IgE免疫複合体
0
5
10
15 (min)

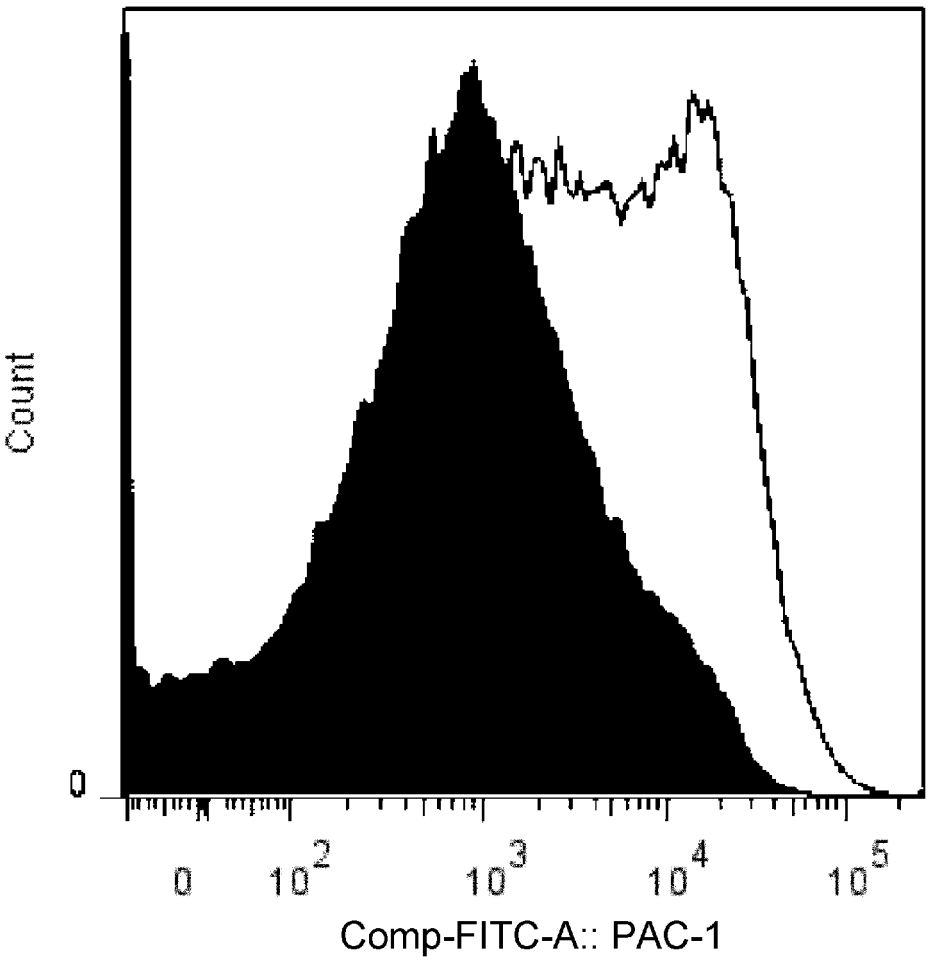
[図18]


•omalizumab-G1d-v3/IgE免疫複合体
•PBS
•omalizumab-IgG1d-v3のみ
•IgEのみ
100%
50%
0%
ADP 30 μM
0
5
10
15 (min)

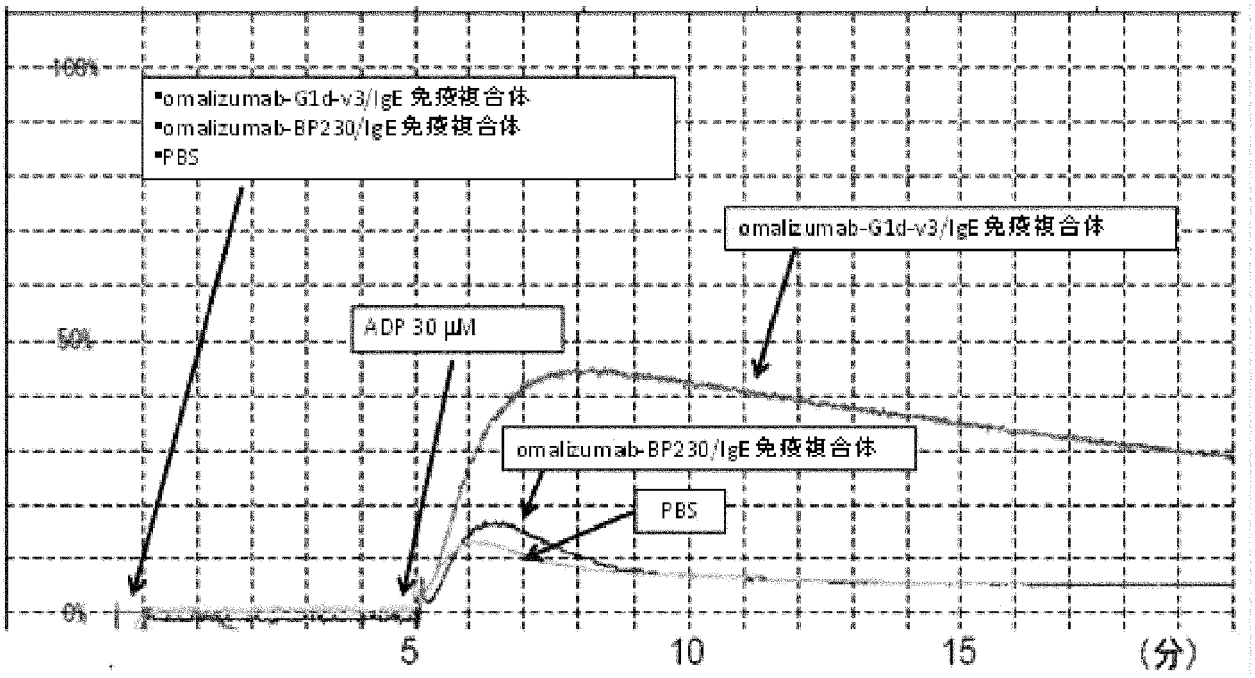
[図19]


Count
0
0
10^2
10^3
10^4
10^5
Comp-PE-A:: CD62p

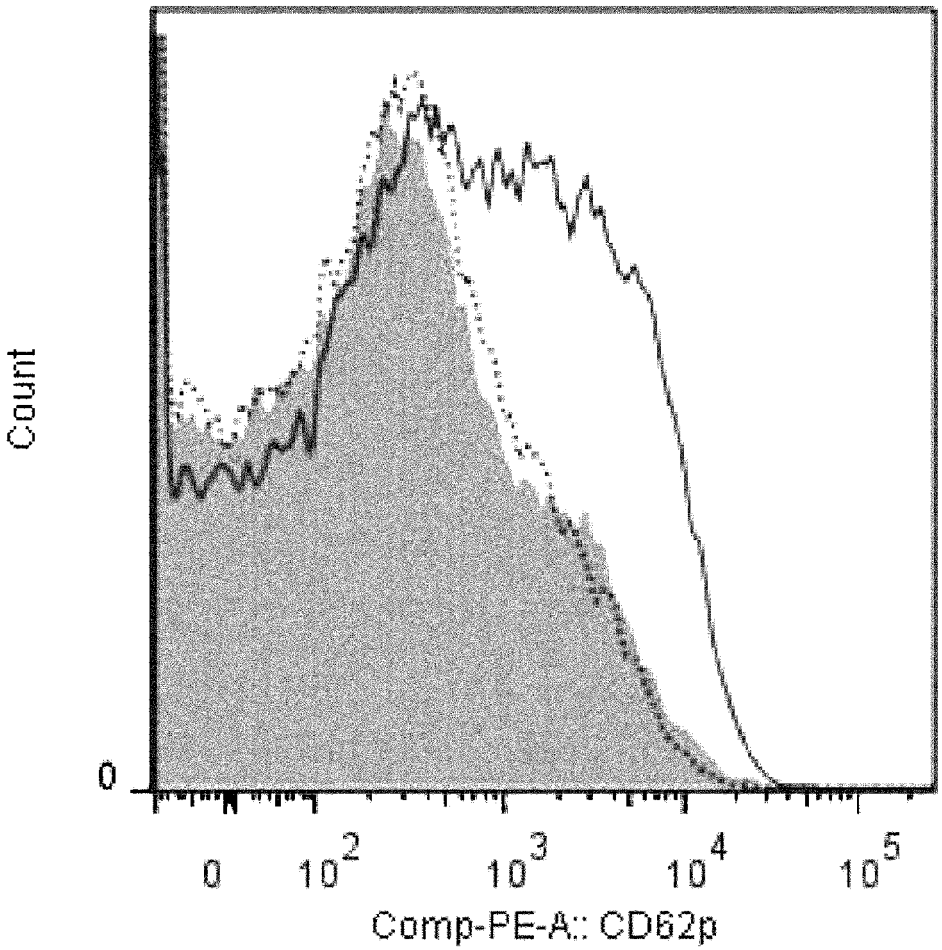
[図20]


Count
0
0
10^2
10^3
10^4
10^5
Comp-FITC-A:: PAC-1

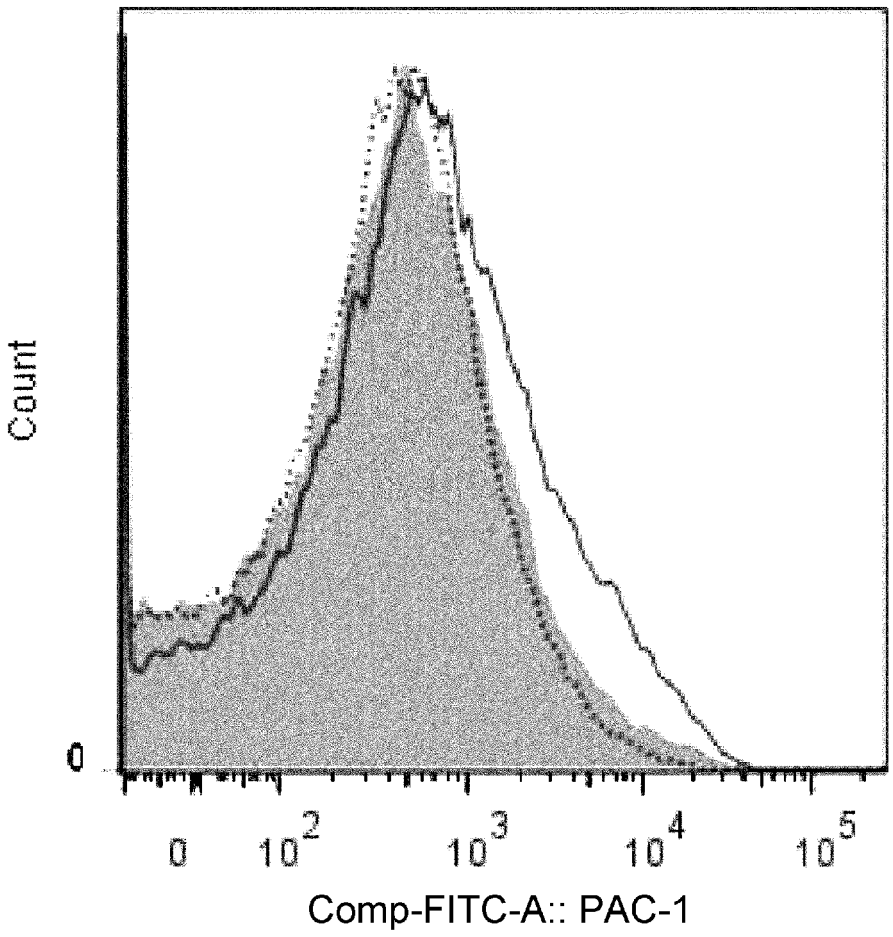
[図21]


100%
50%
0%
•omalizumab-G1d-v3/IgE 免疫複合体
•omalizumab-BP230/IgE免疫複合体
•PBS
ADP 30 μM
omalizumab-G1d-v3/IgE免疫複合体
omalizumab-BP230/IgE免疫複合体
PBS
5
10
15
(分)

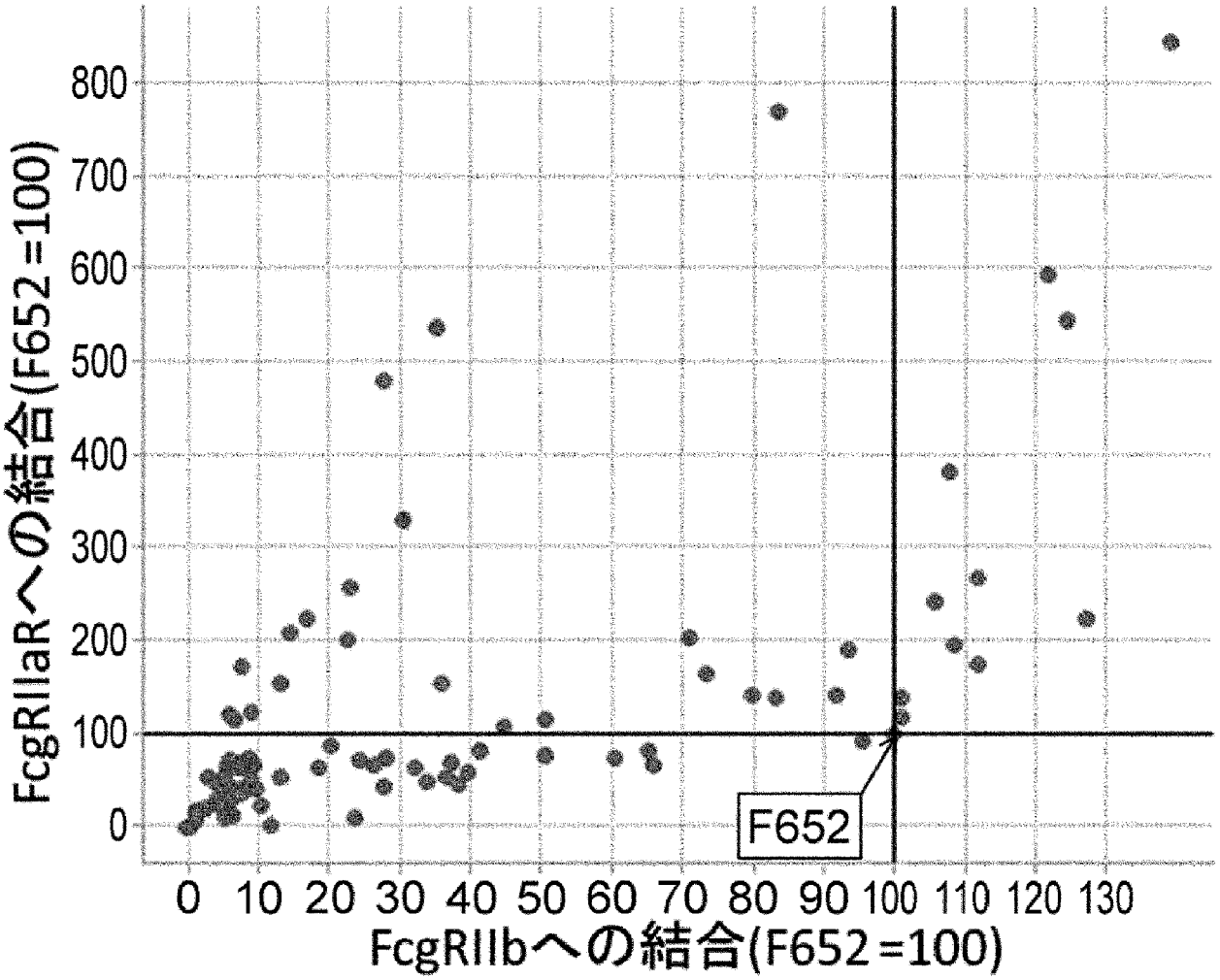
[図22]


Count
0
0
10^2
10^3
10^4
10^5
Comp-PE-A:: CD62p

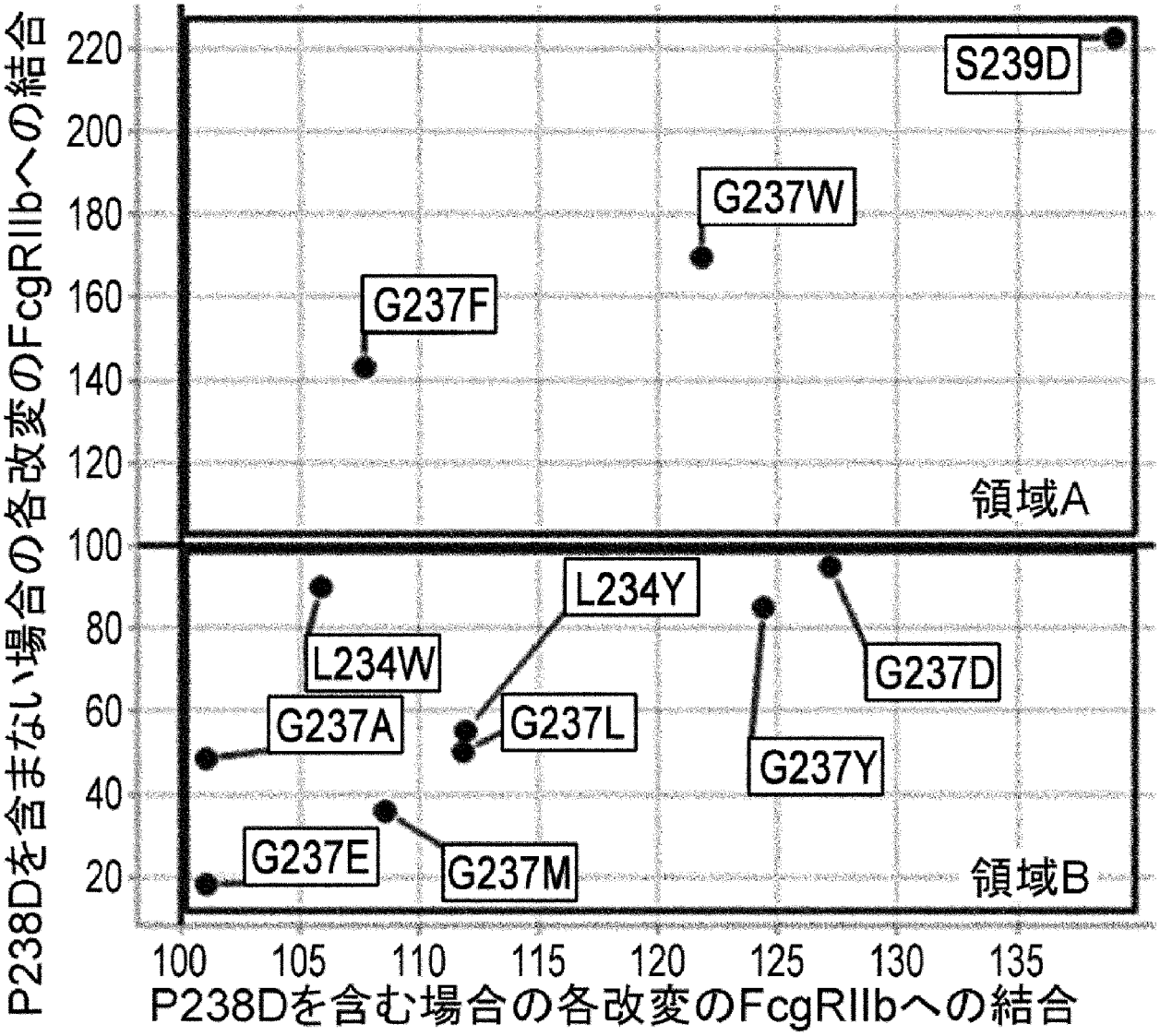
[図23]


Count
0
0
10^2
10^3
10^4
10^5
Comp-FITC-A:: PAC-1

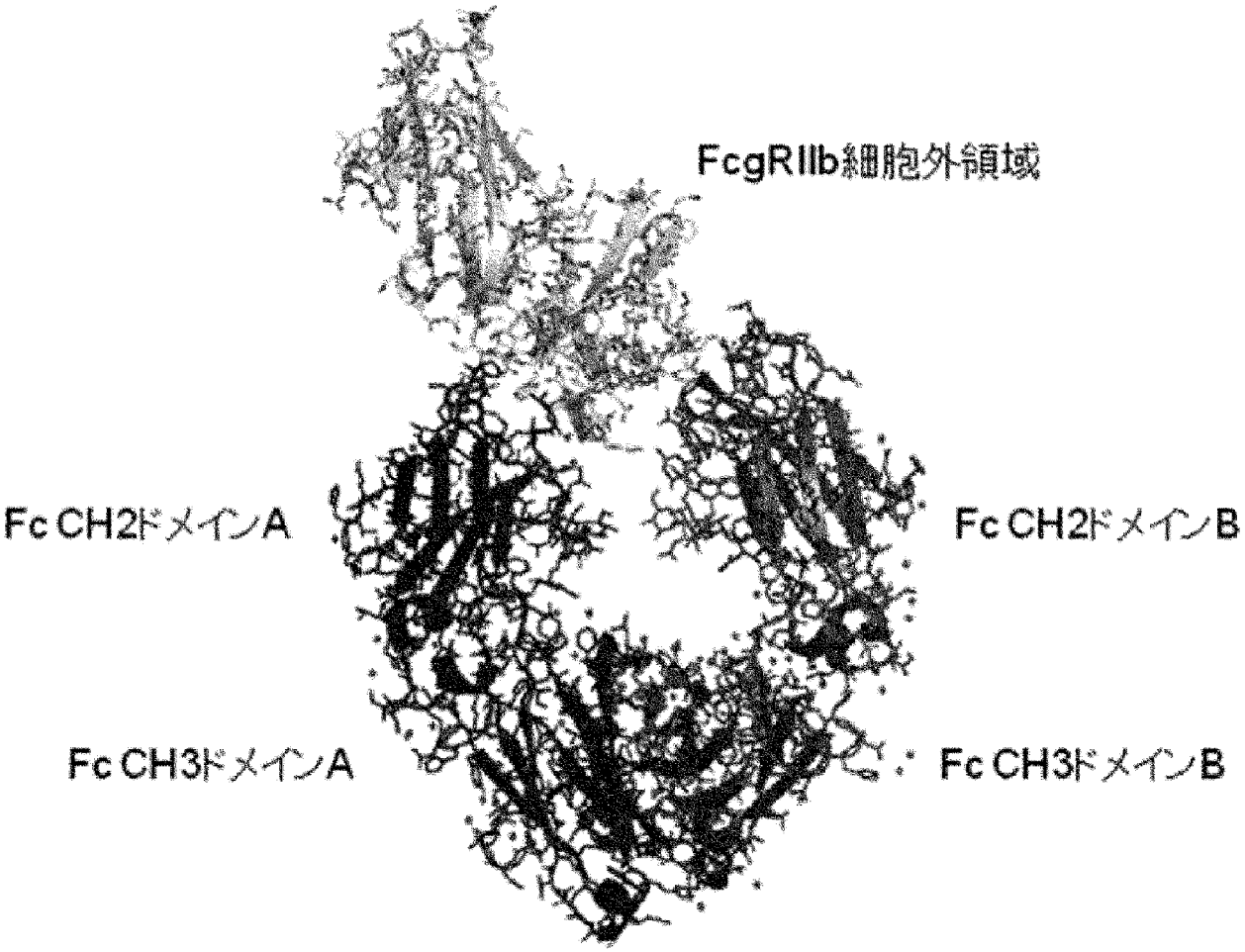
[図24]


FcgRIIaRへの結合(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
FcgRIIbへの結合(F652=100)

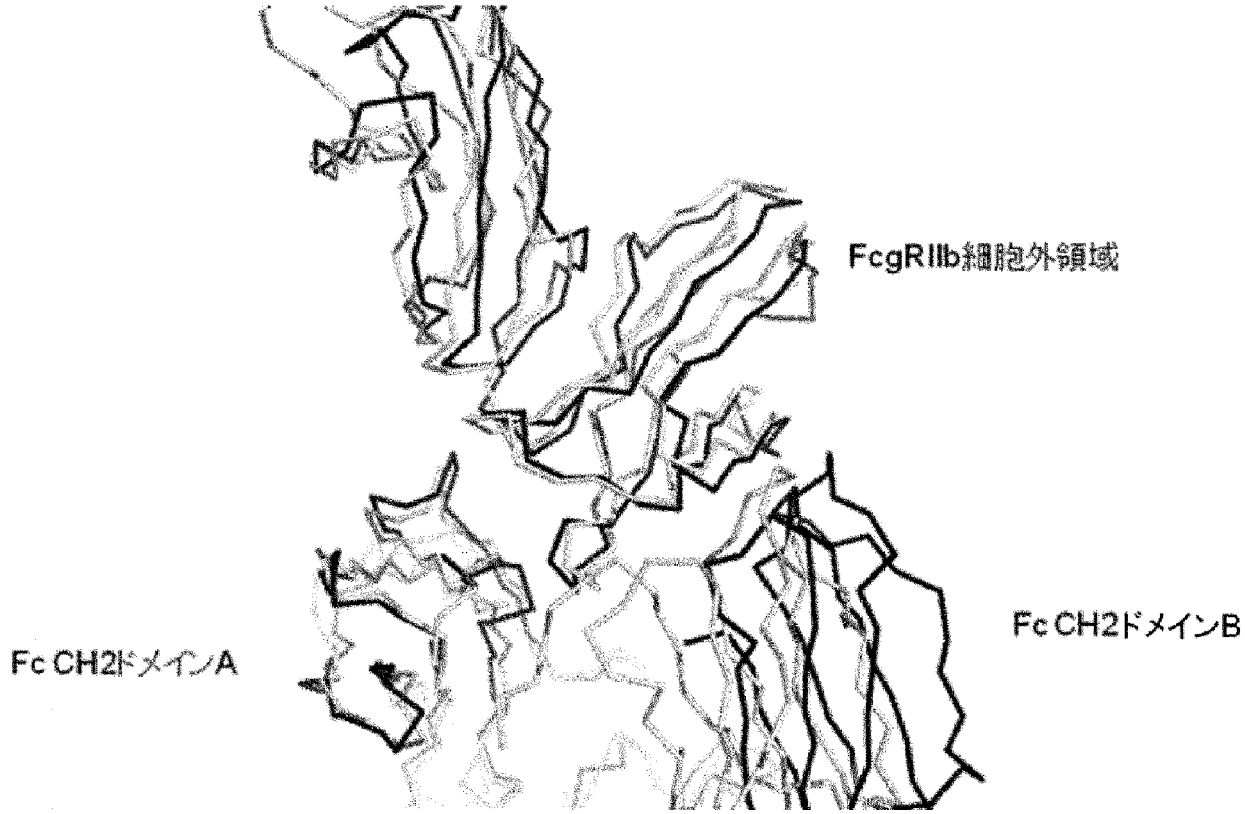
[図25]


S239D
G237W
G237F
領域A
L234Y
L234W
G237D
G237A
G237L
G237Y
G237E
G237M
領域B
220
200
180
160
140
120
100
80
60
40
20
100
105
110
115
120
125
130
135
P238Dを含まない場合の各改変のFcgRIIbへの結合
P238Dを含む場合の各改変のFcgRIIbへの結合

[図26]

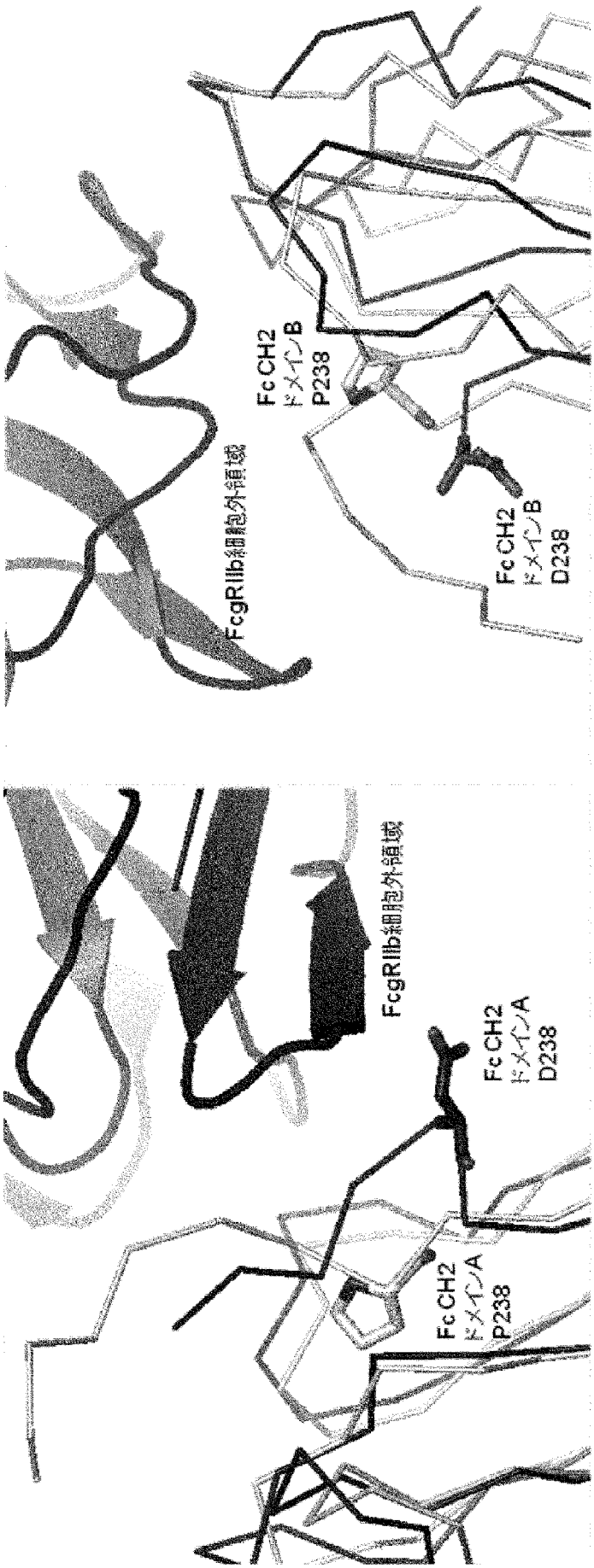


[図27]



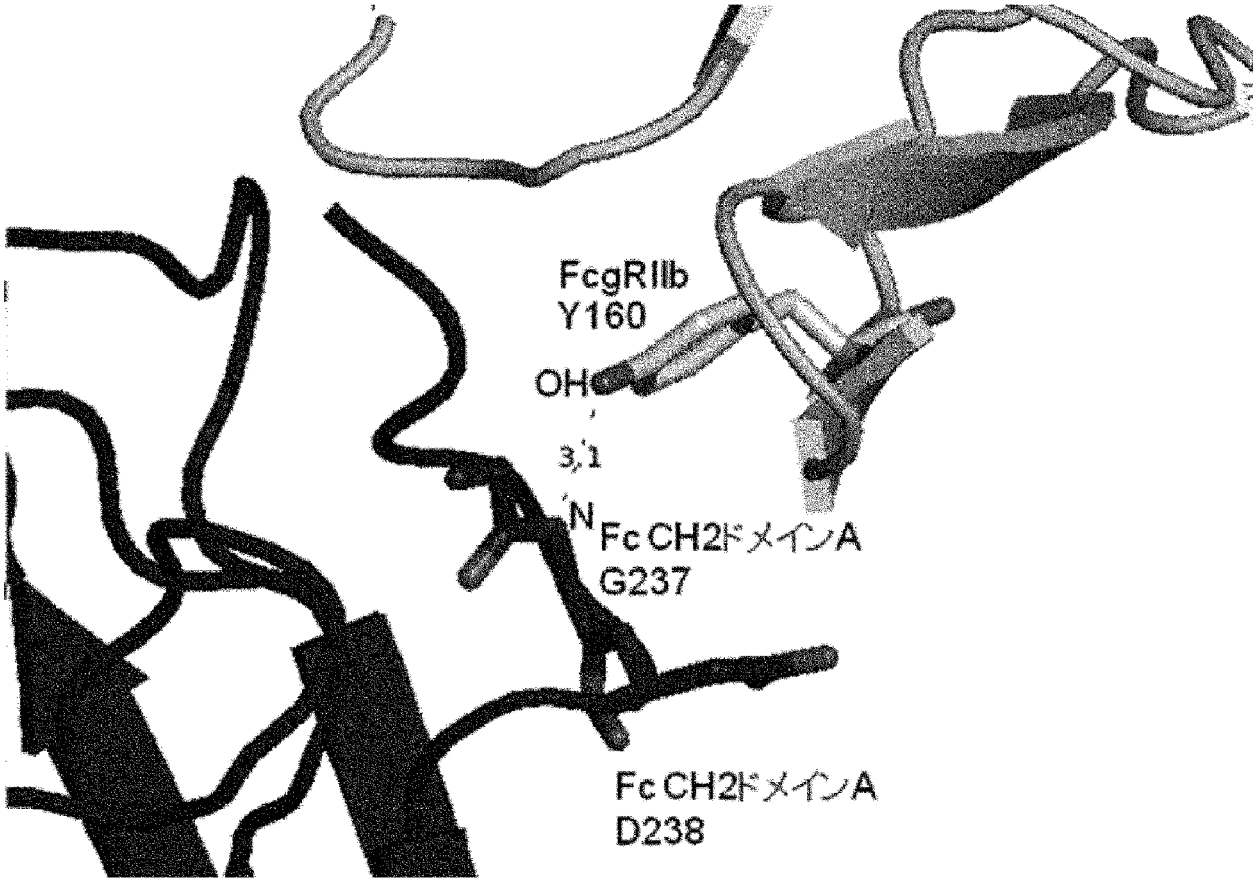
黒　Fc(P238D) / Fcγ RIIb細胞外領域複合体の 結晶構造
灰色　Fc(WT) / Fcγ RIIb 細胞外領域複合体のモデル構造

[図28]

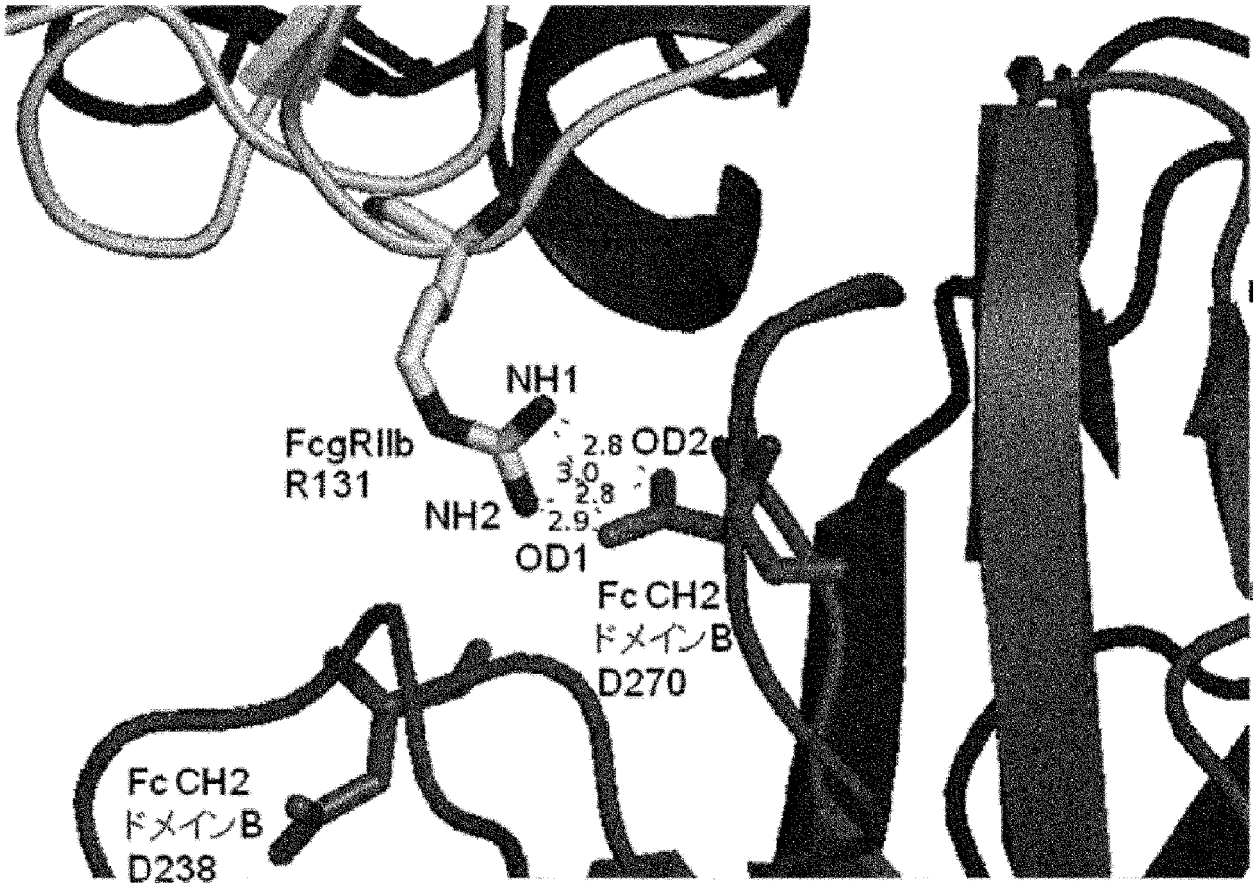


黒　Fc(P238D) / Fc γ RIIb細胞外領域複合体の結晶構造
灰色　Fc(WT) / Fc γ RIIb 細胞外領域複合体のモデル構造

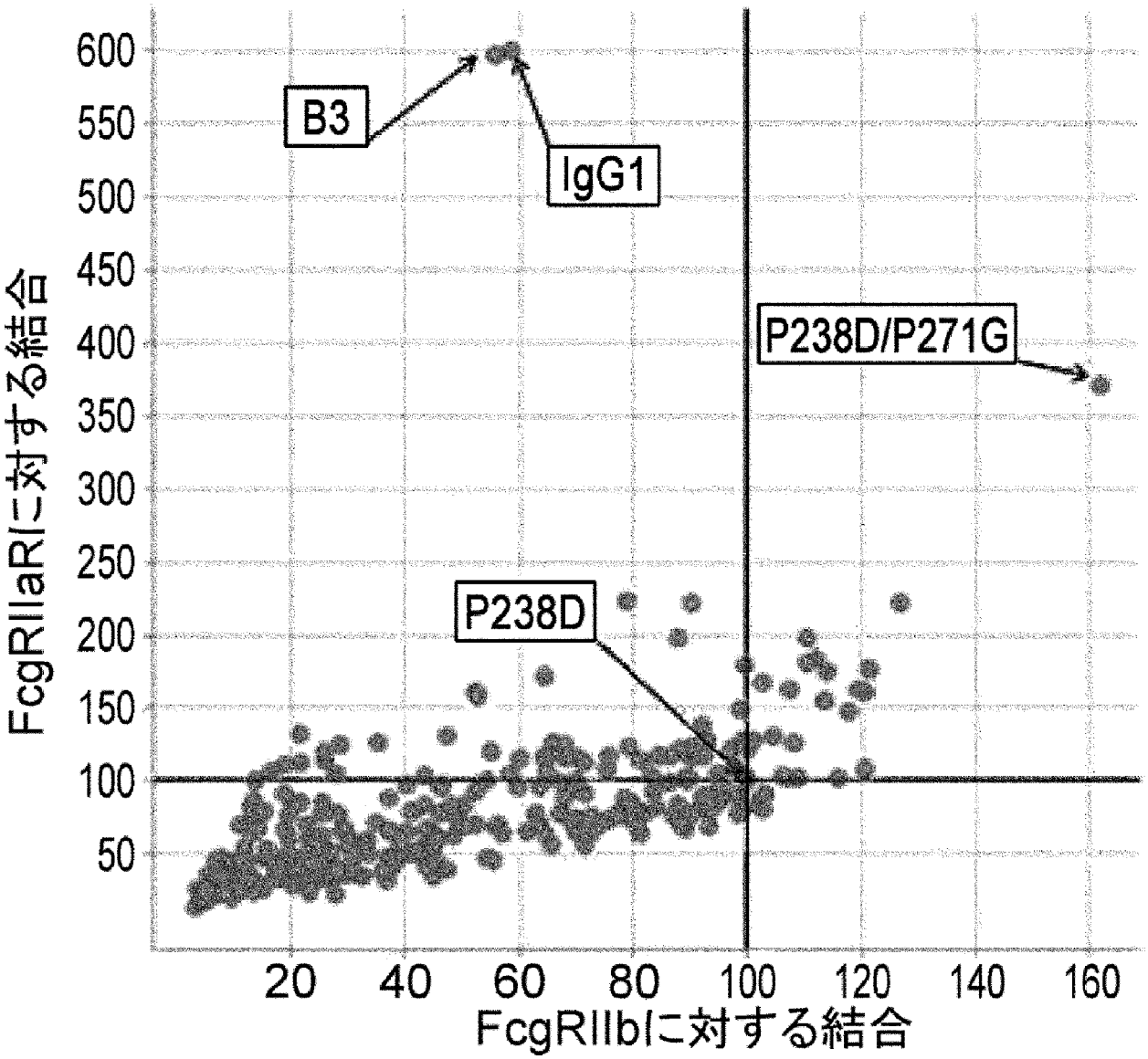
[図29]


FcgRIIb
Y160
OH
3.1
N
Fc CH2ドメインA
G237
Fc CH2ドメインA
D238

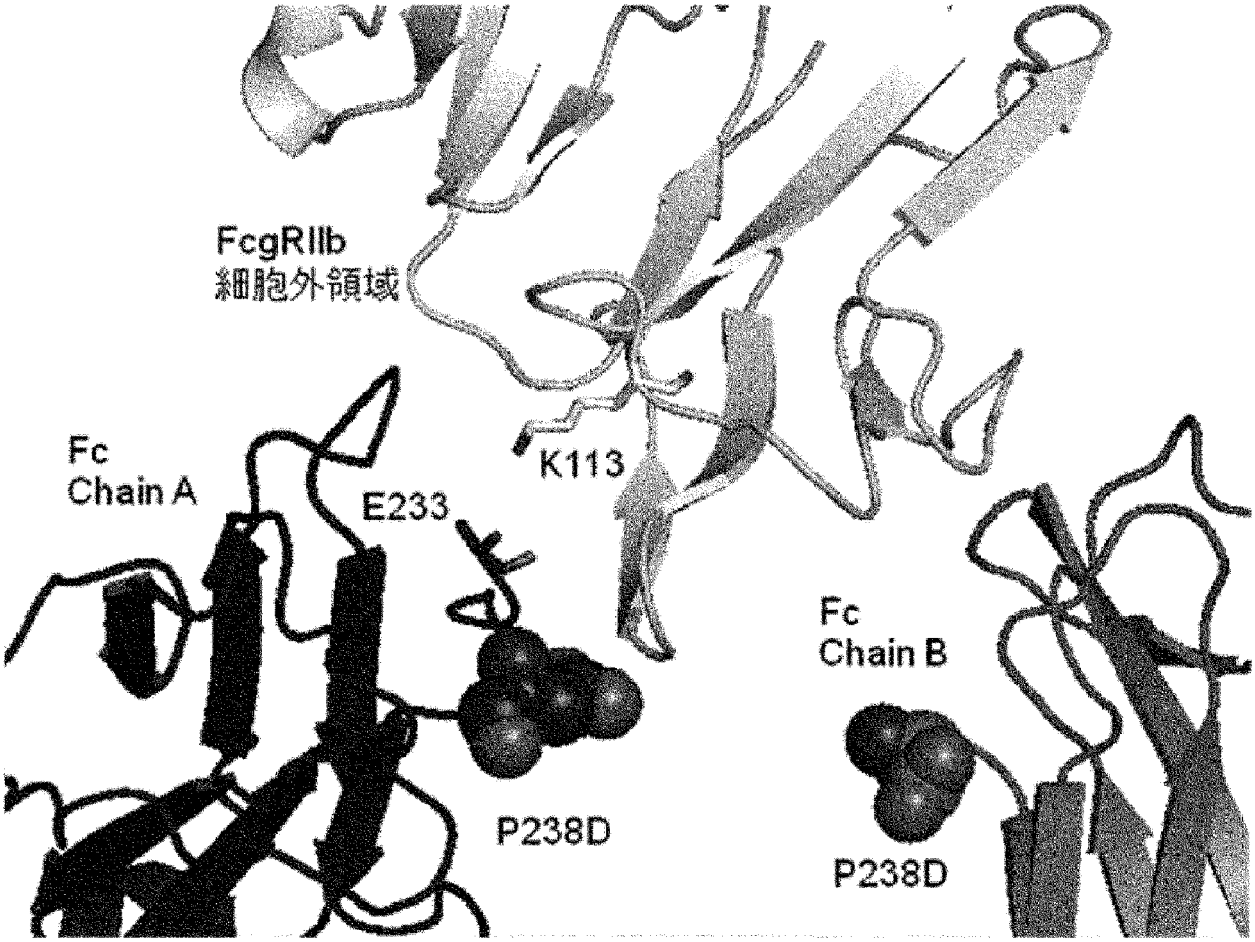
[図30]


NH1
FcgRIIb
R131
2.8
OD2
3.0
2.8
NH2
2.9
OD1
Fc CH2
ドメインB
D270
Fc CH2
ドメインB
D238

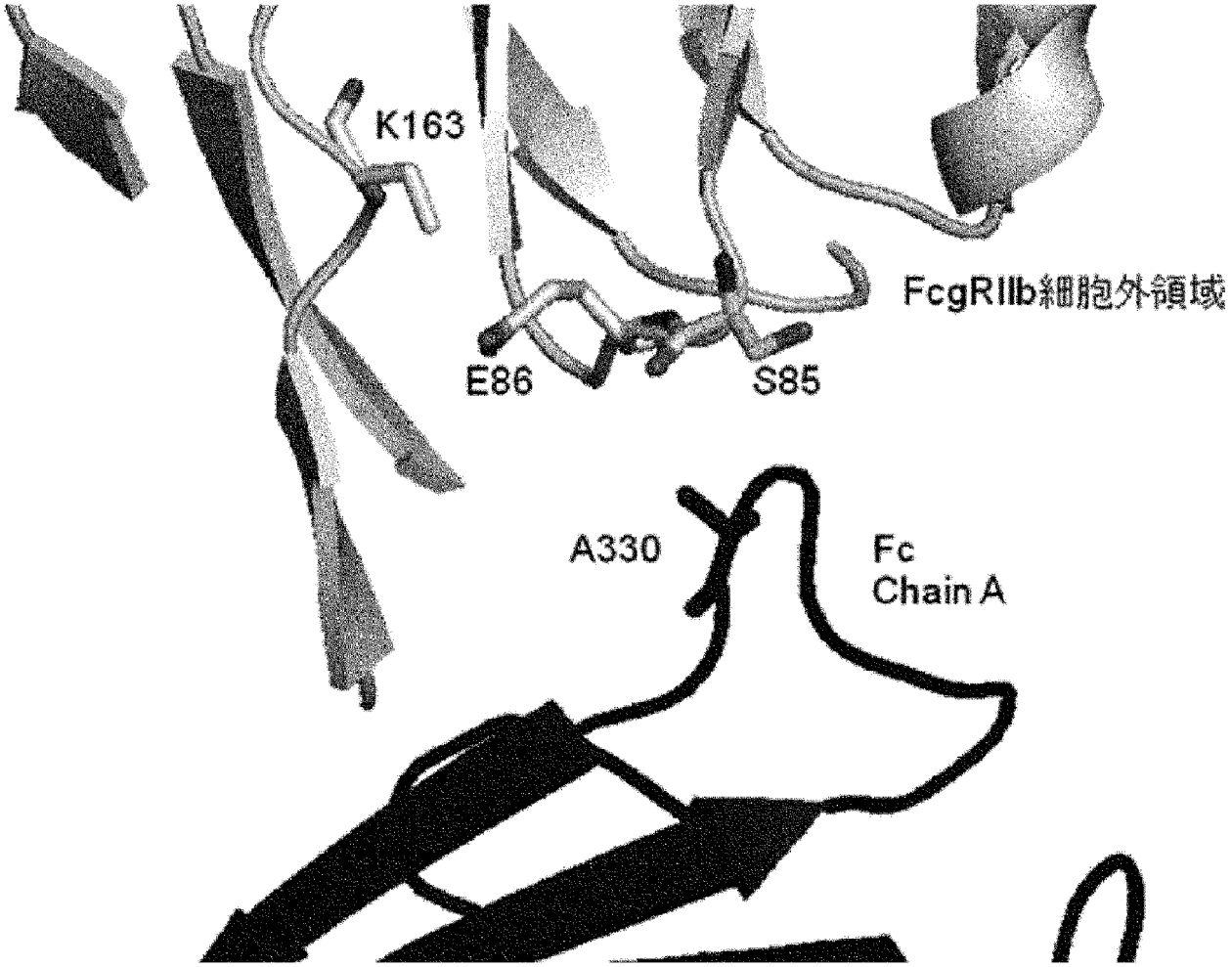
[図31]


600
550
500
450
400
350
300
250
200
150
100
50
FcgRIIaRに対する結合
B3
IgG1
P238D/P271G
P238D
20
40
60
80
100
120
140
160
FcgRIIbに対する結合

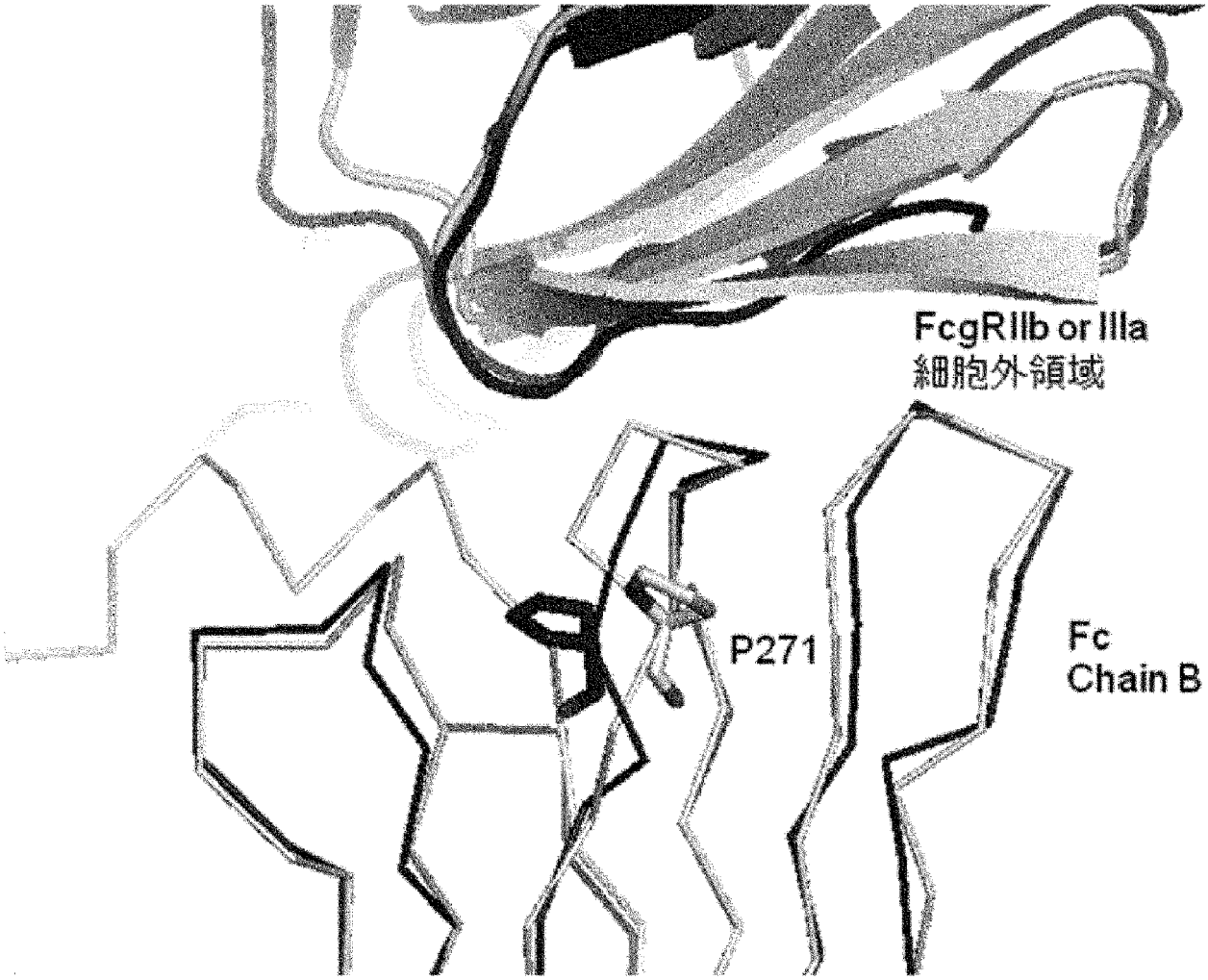
[図32]



[図33]

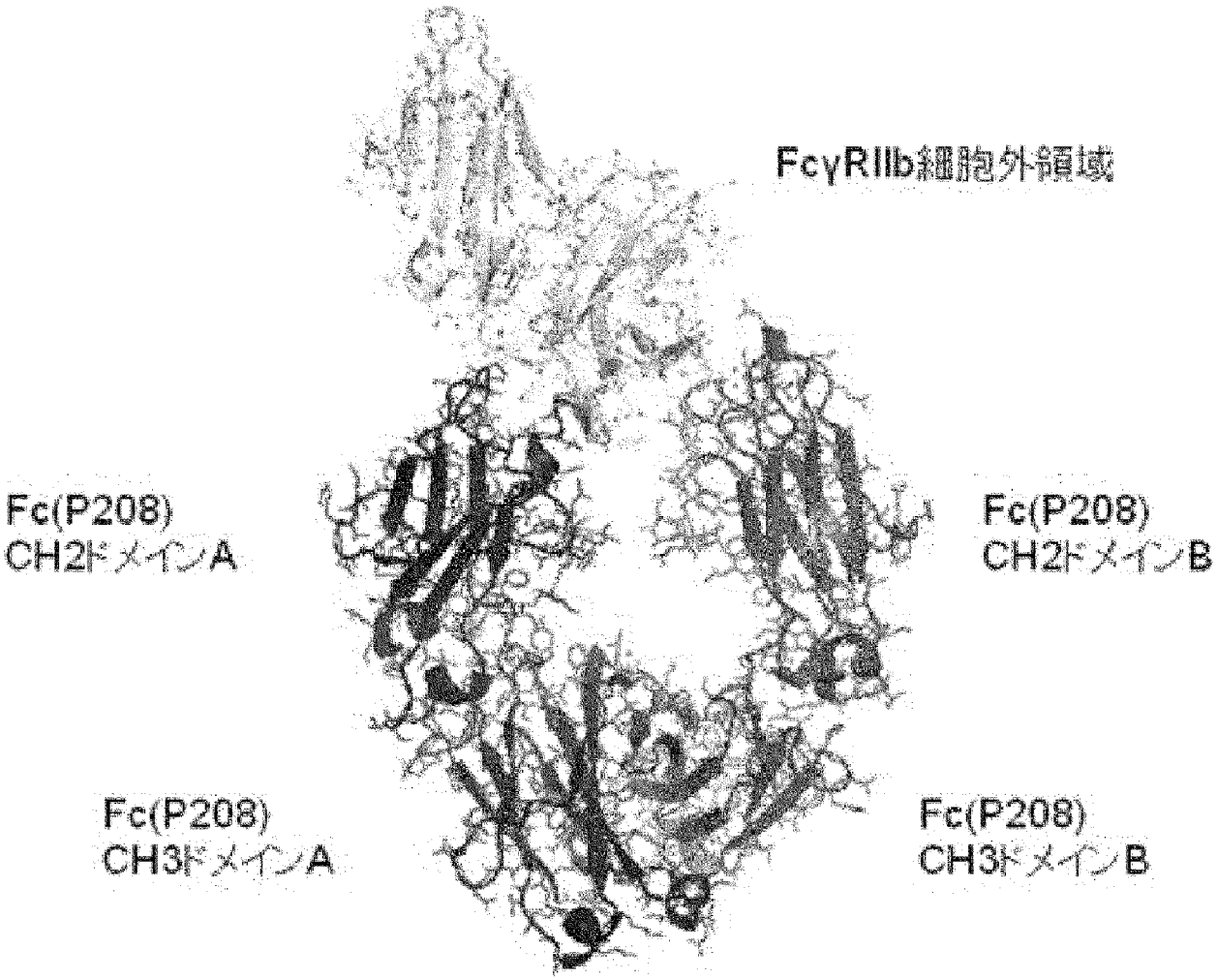


[図34]

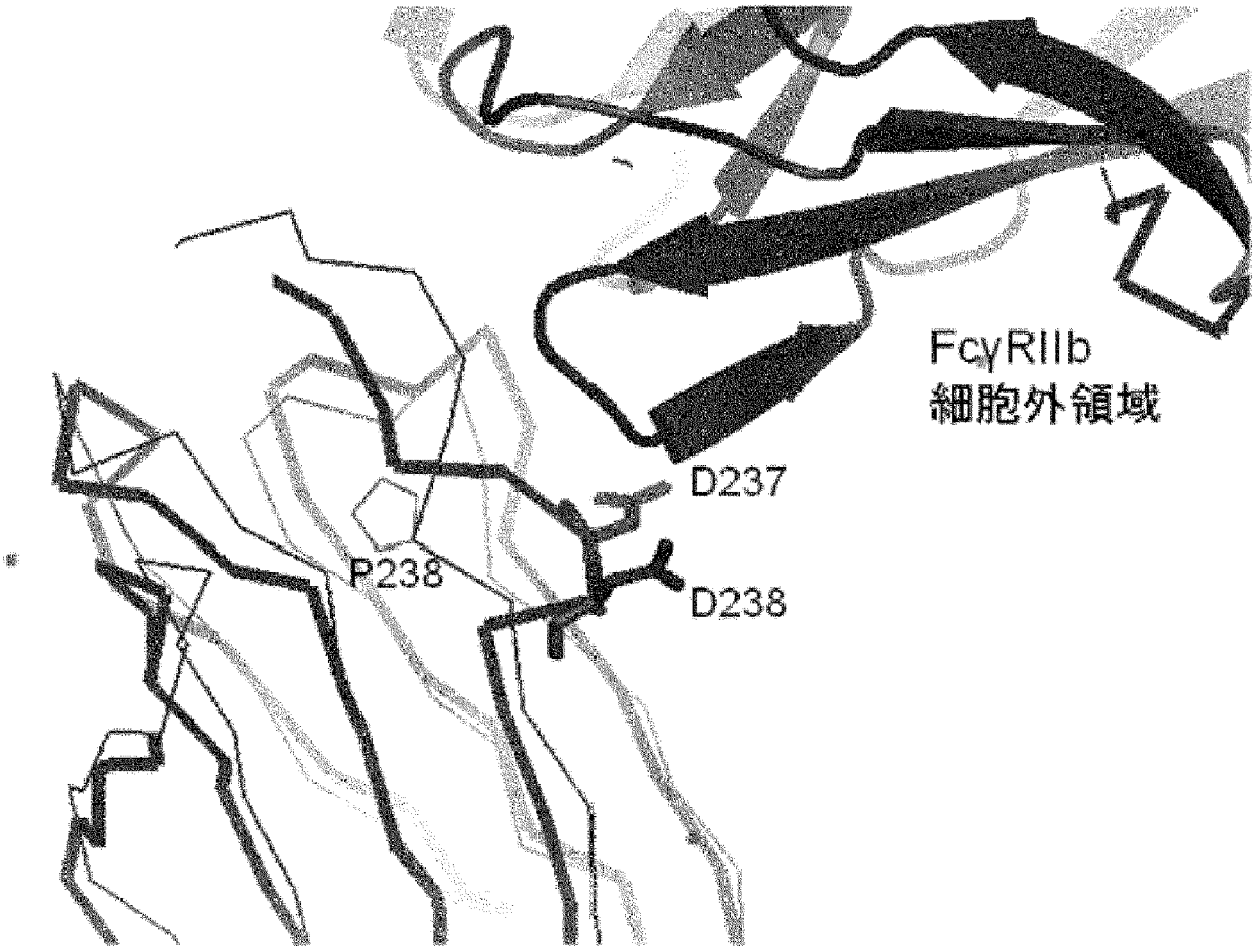


黒　Fc(P238D) / Fcγ RIIb細胞外領域複合体の結晶構造
灰色　Fc(WT) / Fcγ RIIIa細胞外領域複合体の結晶構造(PDB code:3SGJ)

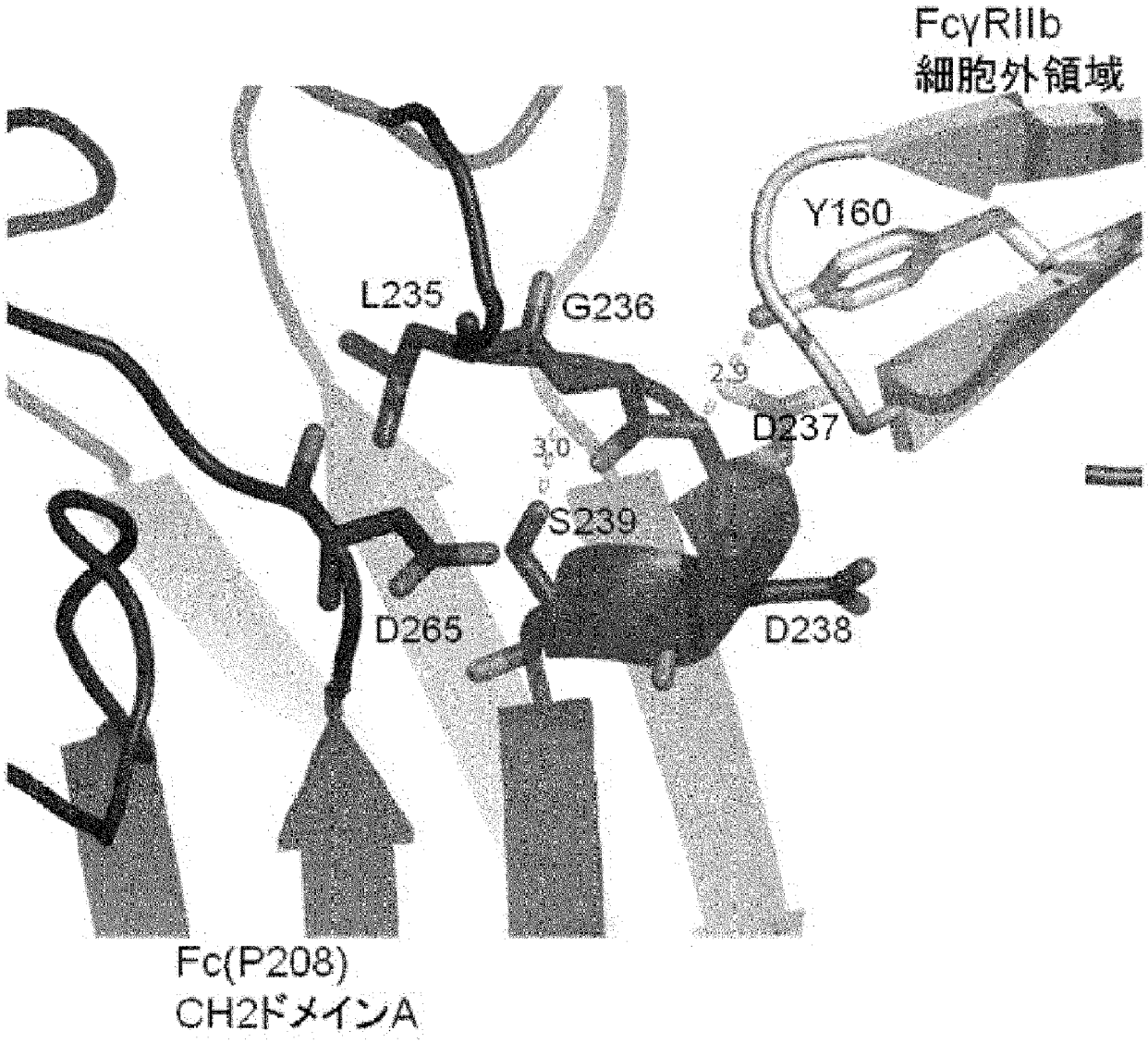
[図35]


FcγRIIb細胞外領域
Fc(P208)
CH2ドメインA
Fc(P208)
CH2ドメインB
Fc(P208)
CH3ドメインA
Fc(P208)
CH3ドメインB

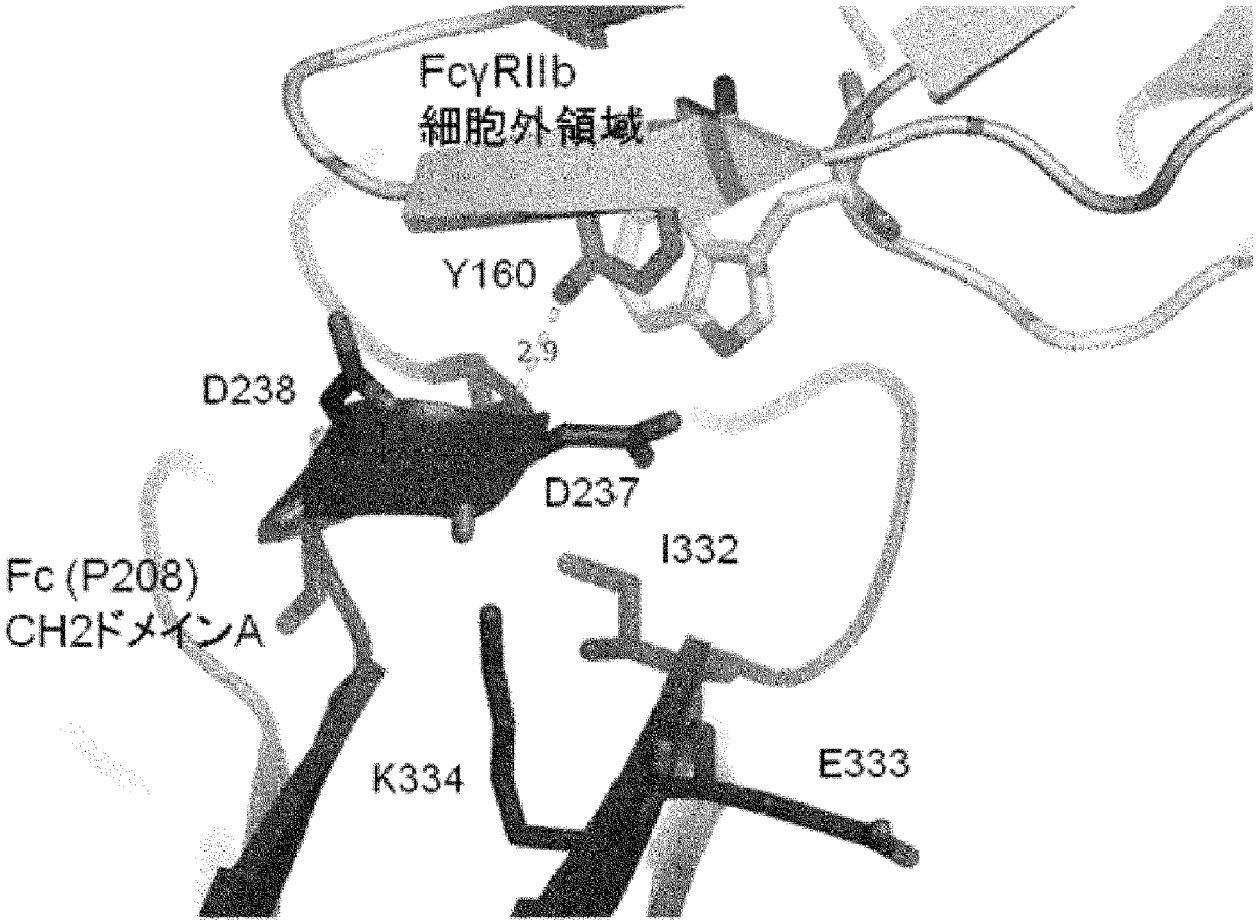
[図36]


FcγRIIb
細胞外領域
D237
P238
D238
細線： Fc(Wt) CH2ドメインA
太線： Fc(P208) CH2ドメインA

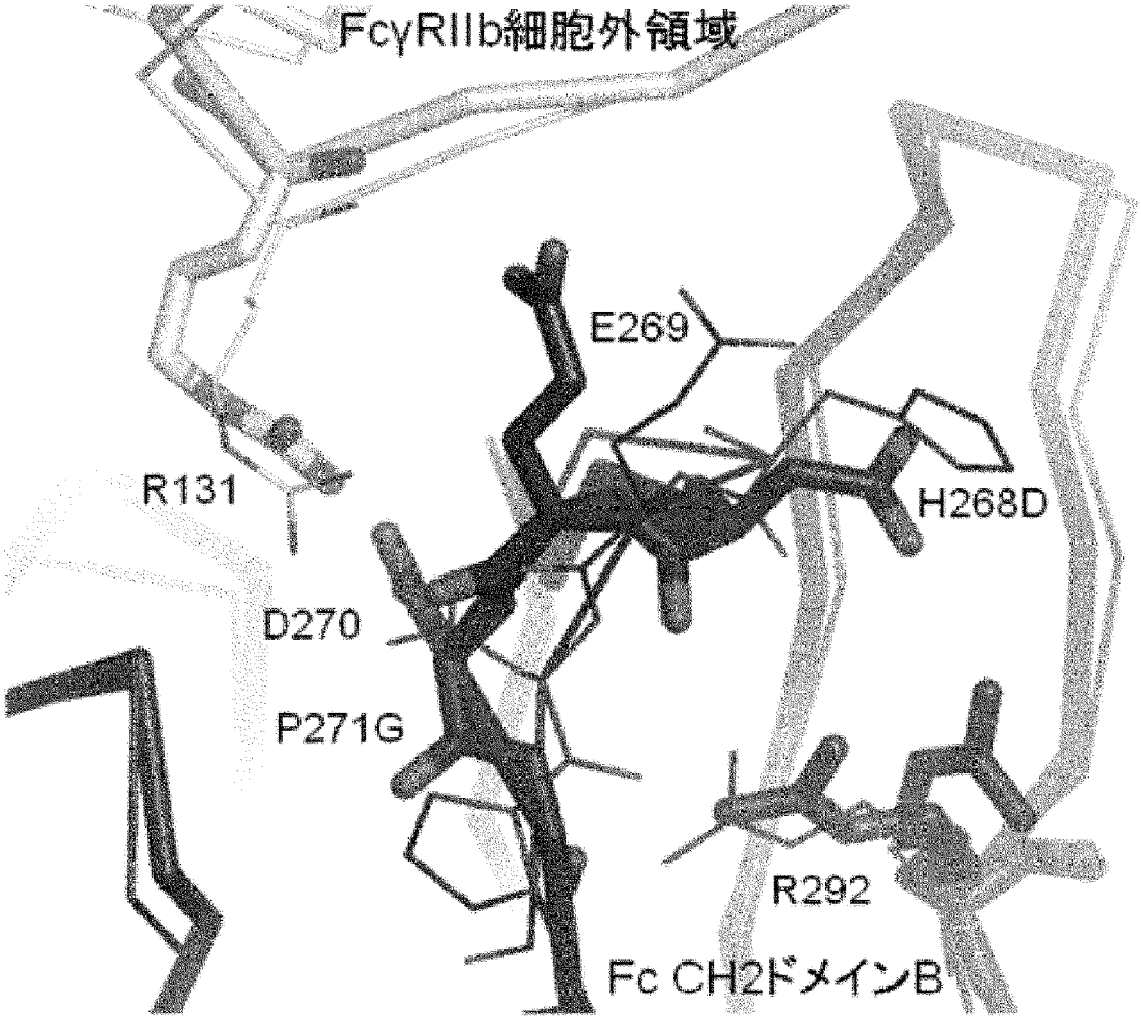
[図37]


FcγRIIb
細胞外領域
Y160
L235
G236
2.9
D237
3.0
S239
D265
D238
Fc(P208)
CH2ドメインA

[図38]

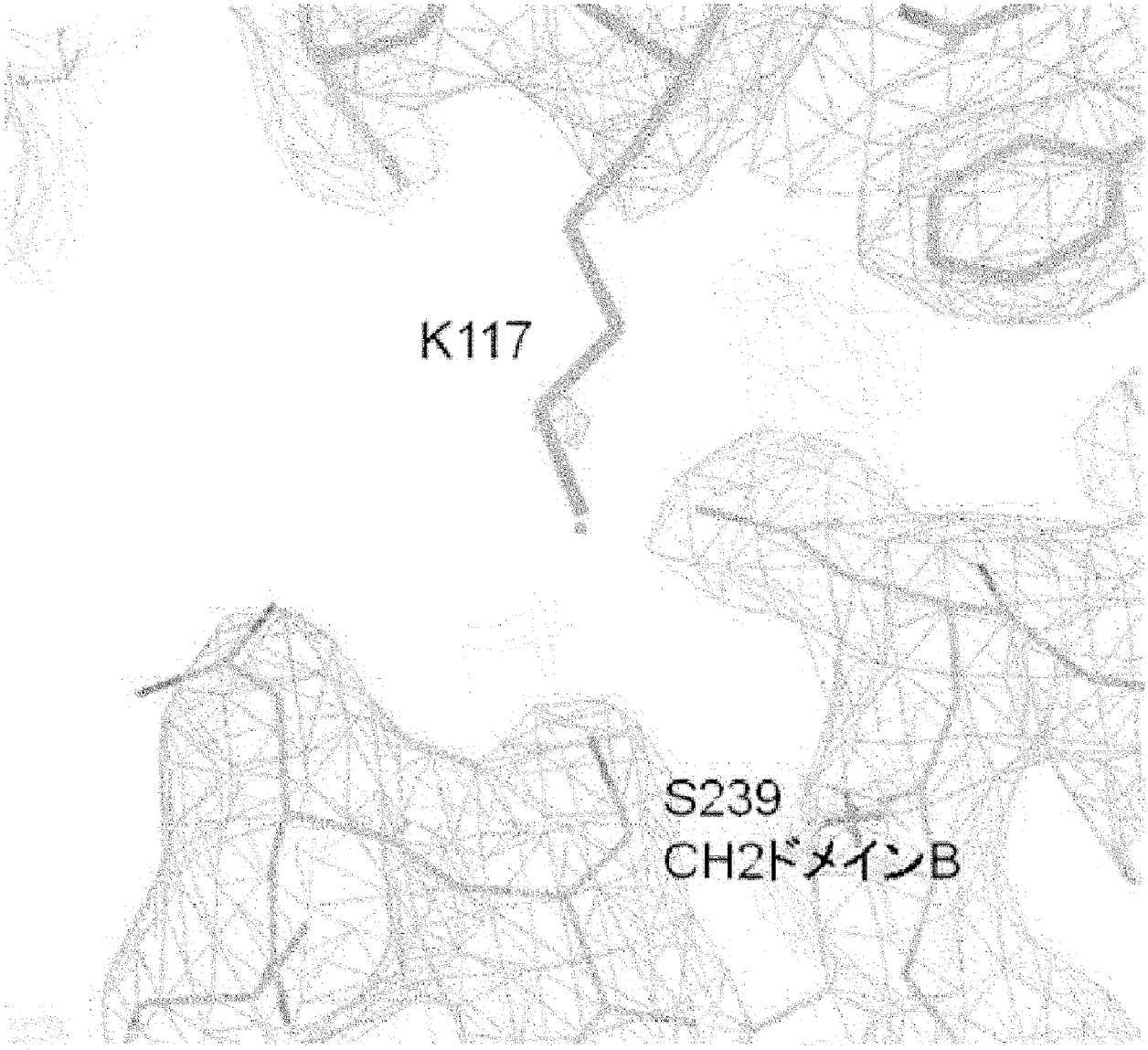

FcγRIIb
細胞外領域
Y160
2.9
D238
D237
I332
Fc (P208)
CH2ドメインA
K334
E333

[図39]



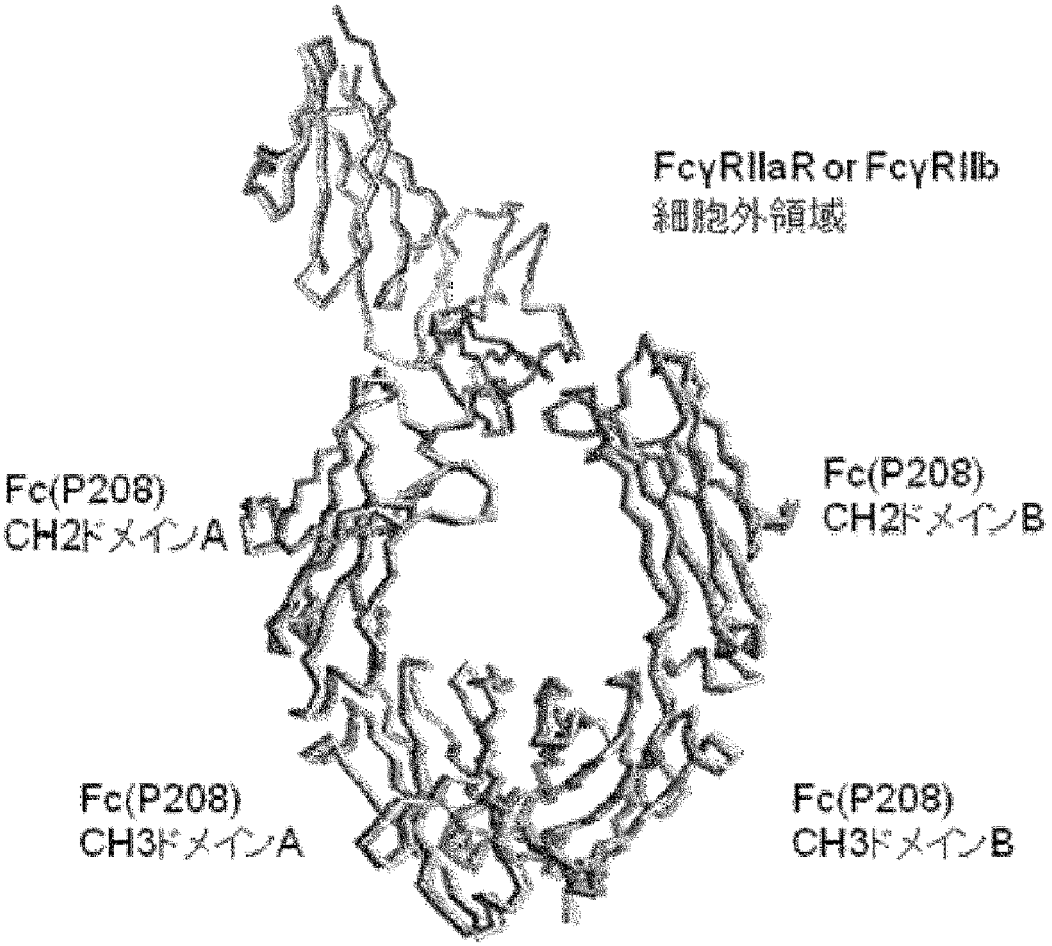
細線：Fc(P238D)/FcγRIIb 細胞外領域複合体X線結晶構造
太線：Fc(P208)/FcγRIIb 細胞外領域複合体X線結晶構造

[図40]



細線：Fc(P208)、太線：FcγRIIb細胞外領域

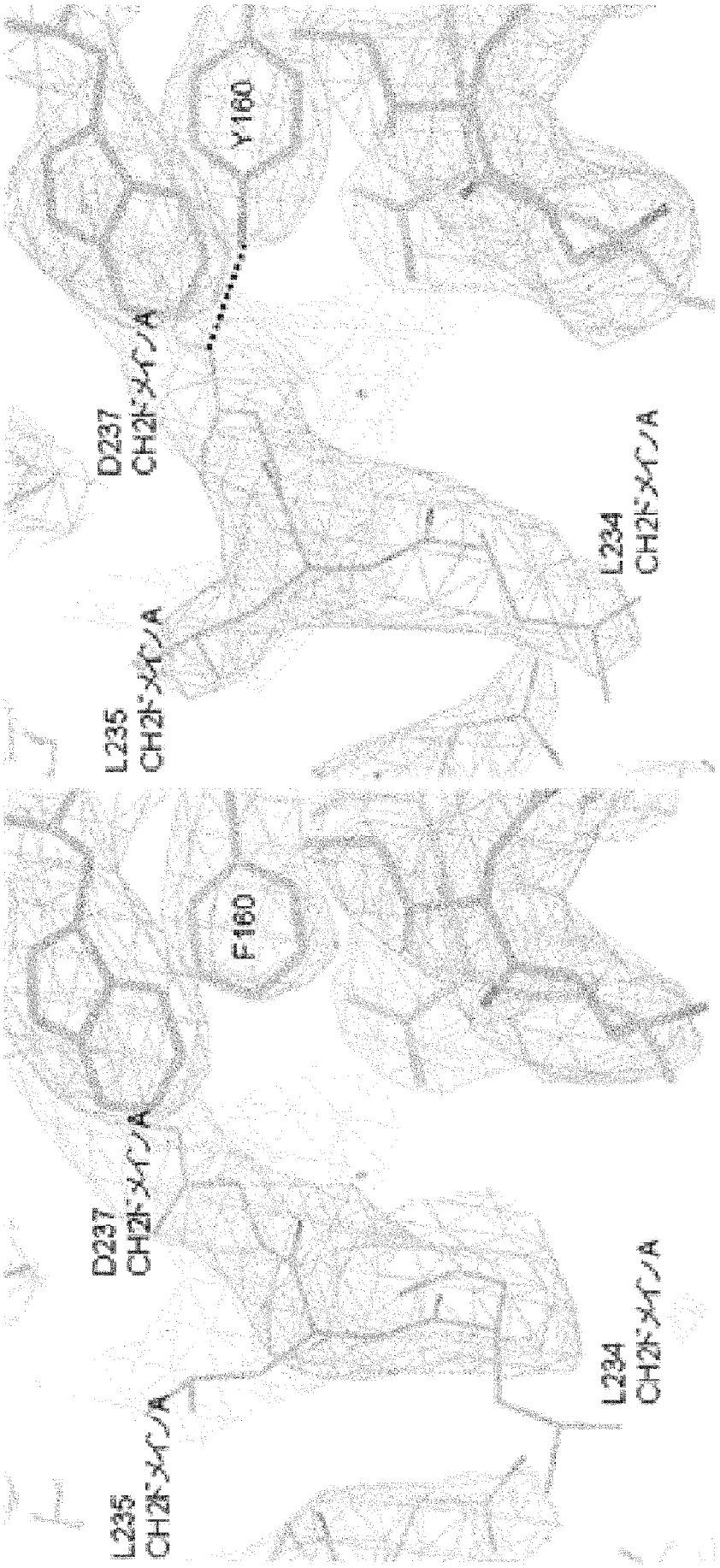
[図41]



黒色： Fc(P208)/FcγRIIaR 細胞外領域複合体X線結晶構造

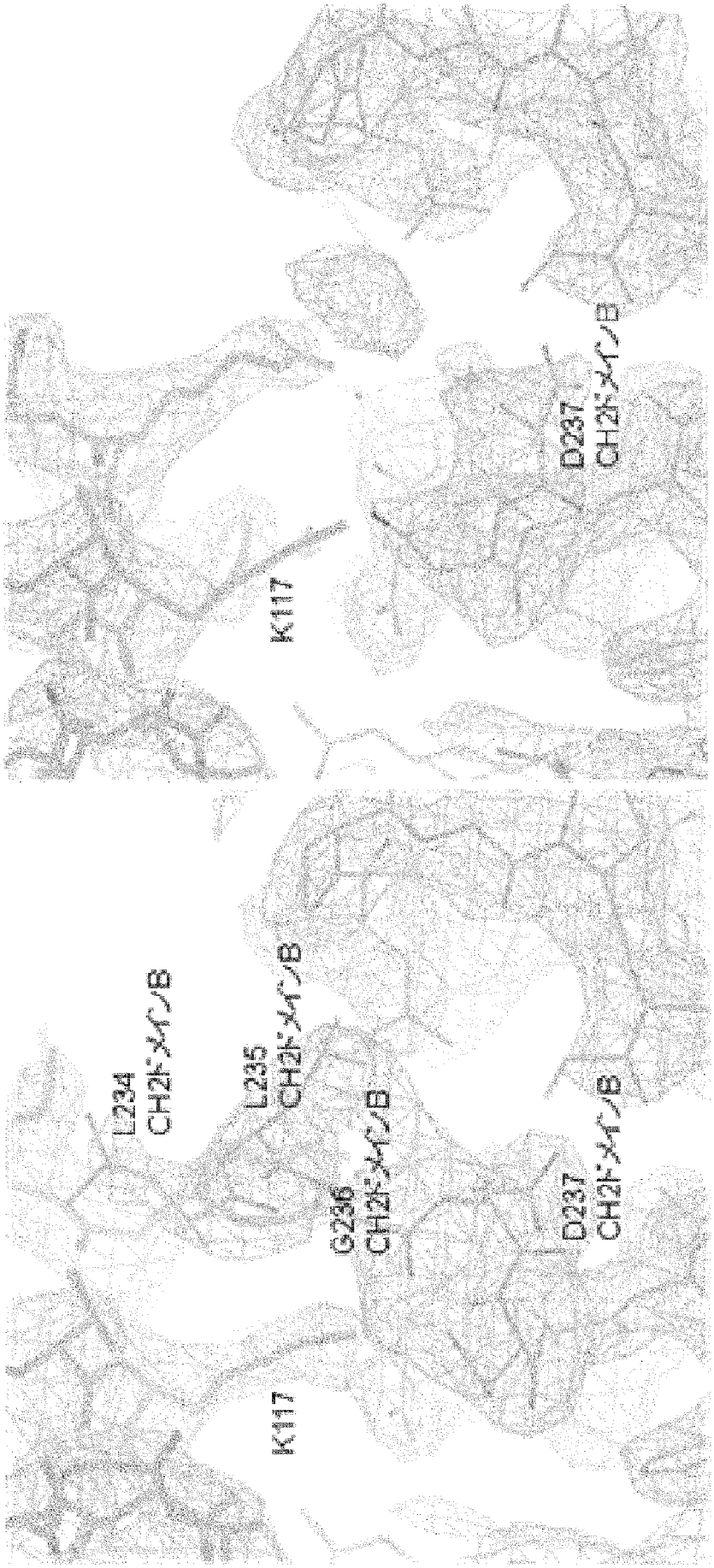
灰色： Fc(P208)/FcγRIIb 細胞外領域複合体X線結晶構造

[図42]



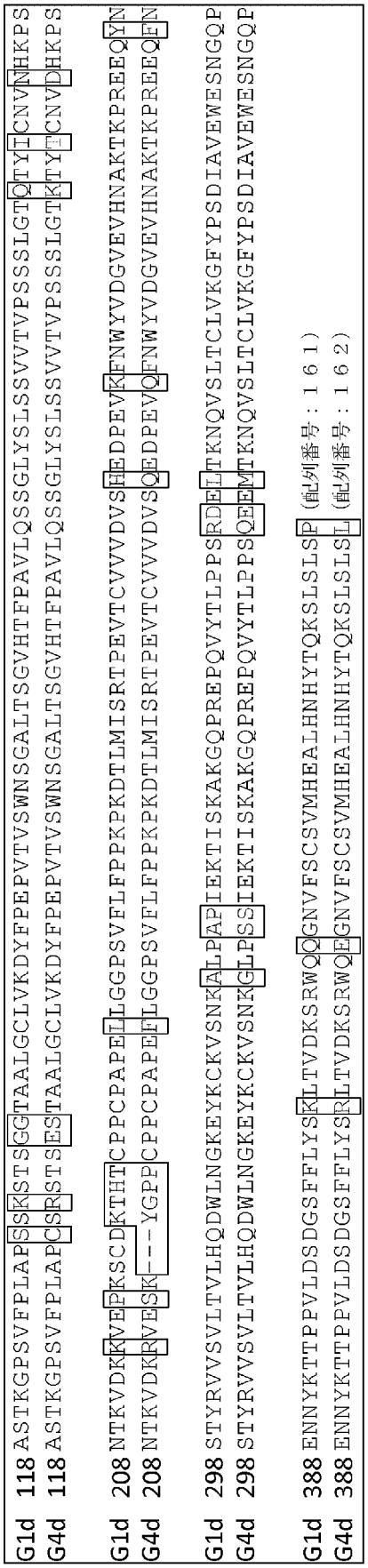
細線：Fc(P208)、太線：FcγRIIaR or FcγRIIb細胞外領域
左：Fc(P208)/FcγRIIaR 細胞外領域複合体X線結晶構造
右：Fc(P208)/FcγRIIb 細胞外領域複合体X線結晶構造

[図43]

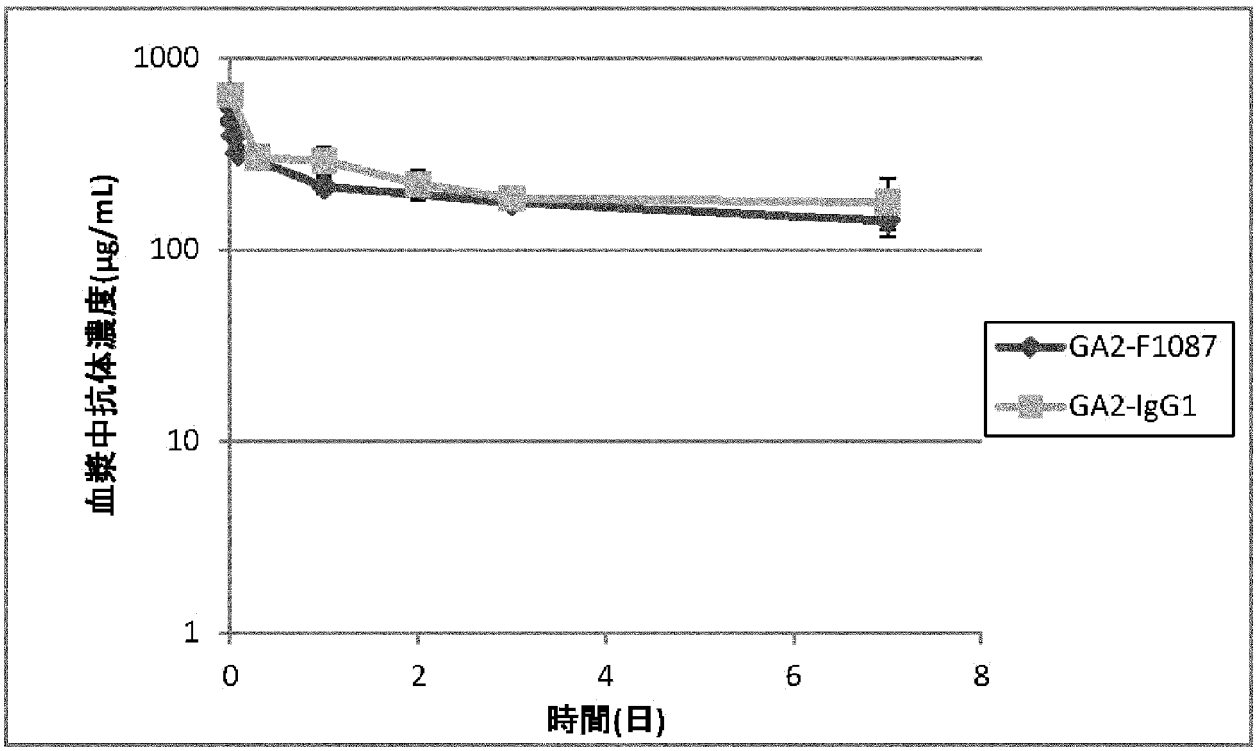


細線：Fc(P208)、太線：FcγRIIaR or FcγRIIb細胞外領域
左： Fc(P208)/FcγRIIaR 細胞外領域複合体X線結晶構造
右： Fc(P208)/FcγRIIb 細胞外領域複合体X線結晶構造

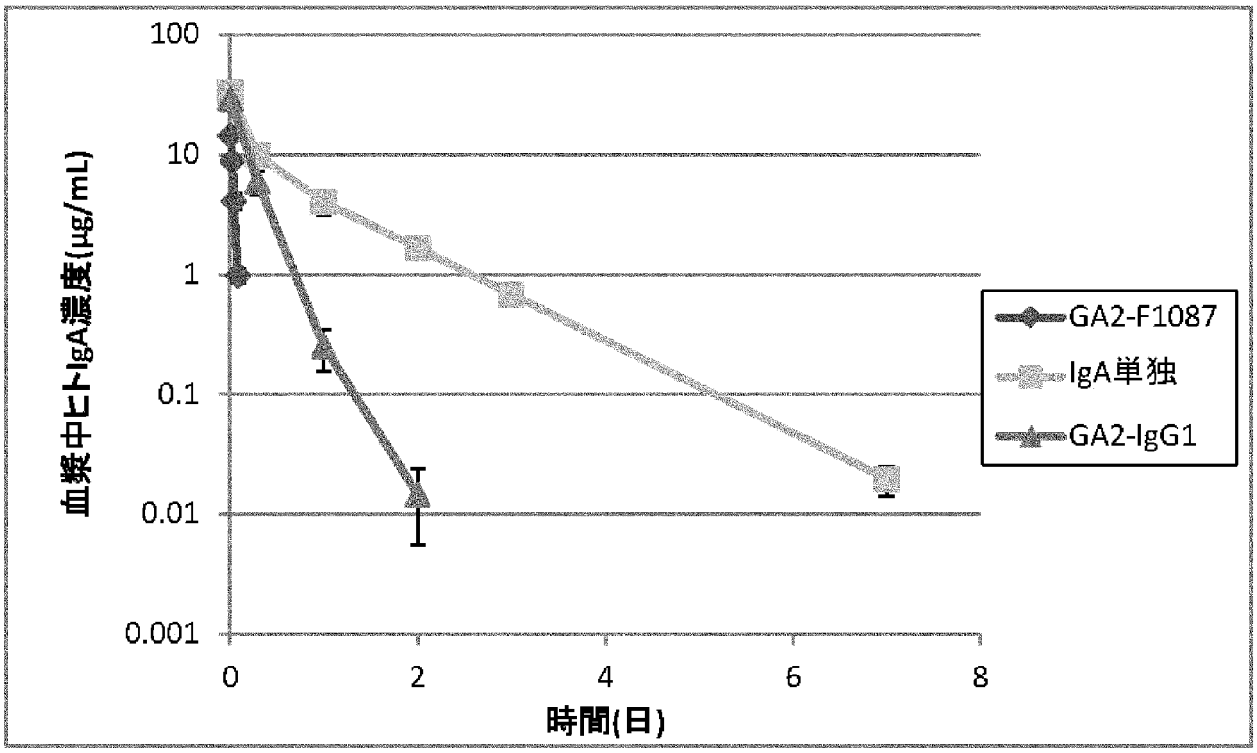
[図44]


G1d 118 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
G4d 118 ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPS
G1d 208 NTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
G4d 208 NTKVDKRVESK---YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN
G1d 298 STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
G4d 298 STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
G1d 388 ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP (配列番号：161)
G4d 388 ENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSL (配列番号：162)

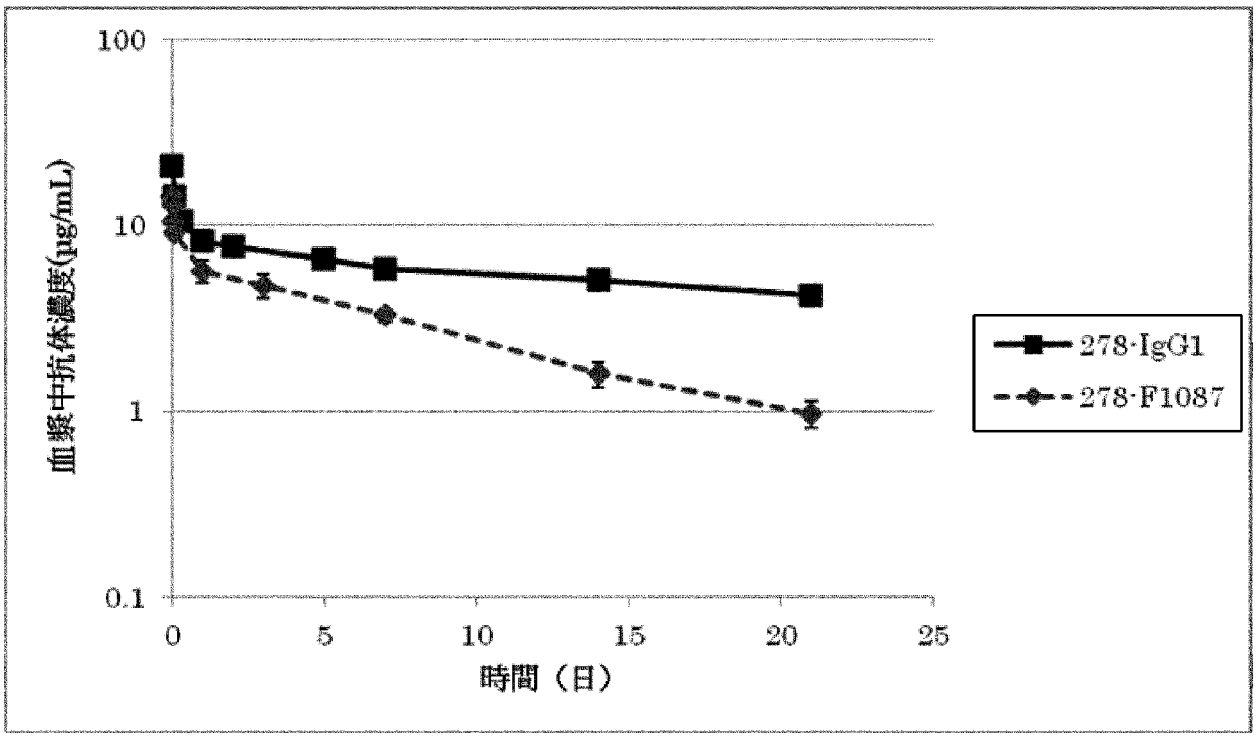
[図45]


1000
100
10
1
血漿中抗体濃度(μg/mL)
0
2
4
6
8
時間(日)
GA2-F1087
GA2-IgG1

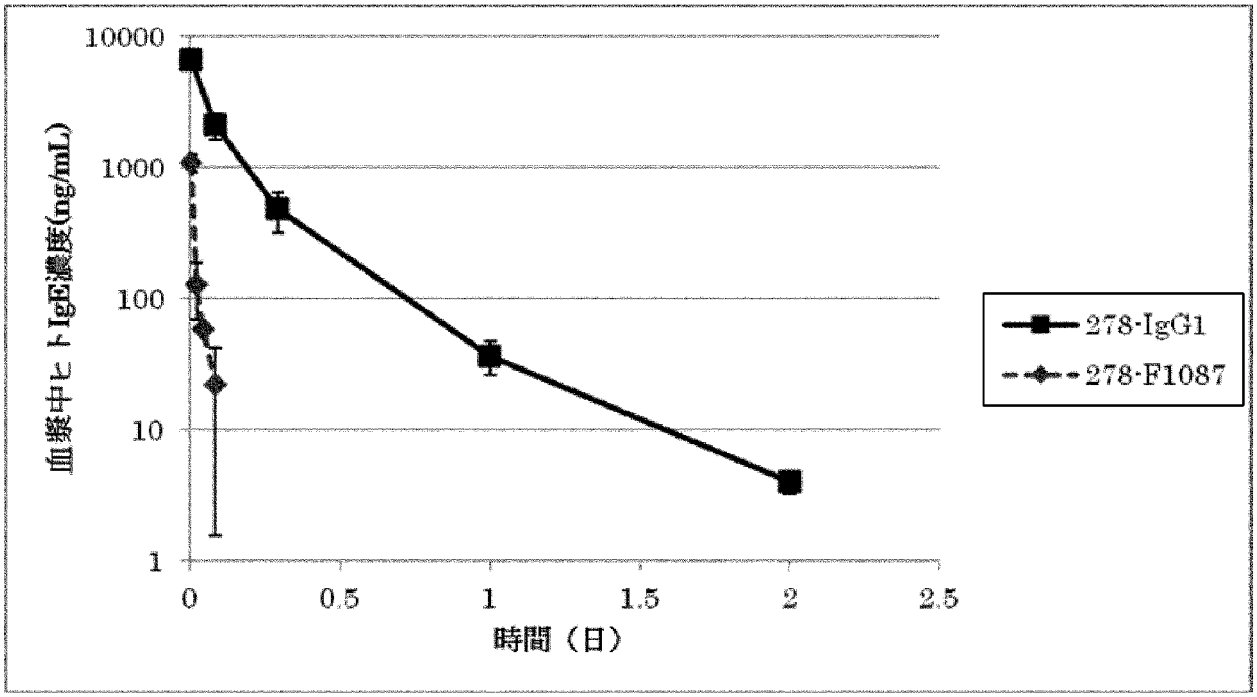
[図46]


100
10
1
0.1
0.01
0.001
血漿中ヒトIgA濃度(μg/mL)
0
2
4
6
8
時間(日)
GA2-F1087
IgA単独
GA2-IgG1

[図47]

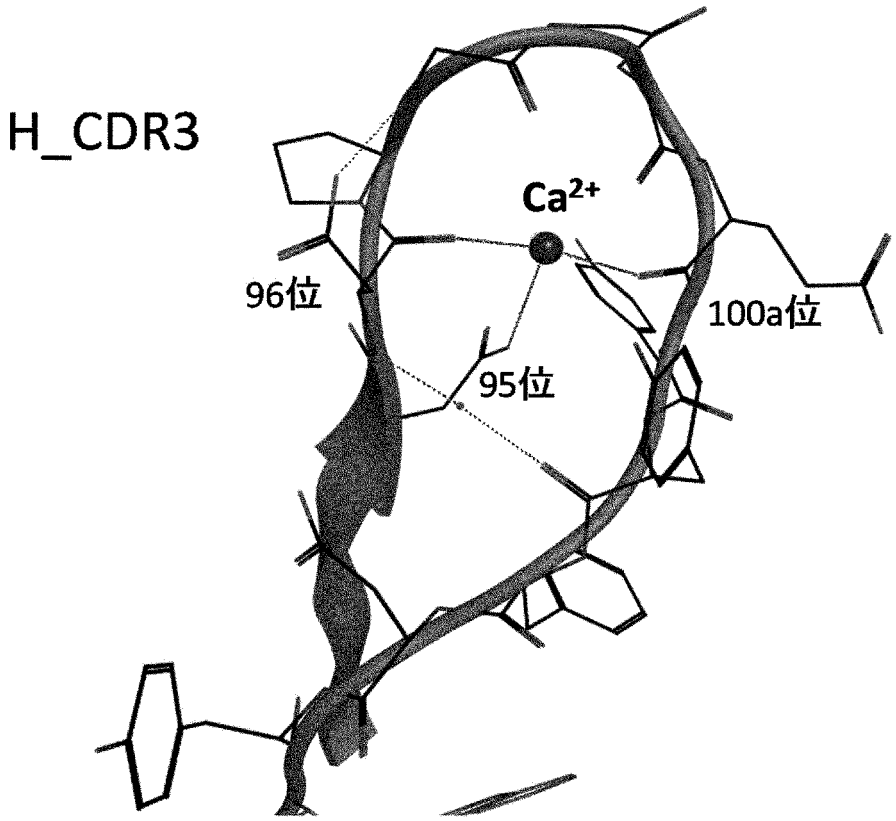

100
10
1
0.1
血漿中抗体濃度(μg/mL)
0
5
10
15
20
25
時間（日）
278-IgG1
278-F1087

[図48]

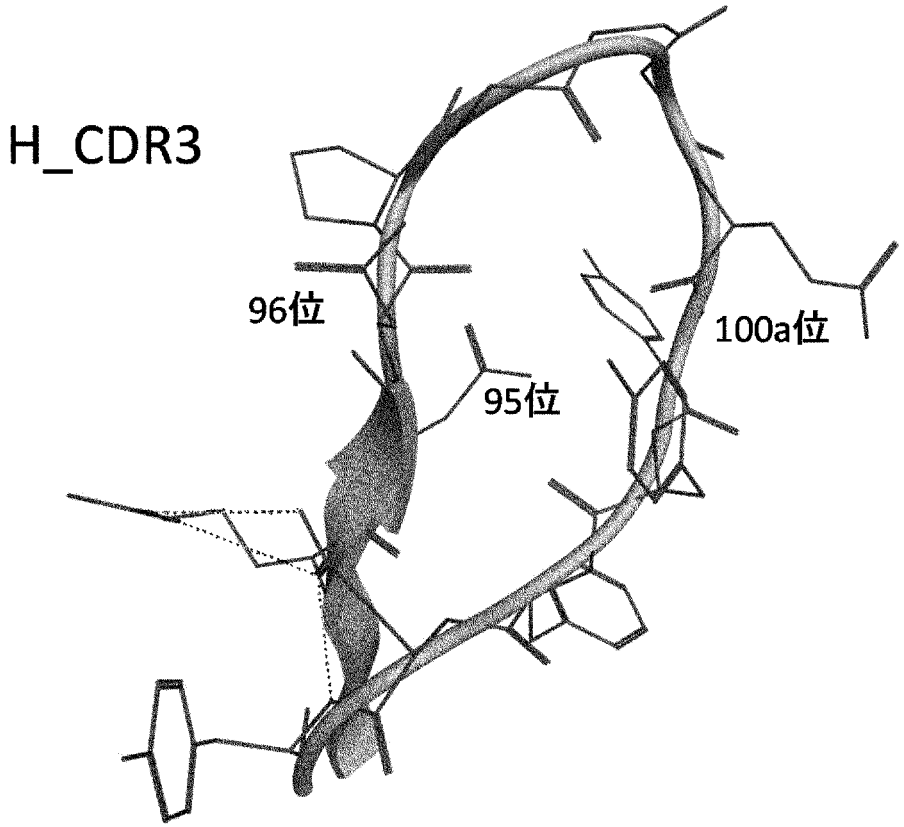

10000
1000
100
10
1
血漿中ヒトIgE濃度(ng/mL)
0
0.5
1
1.5
2
2.5
時間（日）
278-IgG1
278-F1087

[図49]

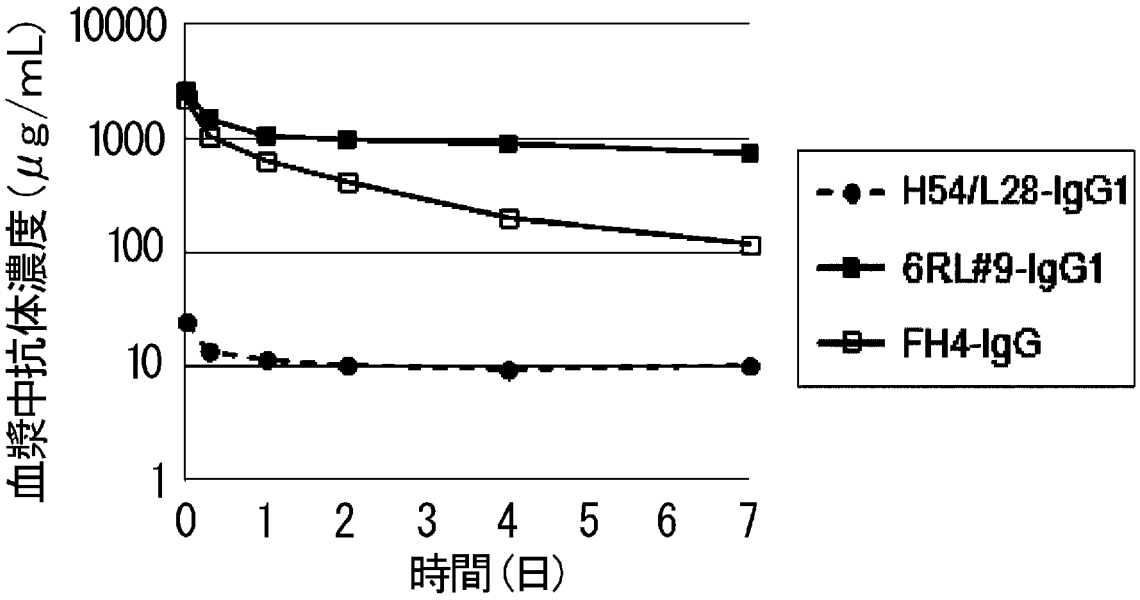
(i) カルシウムイオン存在下での結晶の構造



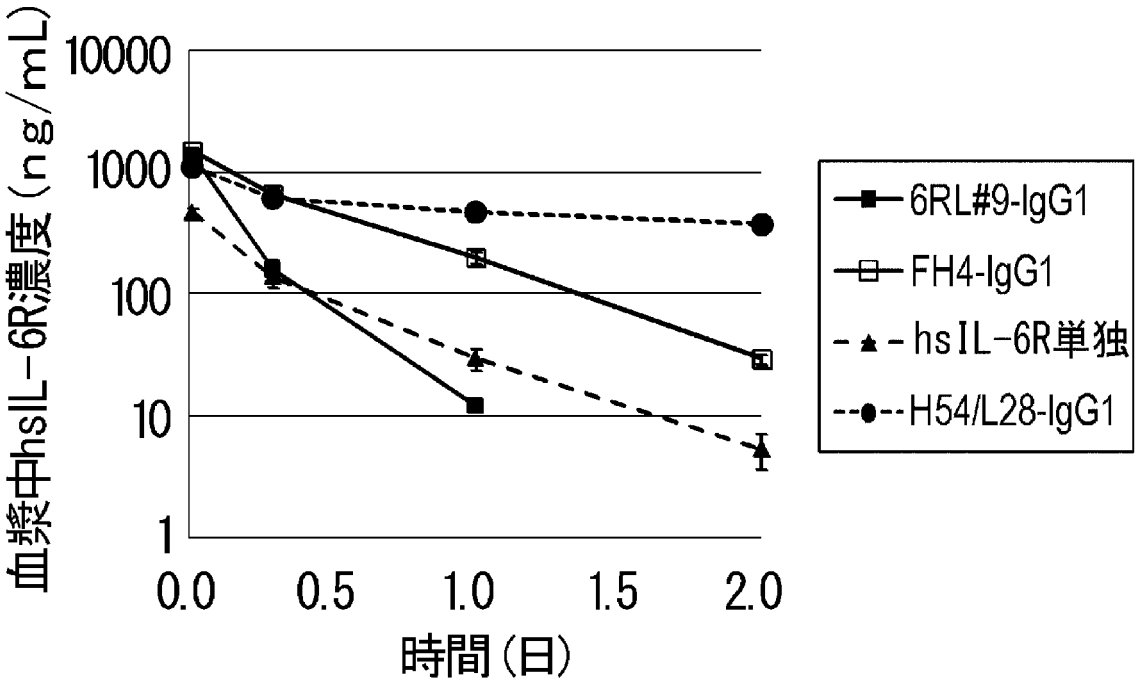
(ii) カルシウムイオン非存在下での結晶の構造



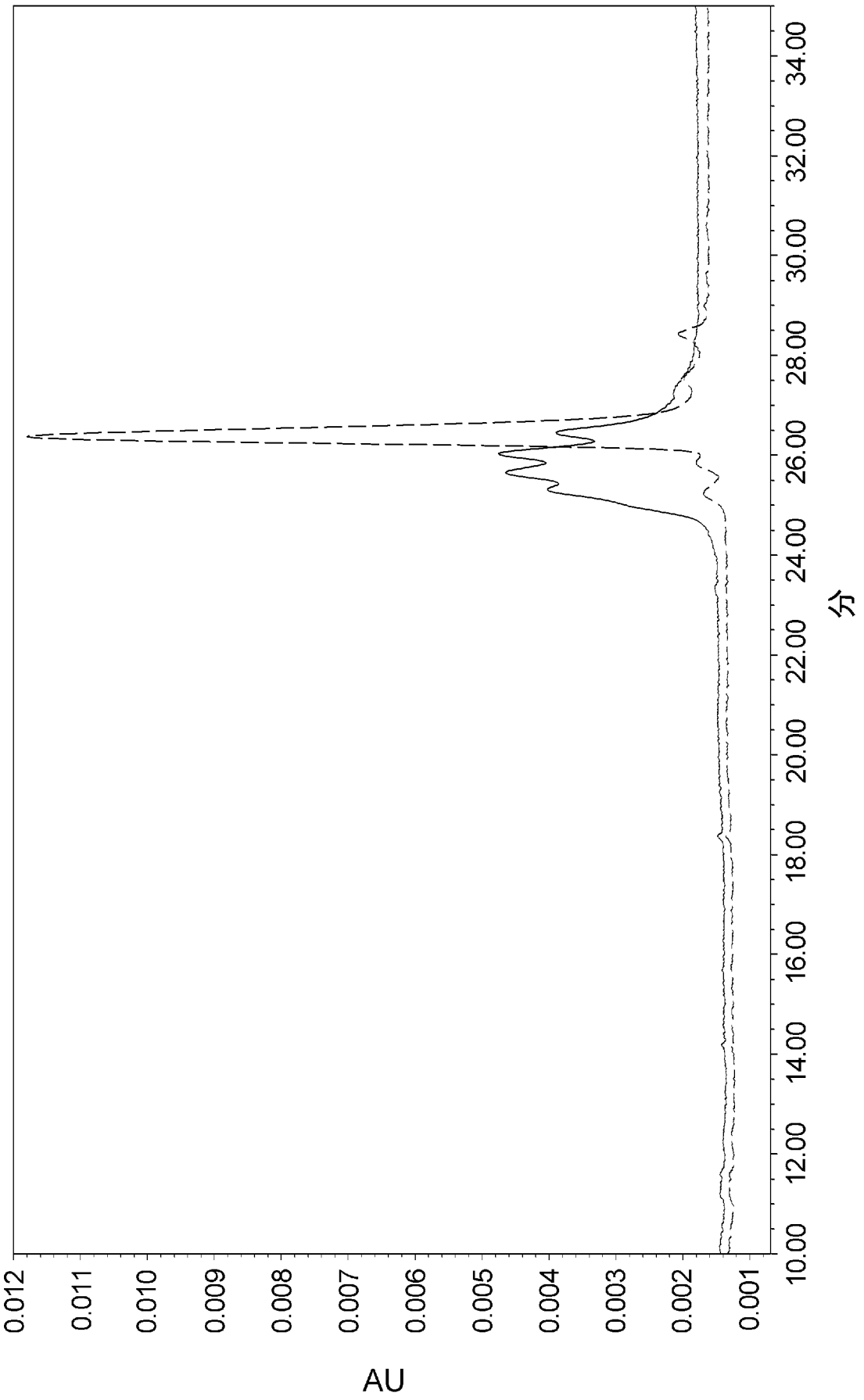
[図50]


血漿中抗体濃度（μg/mL）
10000
1000
100
10
1
0
1
2
3
4
5
6
7
時間（日）
H54/L28-IgG1
6RL#9-IgG1
FH4-IgG

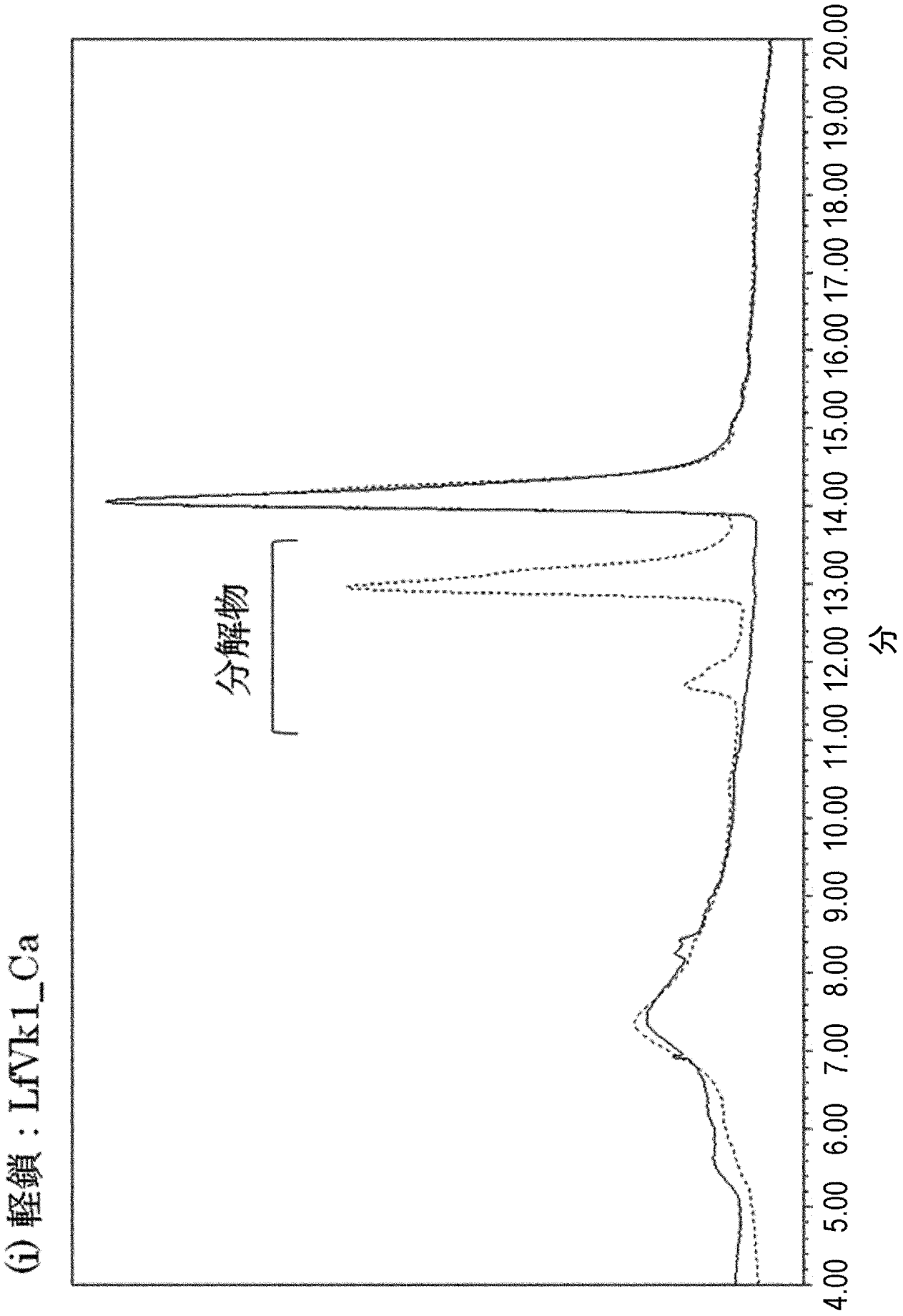
[図51]


血漿中hsIL-6R濃度（ng/mL）
10000
1000
100
10
1
0.0
0.5
1.0
1.5
2.0
時間（日）
6RL#9-IgG1
FH4-IgG1
hsIL-6R単独
H54/L28-IgG1

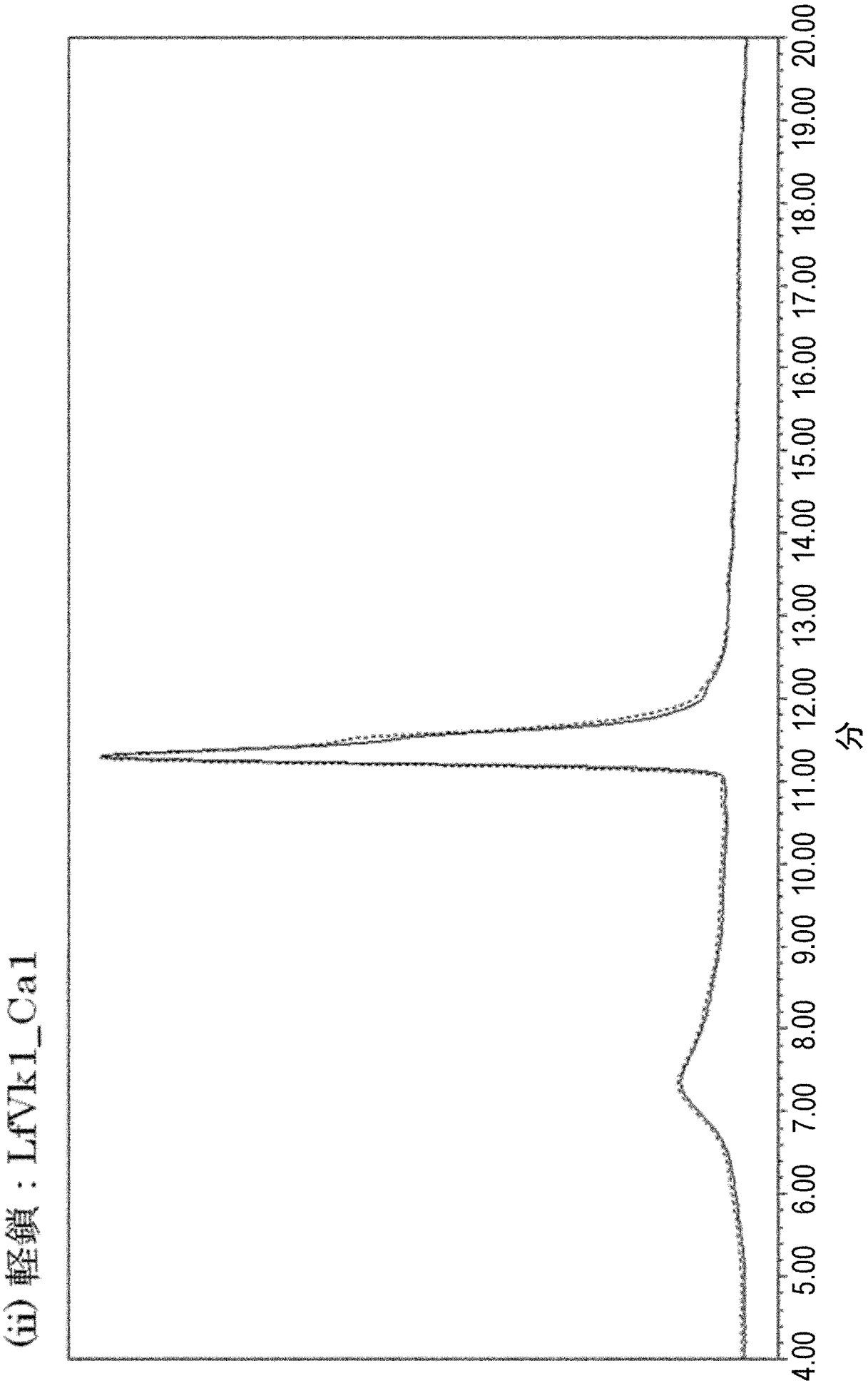
[図52]


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
分

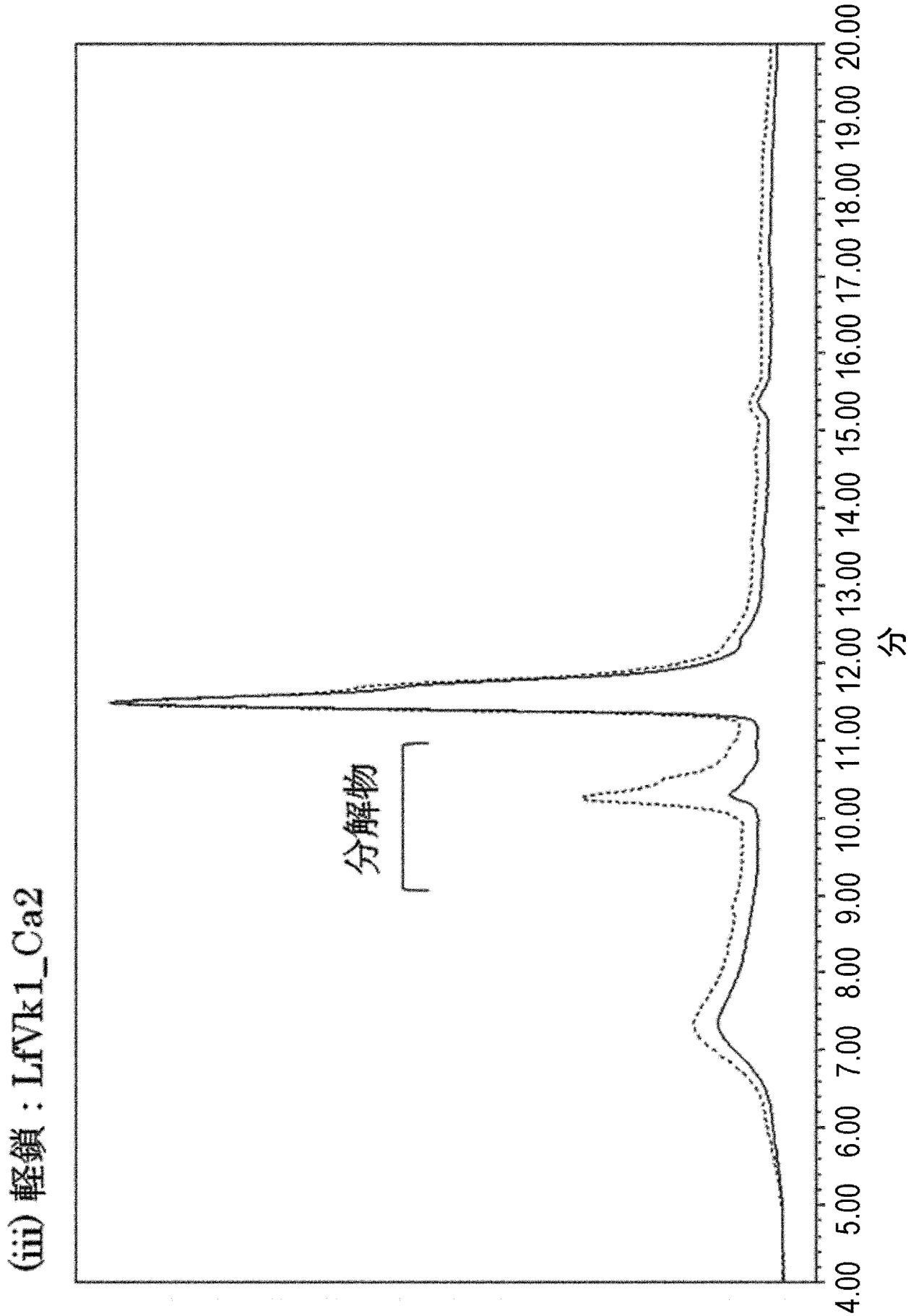
[図53A]


(i) 軽鎖：LfVk1_Ca
分解物
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
分

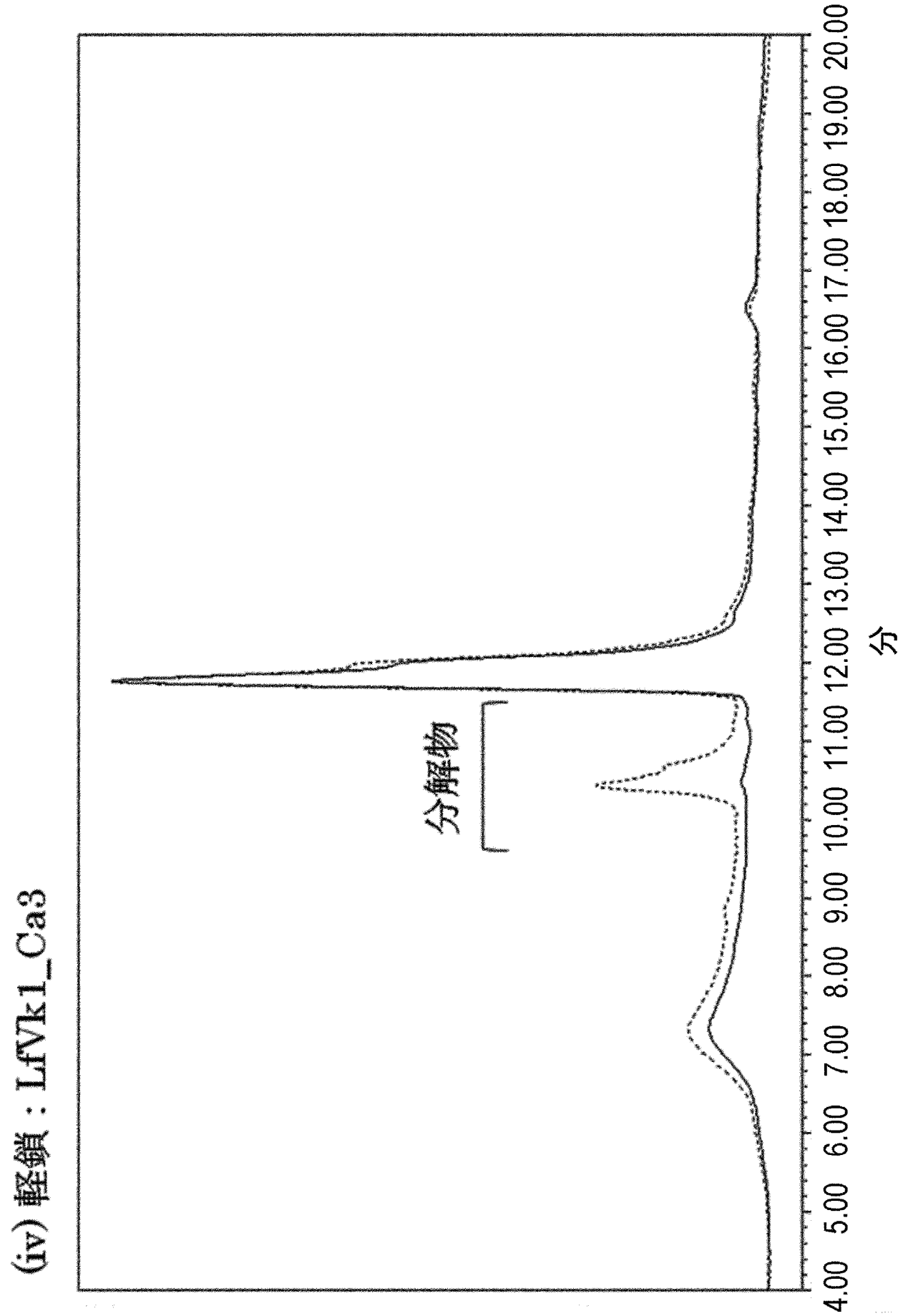
[図53B]


(ii) 軽鎖：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
分

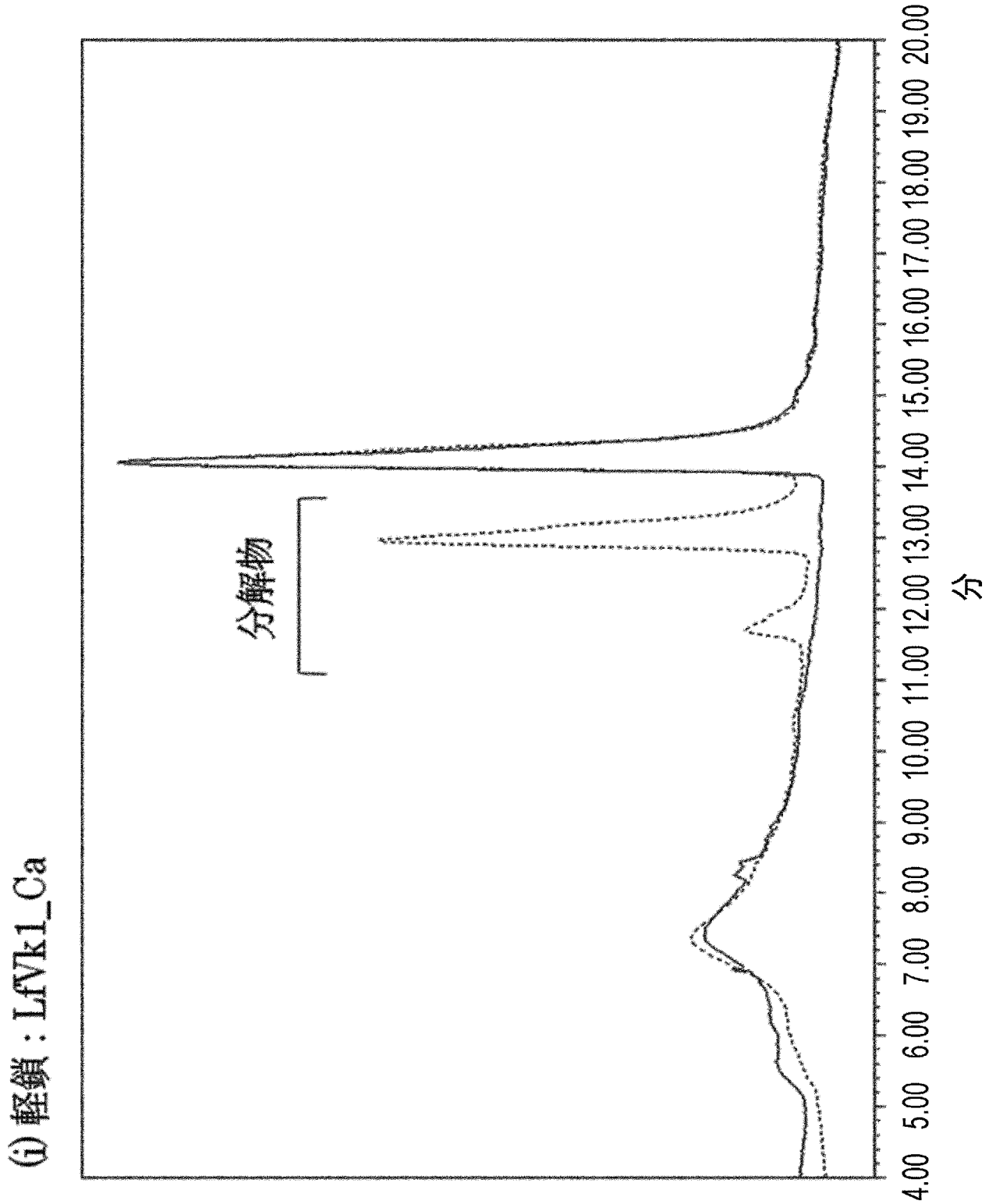
[図53C]


(iii) 軽鎖：LfVk1_Ca2
分解物
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
分

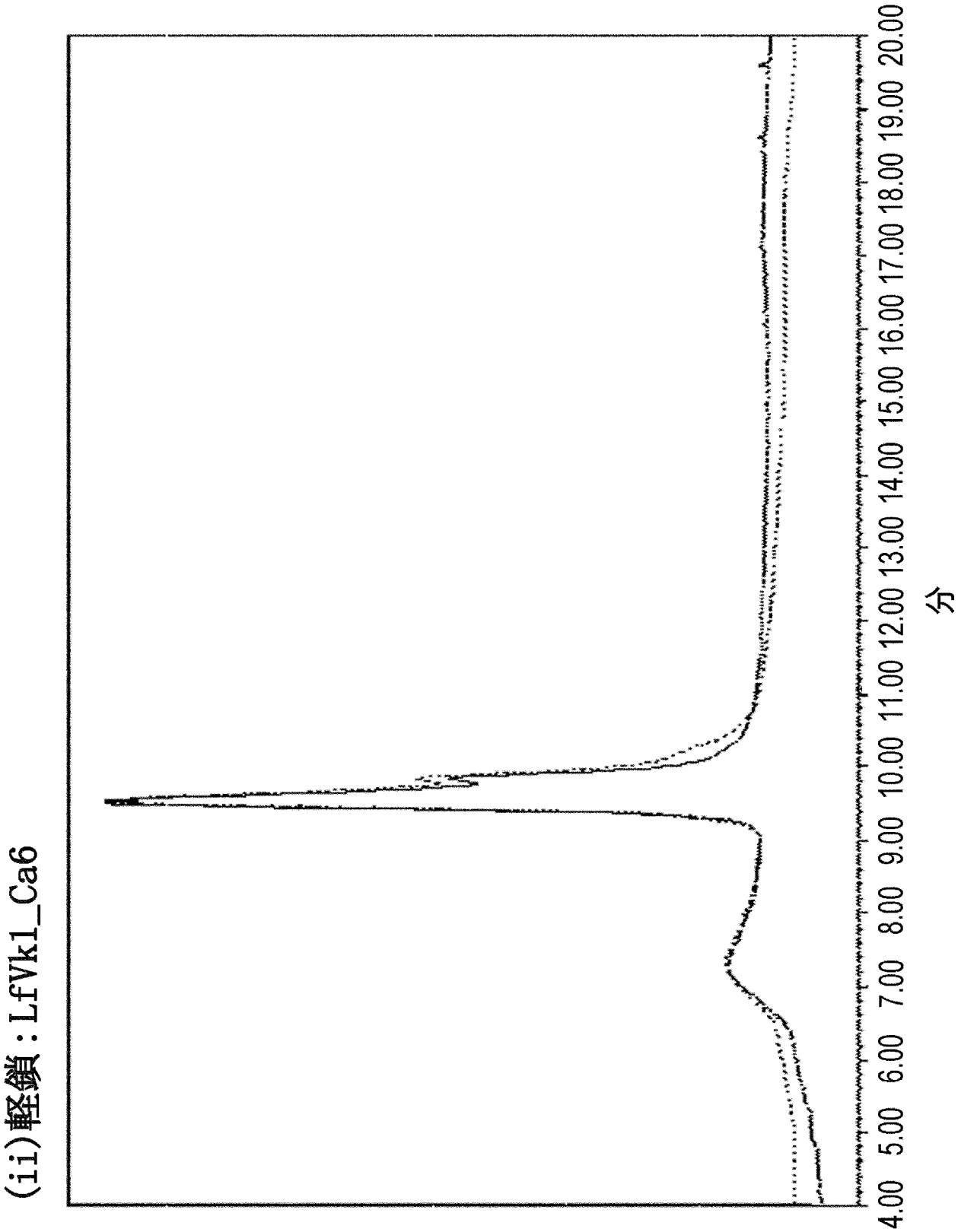
[図53D]


(iv) 軽鎖：LfVk1_Ca3
分解物
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
分

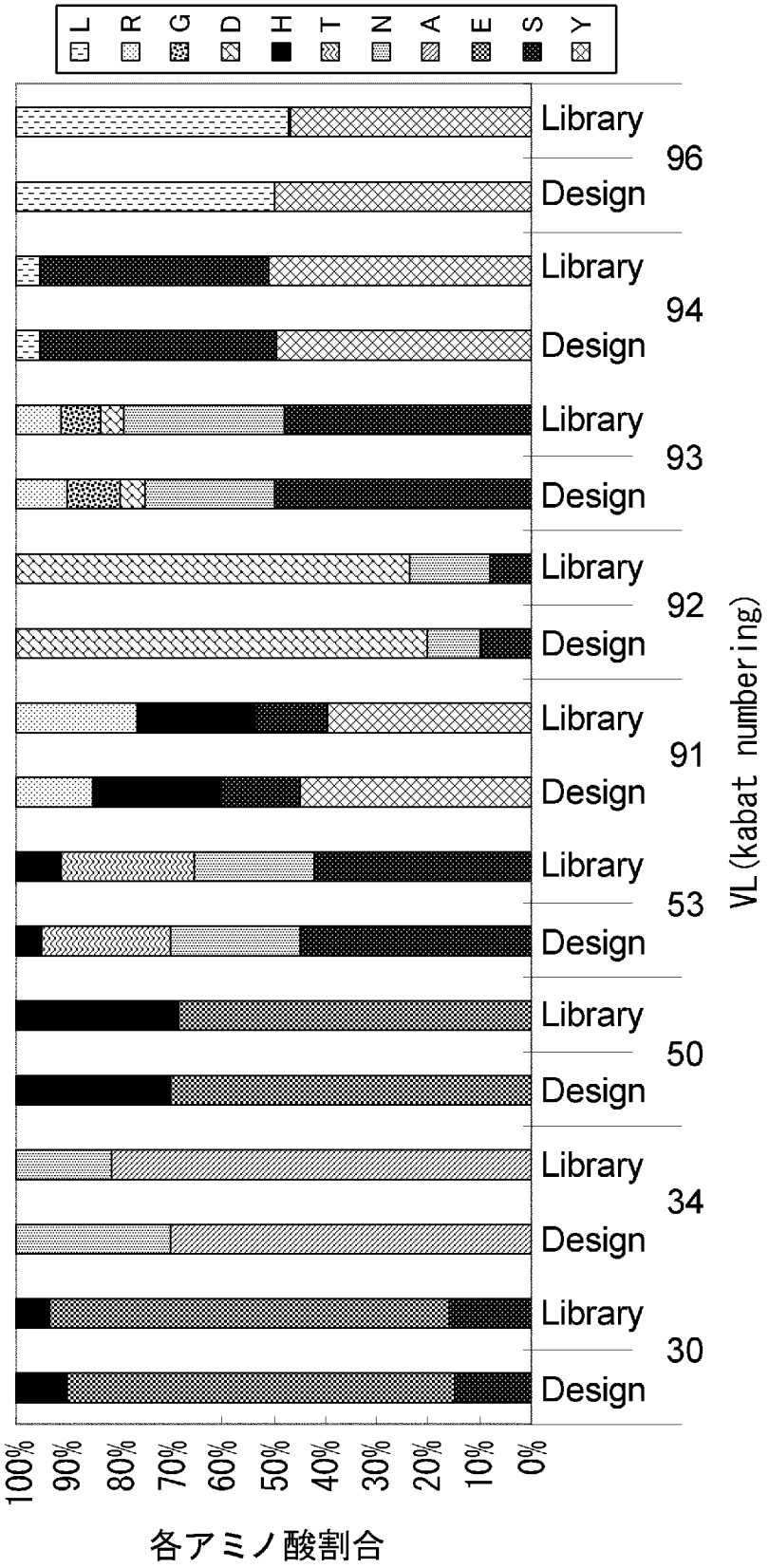
[図54A]


(i) 軽鎖：LfVk1_Ca
分解物
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
分

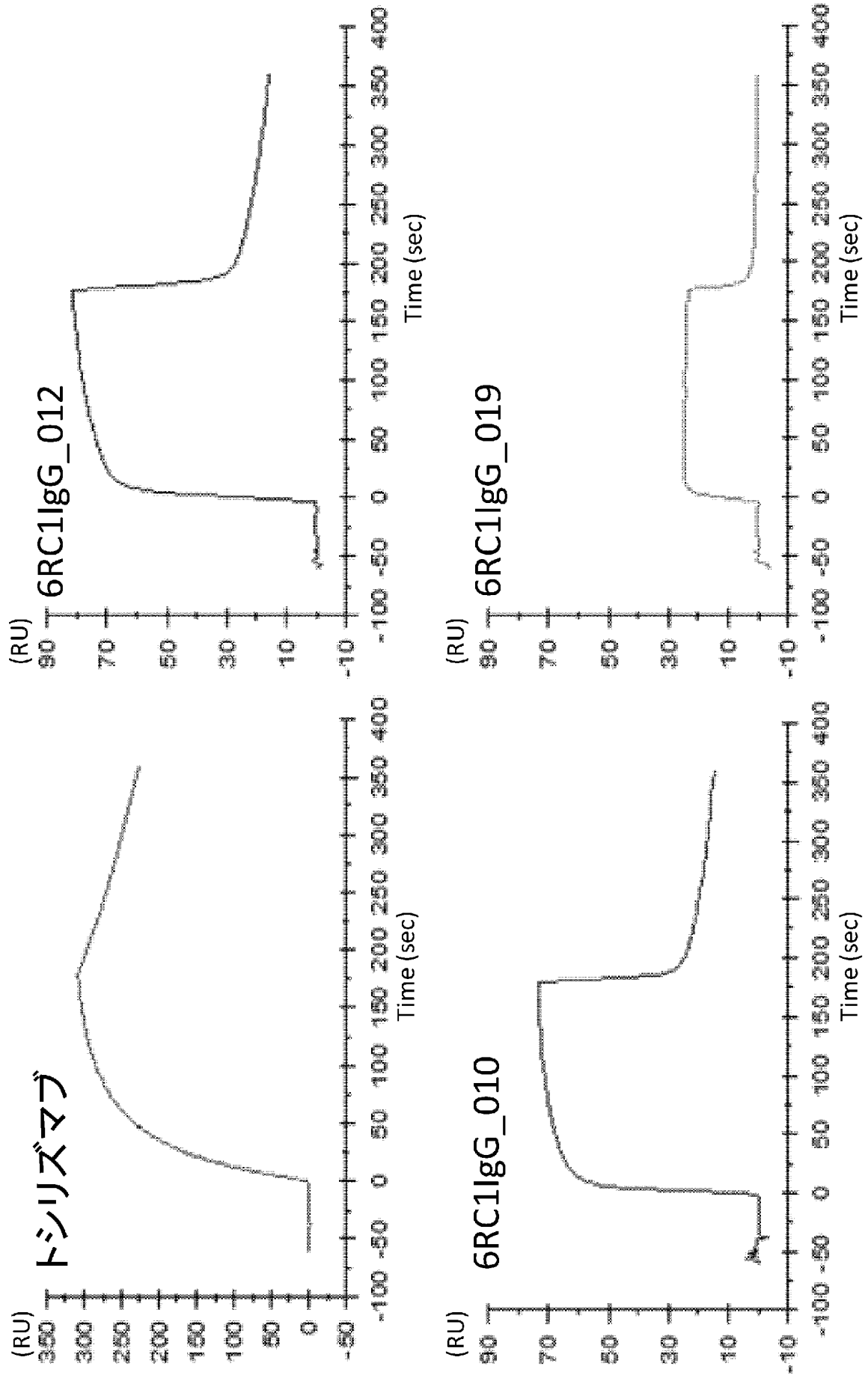
[図54B]


(ii)軽鎖：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
分

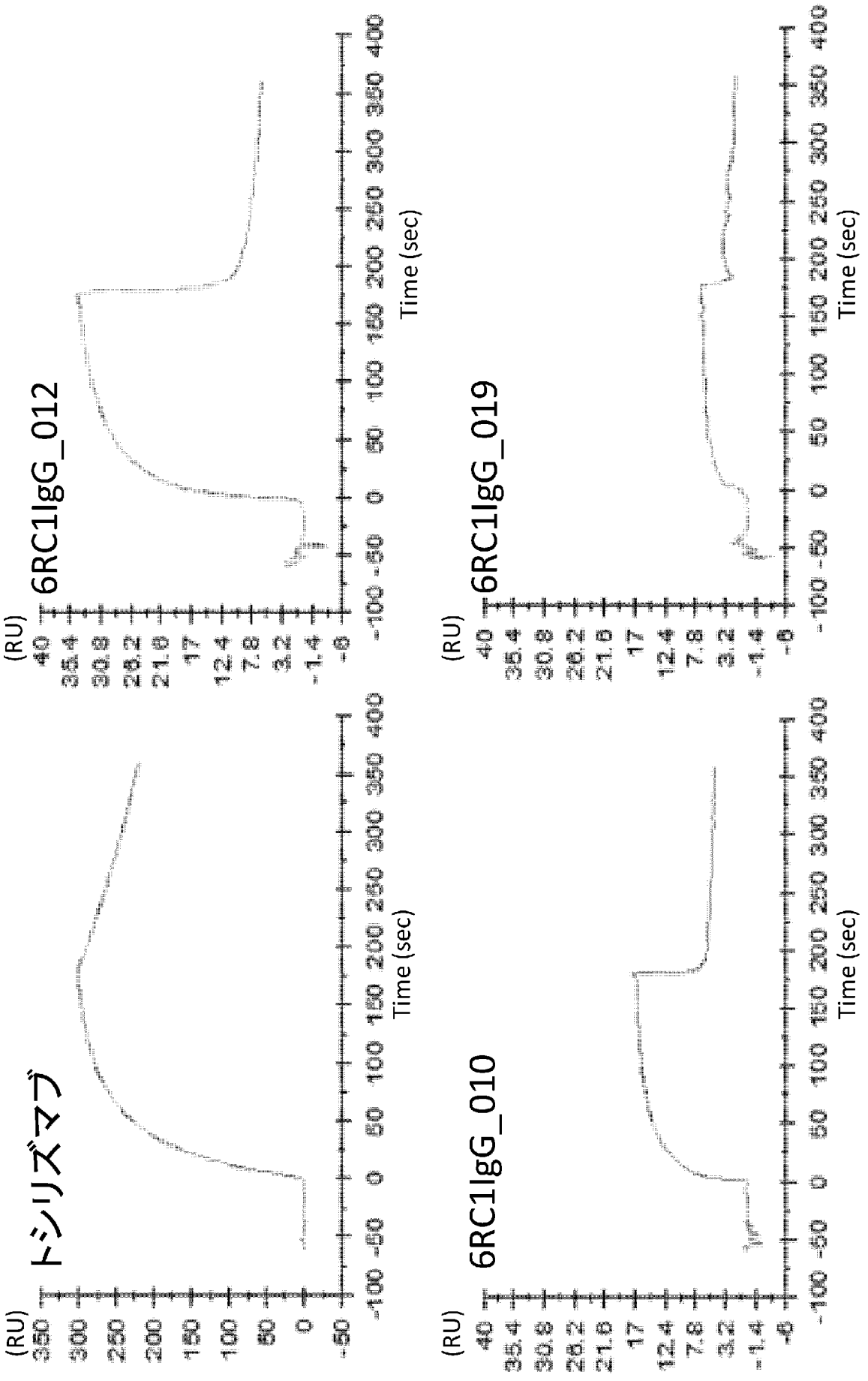
[図55]


L
R
G
D
H
T
N
A
E
S
Y
Library
Design
96
94
93
92
91
53
50
34
30
VL(kabat numbering)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
各アミノ酸割合

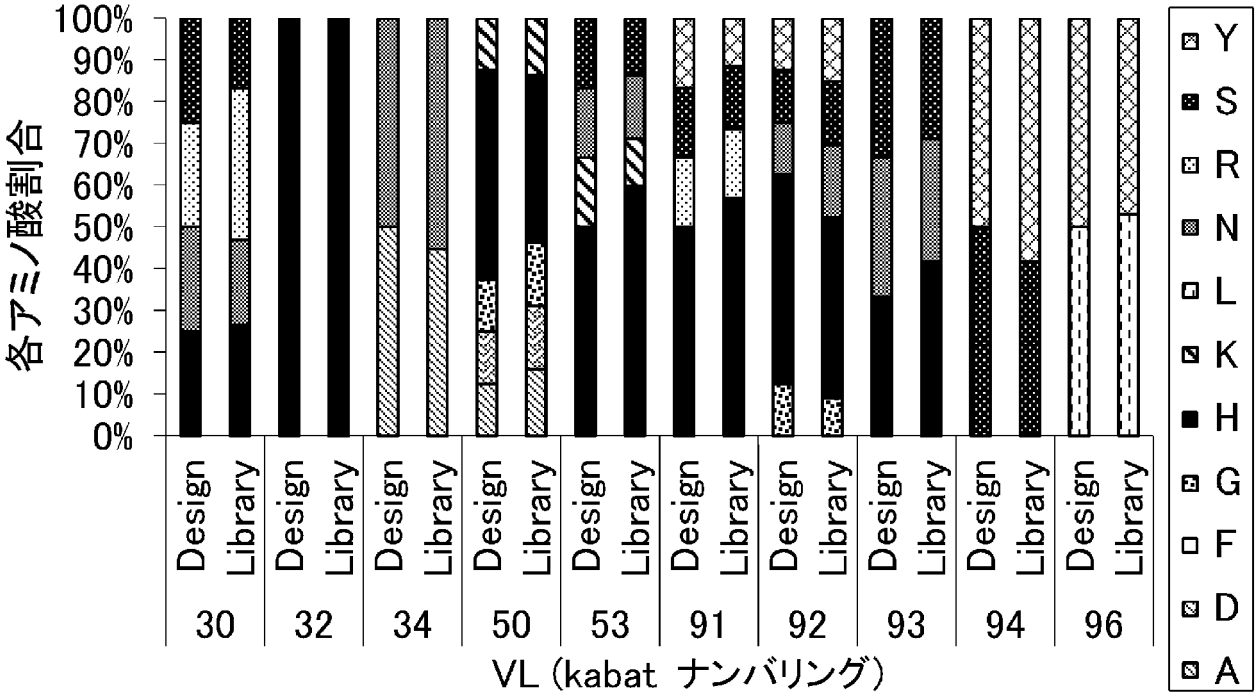
[図56]



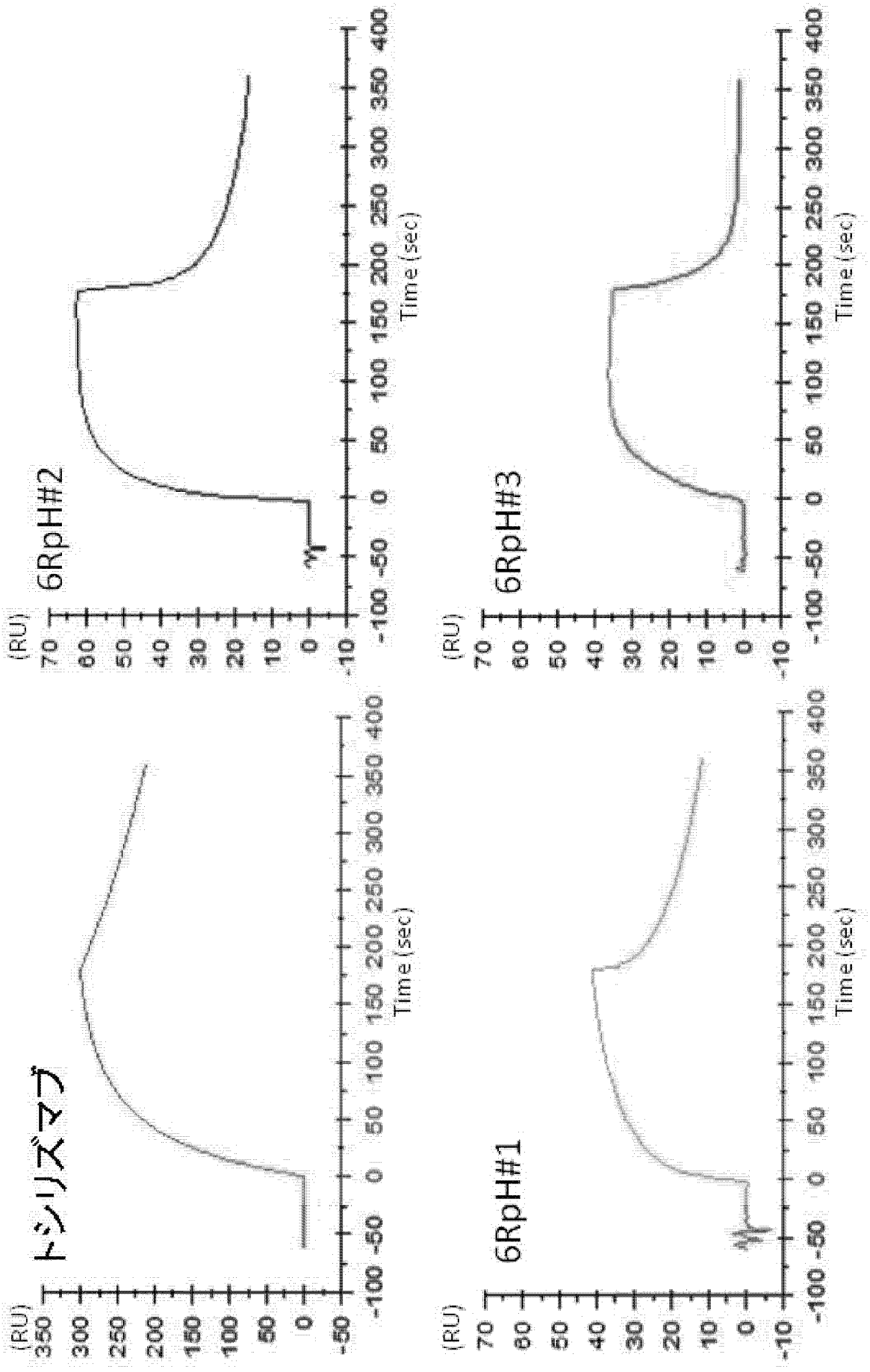
[図57]



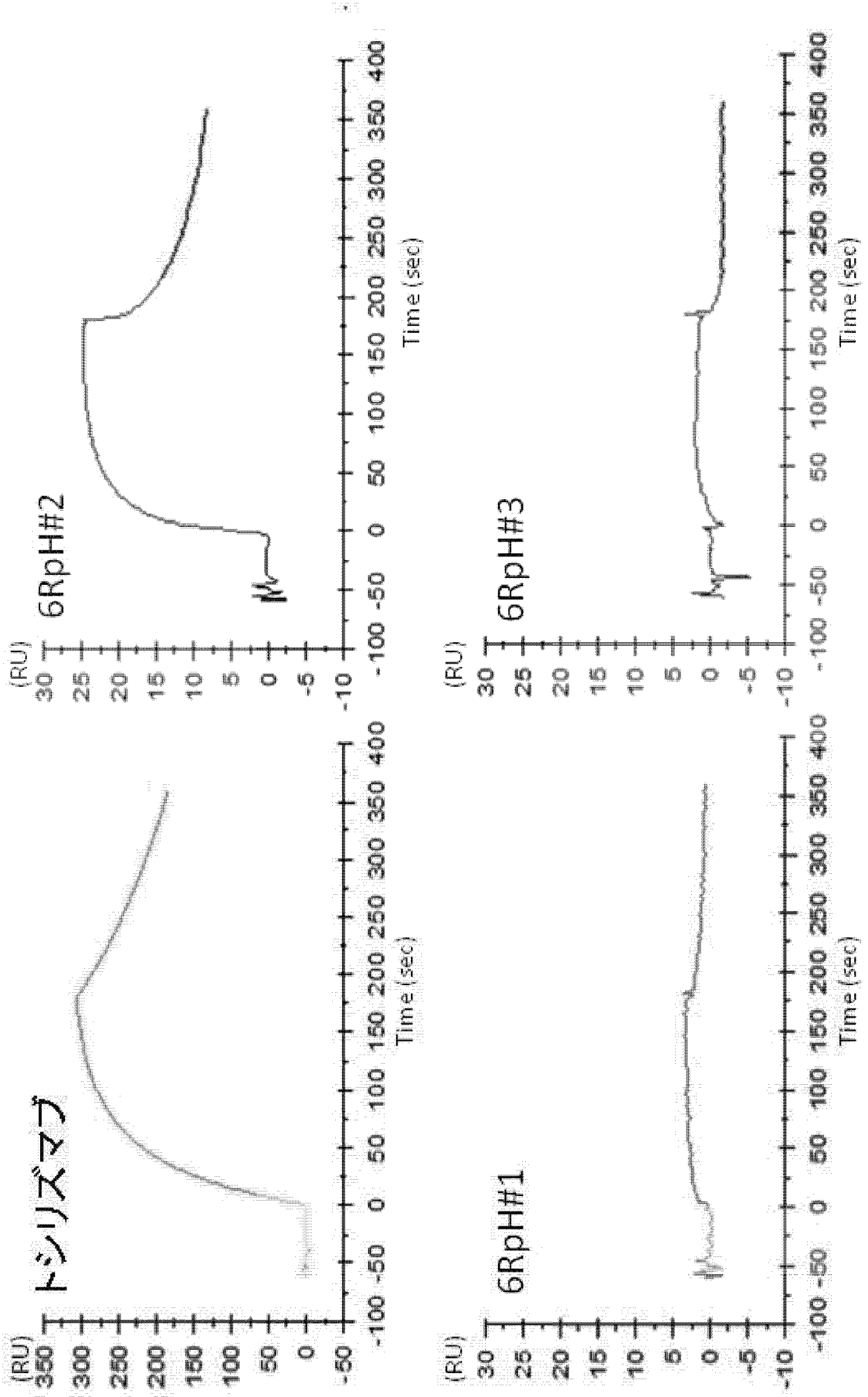
[図58]


各アミノ酸割合
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 ナンバリング)
Y
S
R
N
L
K
H
G
F
D
A

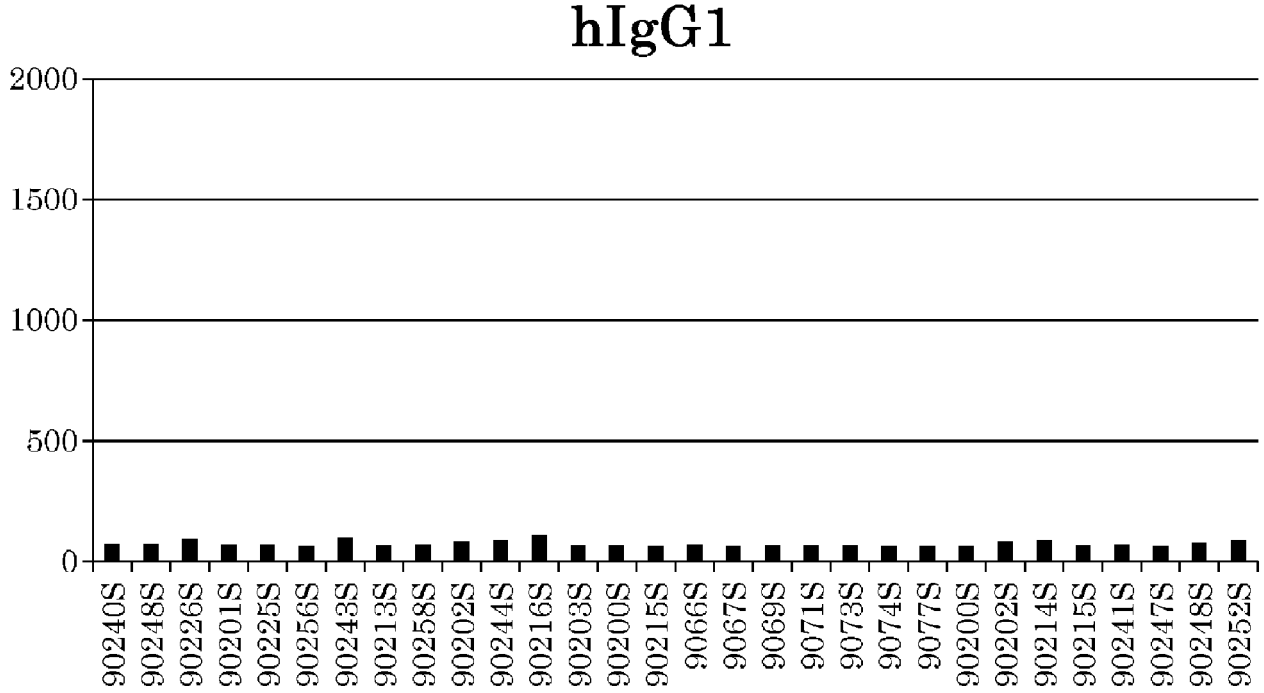
[図59]



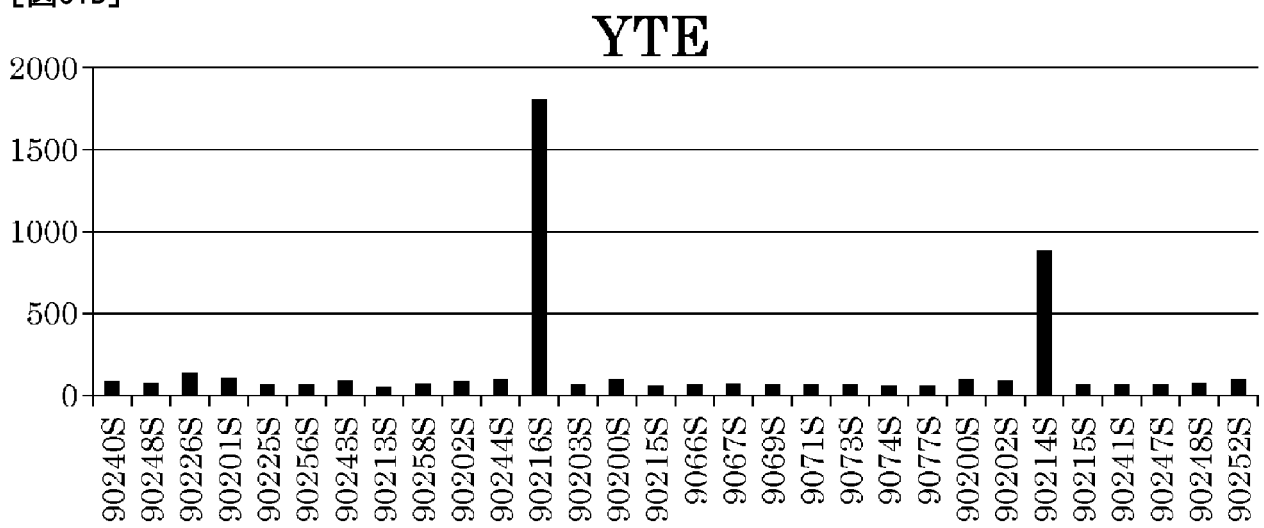
[図60]



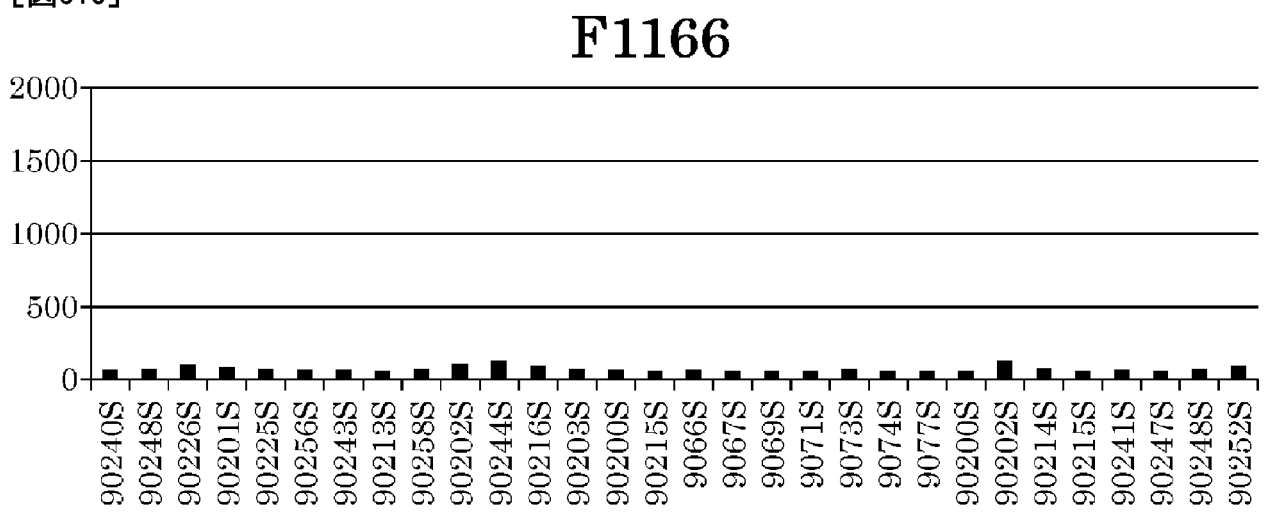
[図61A]



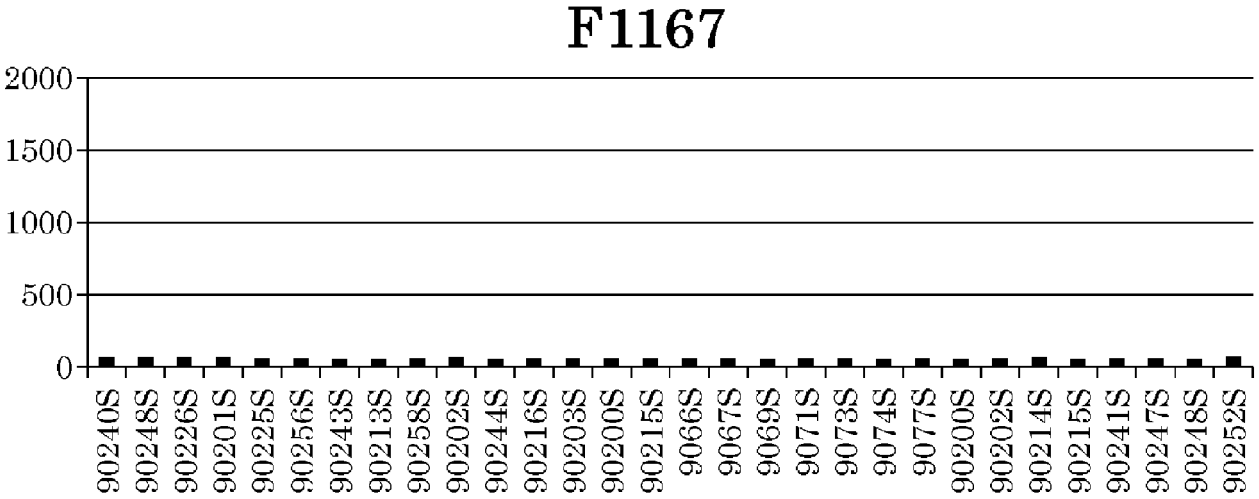
[図61B]



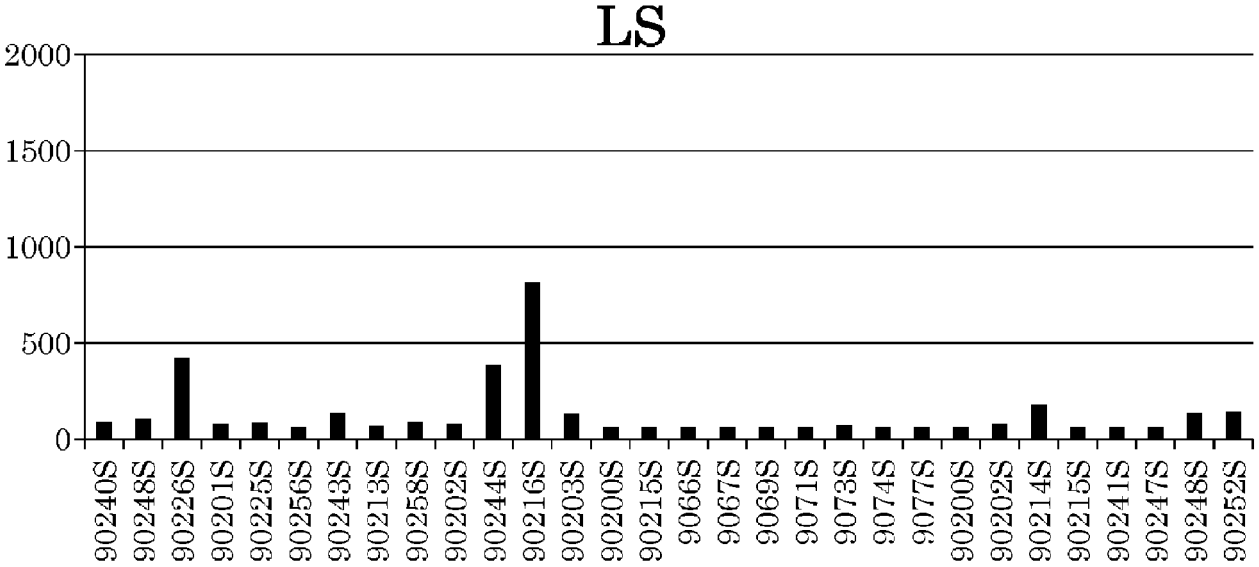
[図61C]



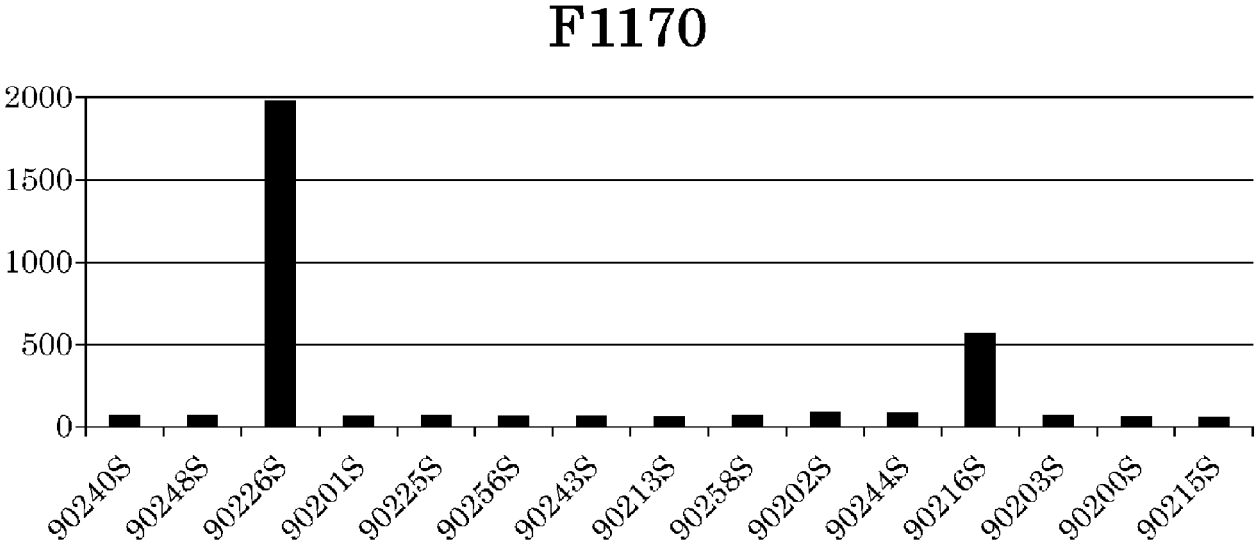
[図61D]



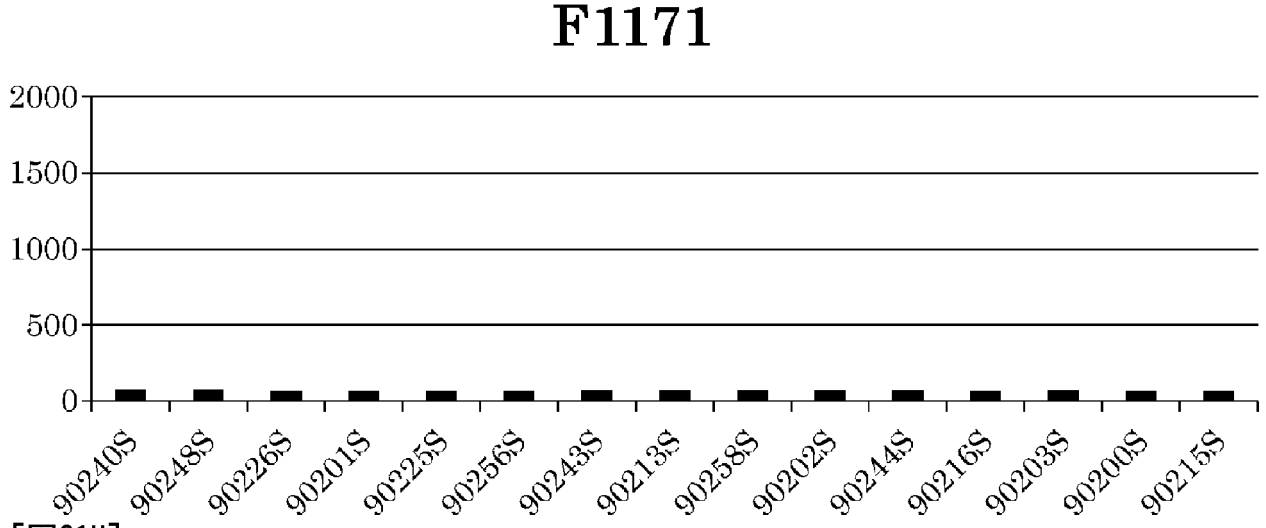
[図61E]



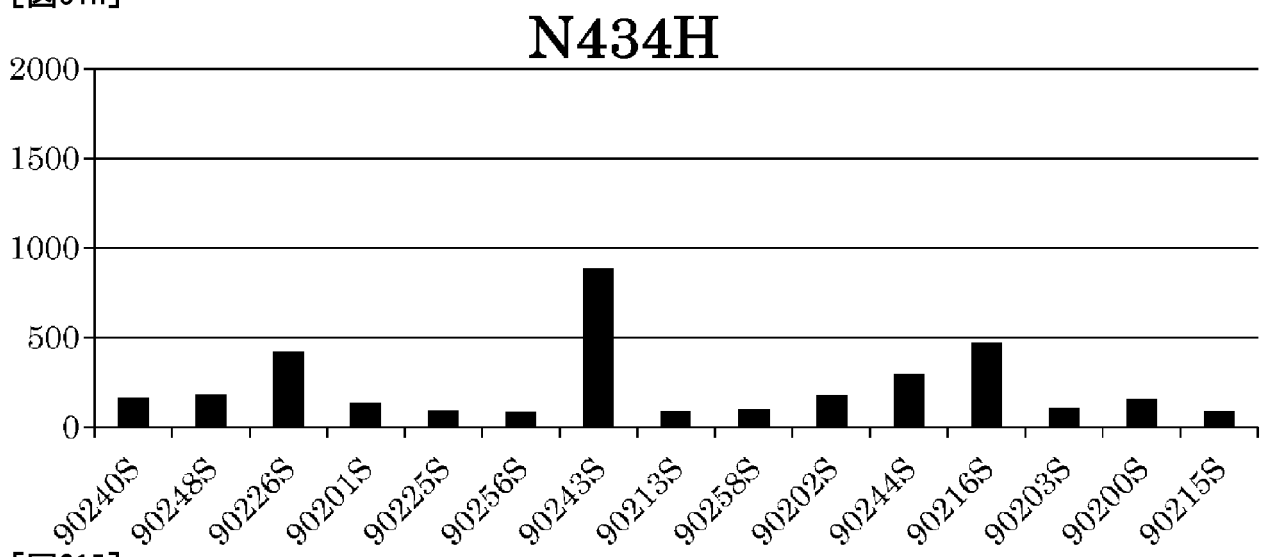
[図61F]



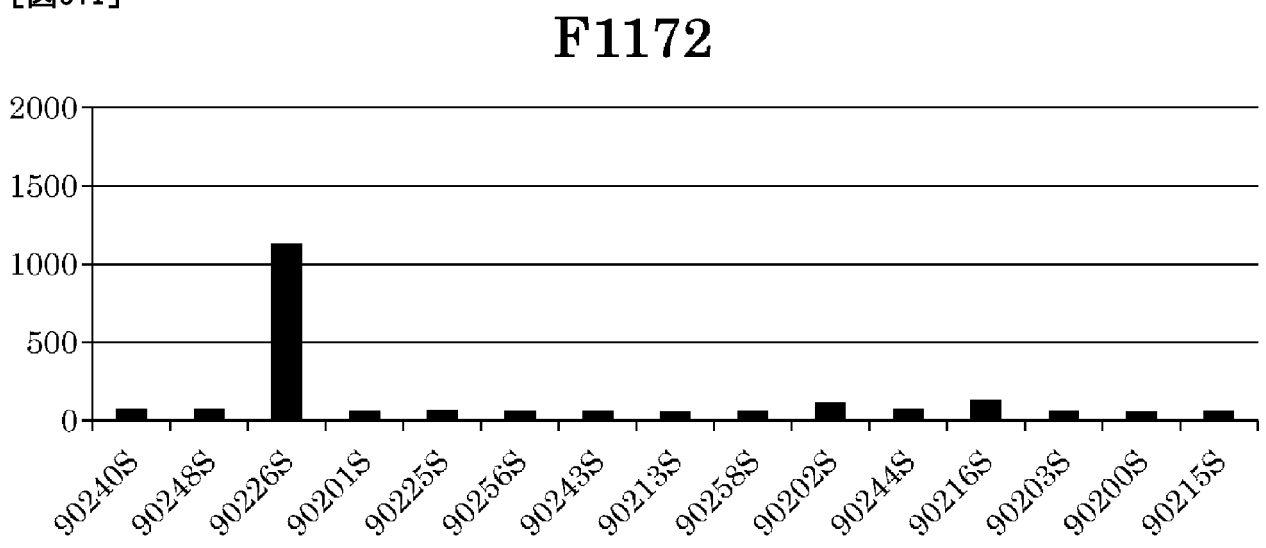
[図61G]



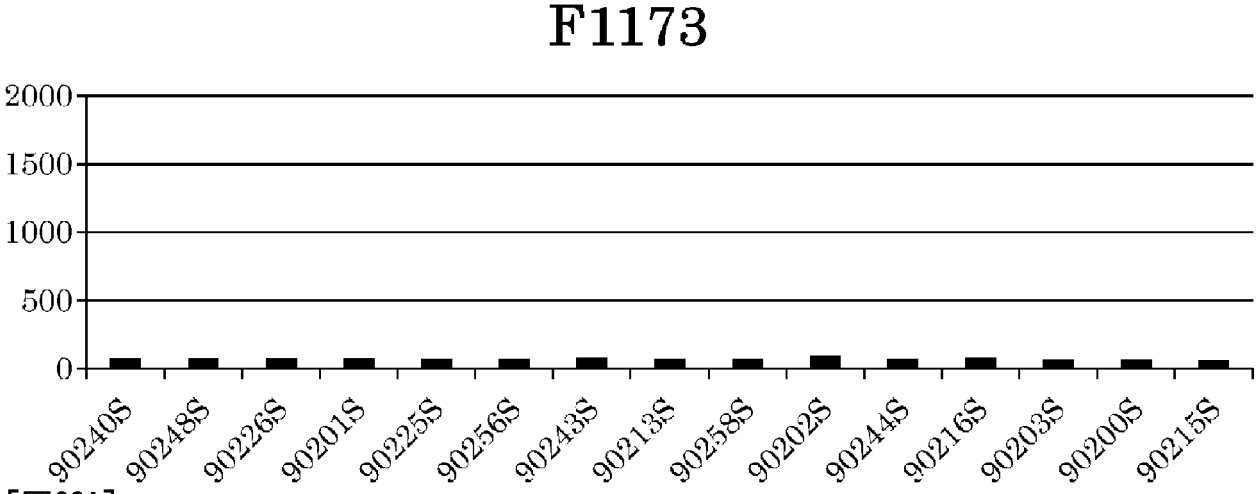
[図61H]



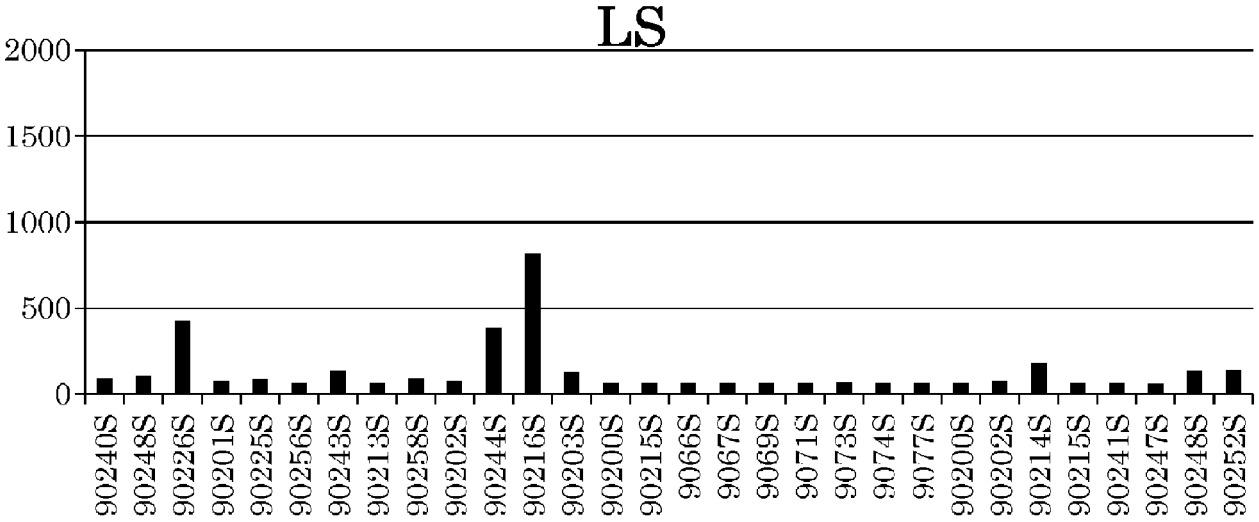
[図61I]



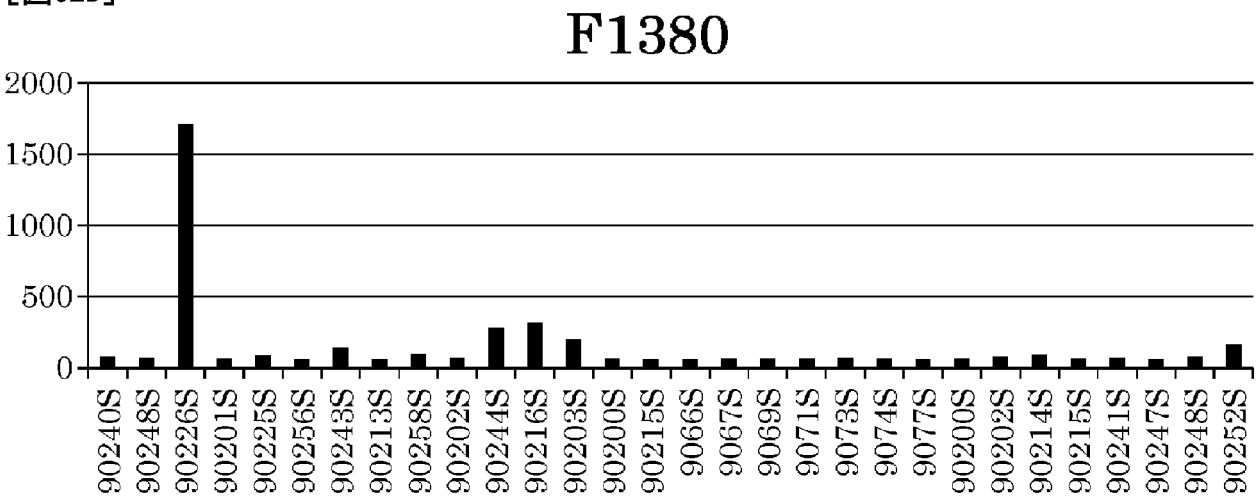
[図61J]



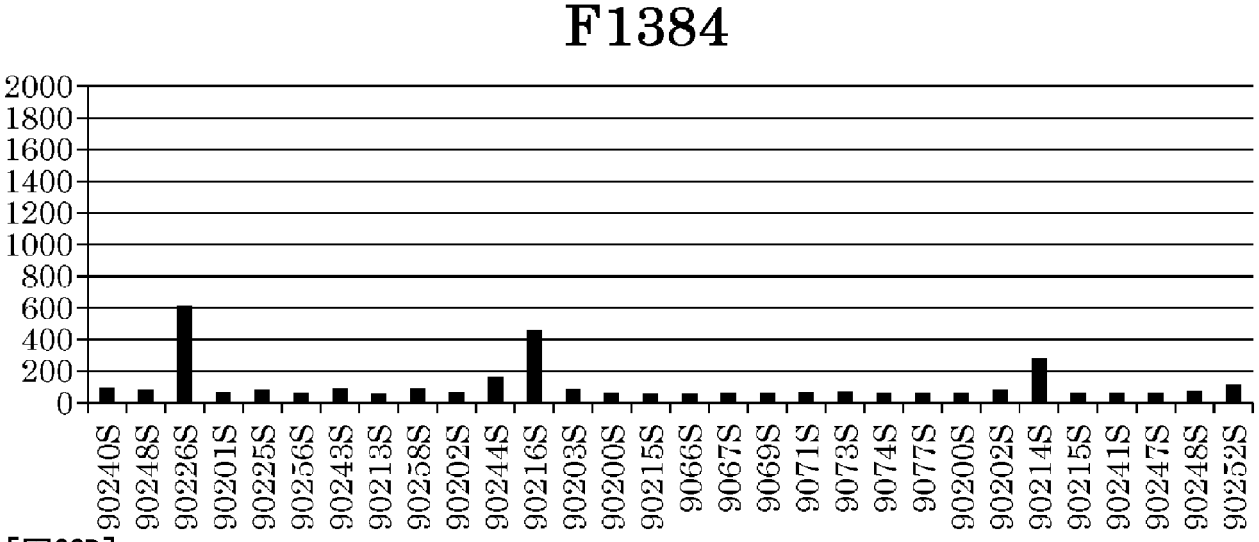
[図62A]



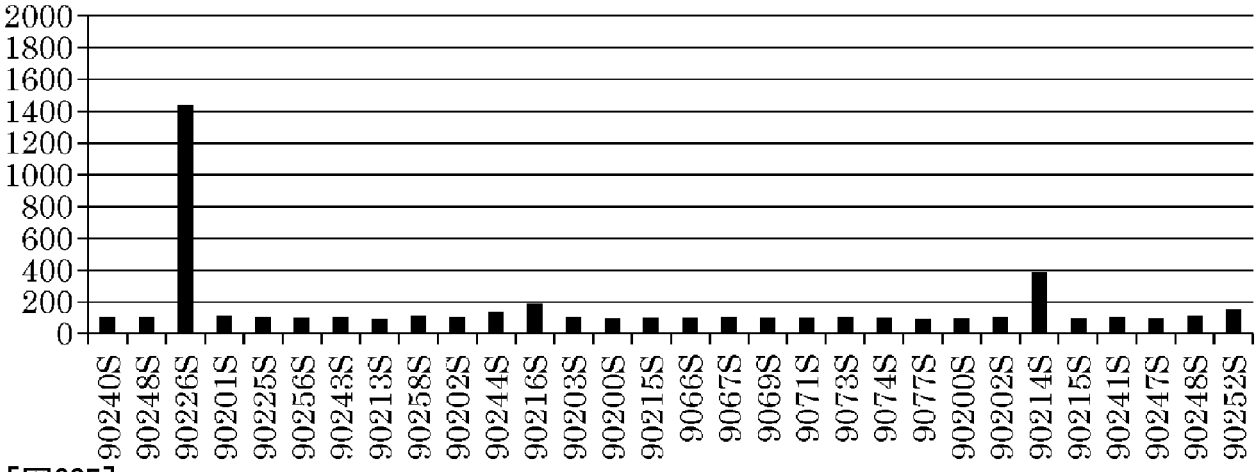
[図62B]



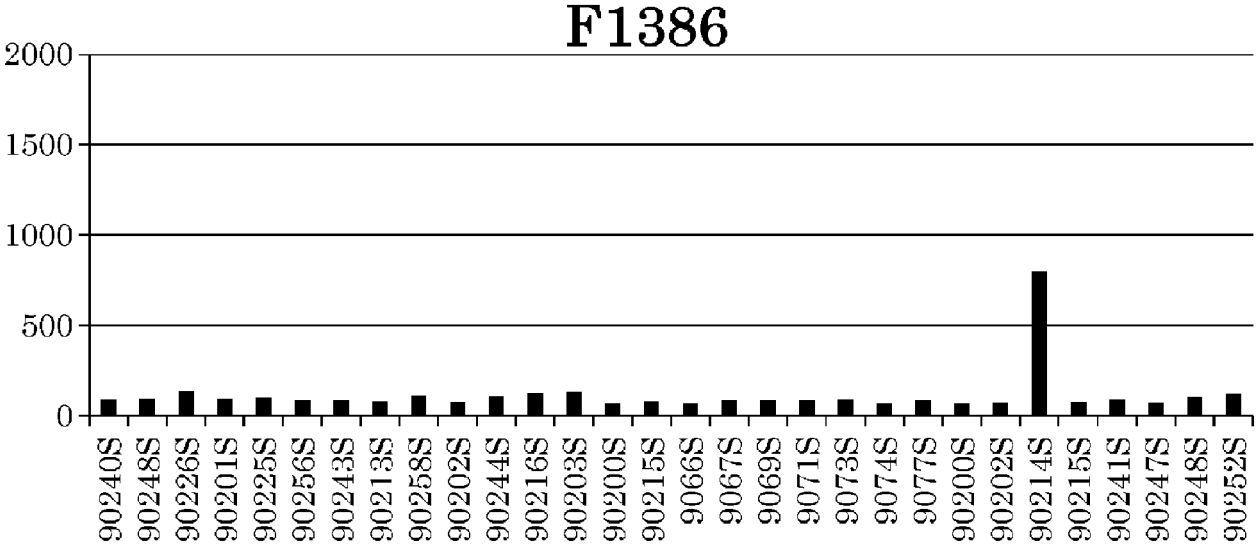
[図62C]



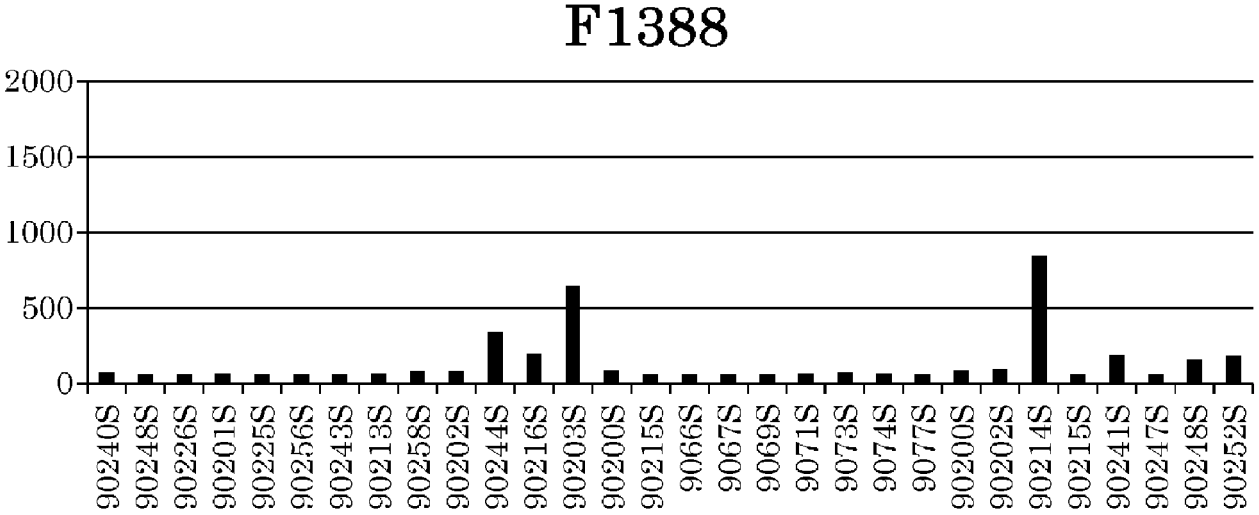
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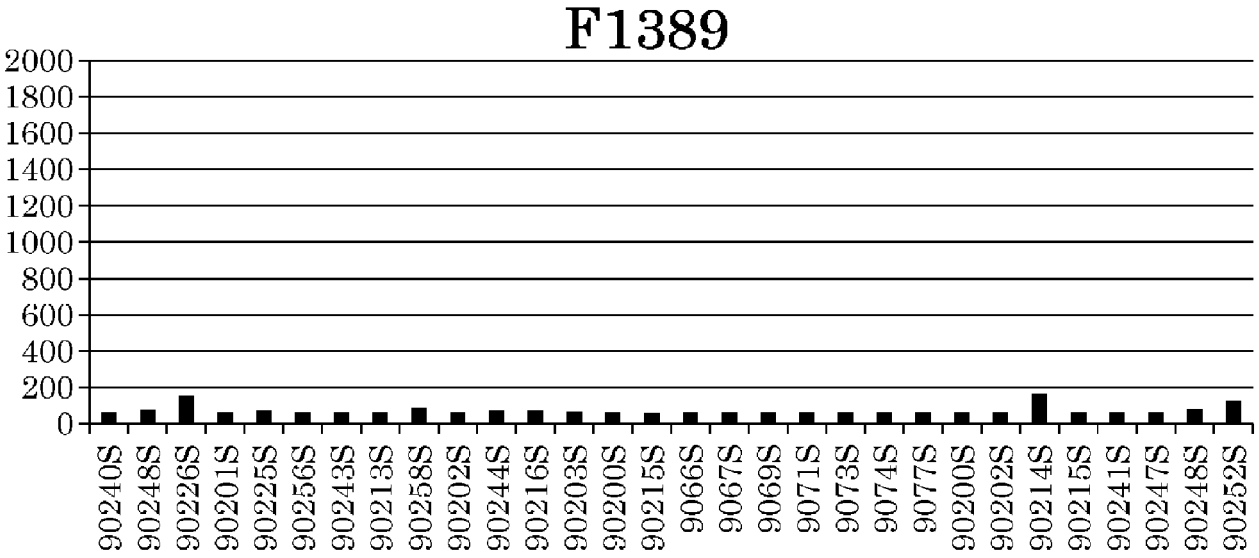
[図62E]



[図62F]


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500
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90256S
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9067S
9069S
9071S
9073S
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9077S
90200S
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90214S
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90241S
90247S
90248S
90252S

[図62G]


F1389
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