FIG. 1

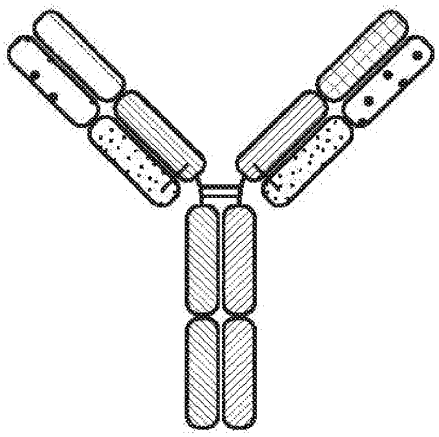


FIG. 2A

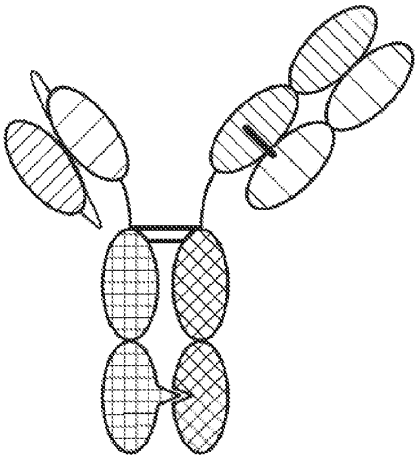


FIG. 2B

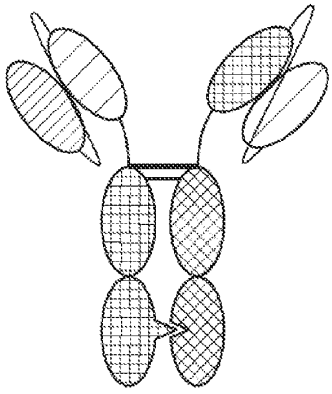


FIG. 2C

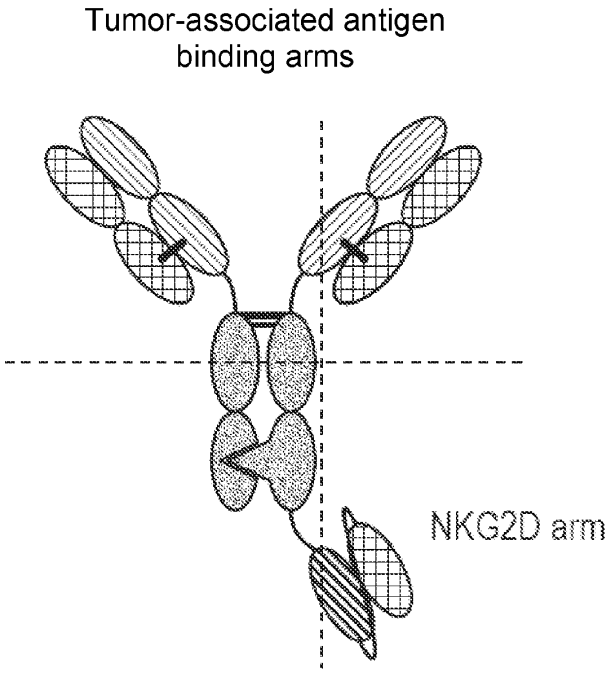

Tumor-associated antigen
binding arms
NKG2D arm

FIG. 2D

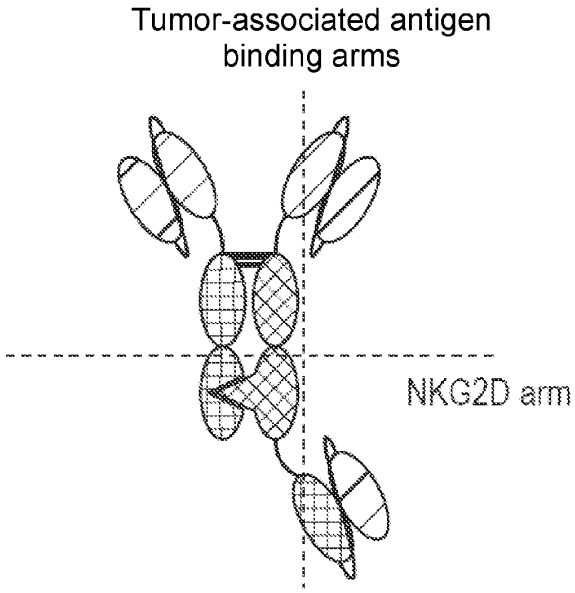

Tumor-associated antigen
binding arms
NKG2D arm

FIG. 2E

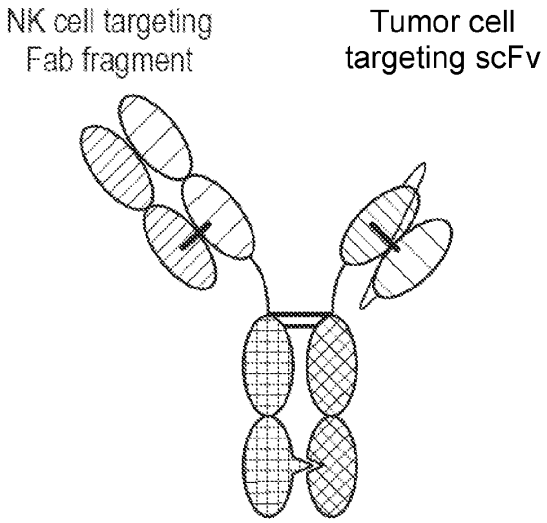


FIG. 3

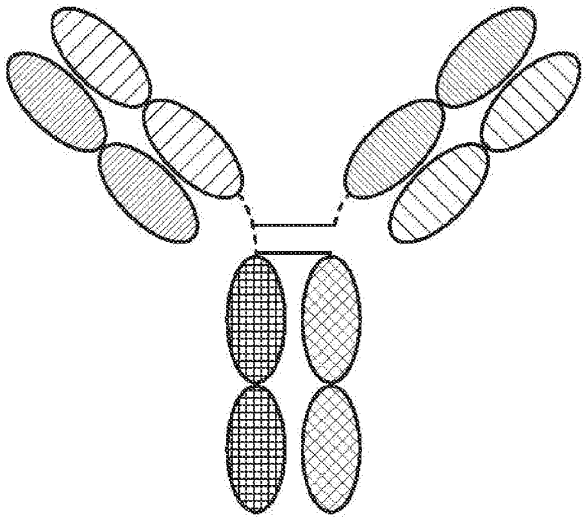


FIG. 4

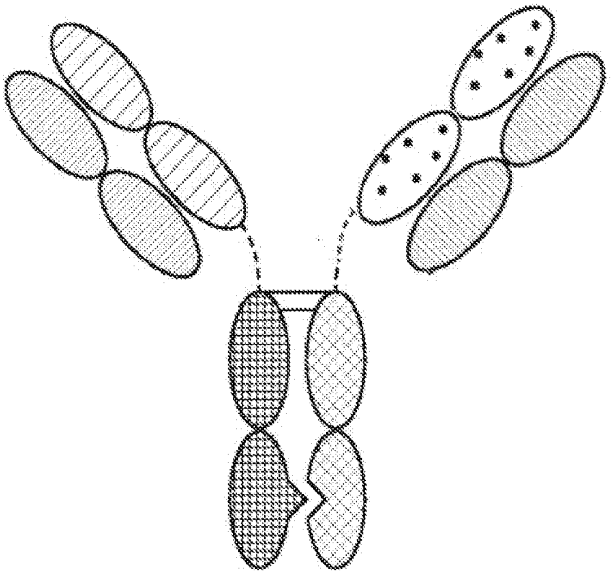


FIG. 5

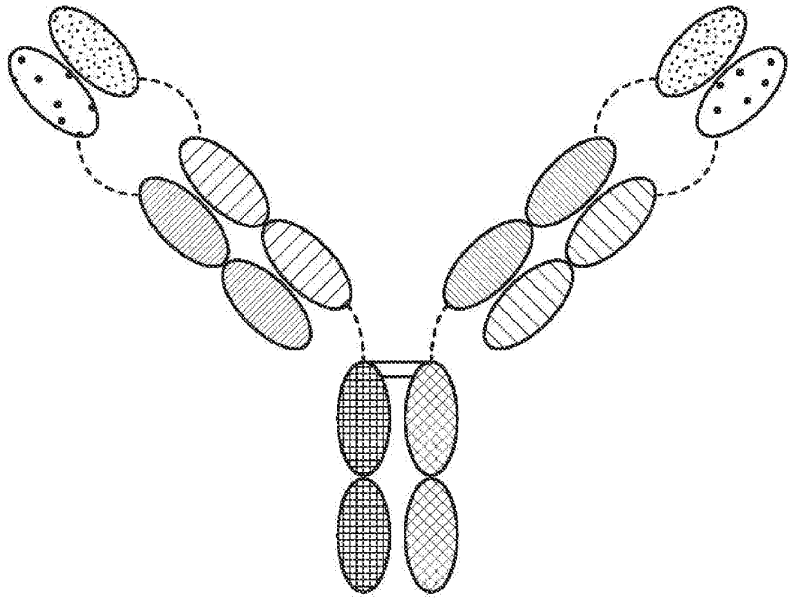


FIG. 6

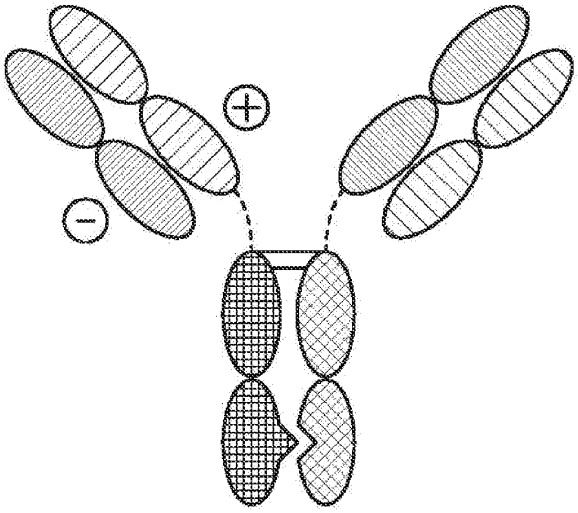


FIG. 7

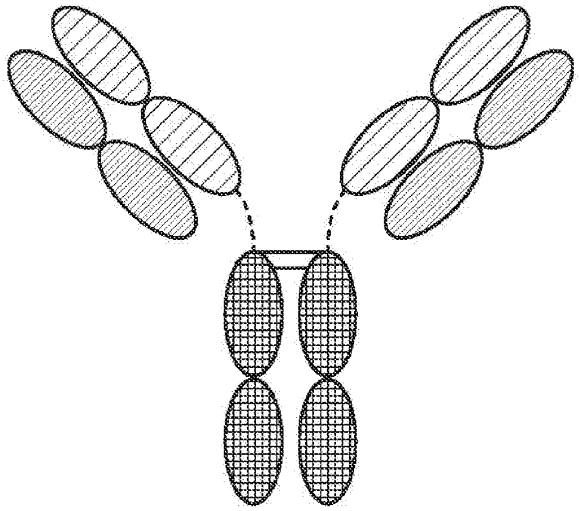


FIG. 8

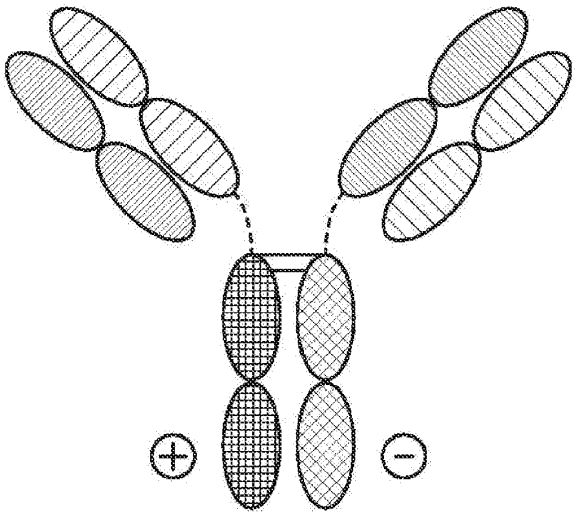


FIG. 9

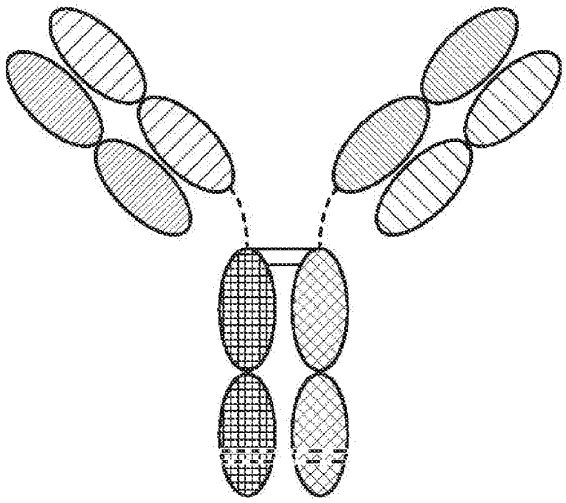


FIG. 10

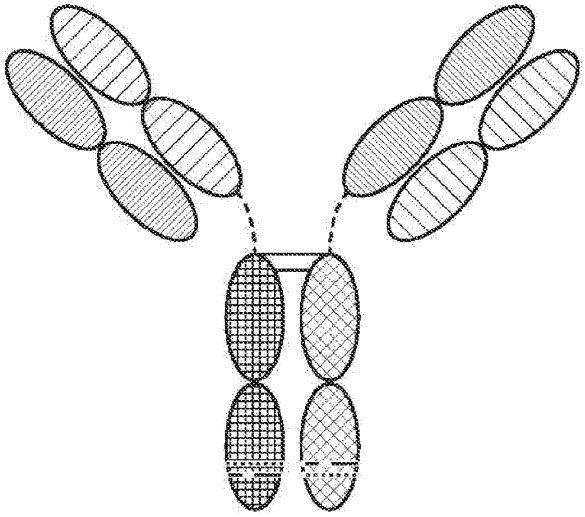


FIG. 11

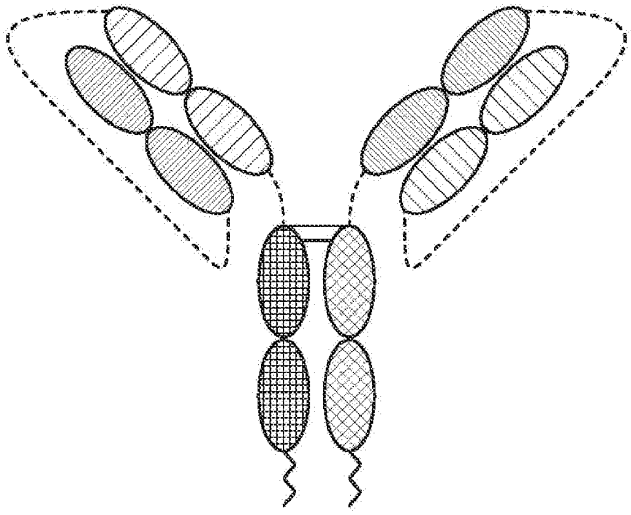


FIG. 12

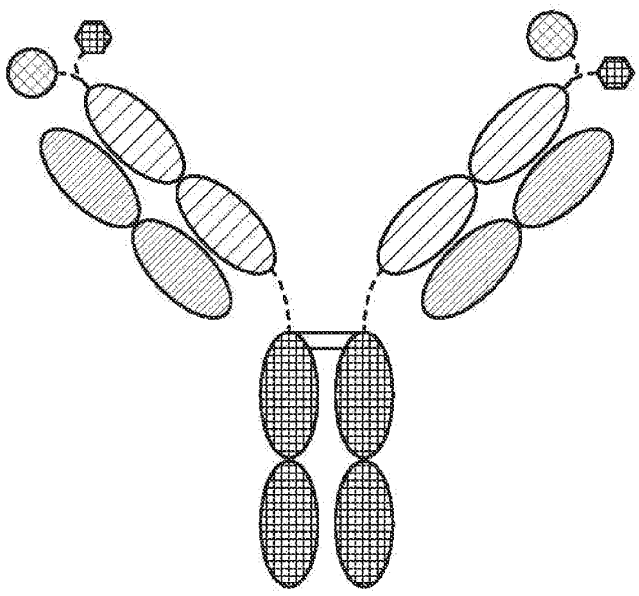


FIG. 13A

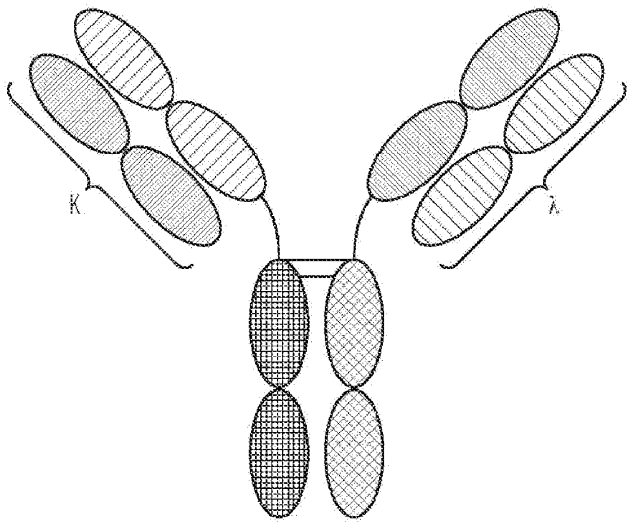


FIG. 13B

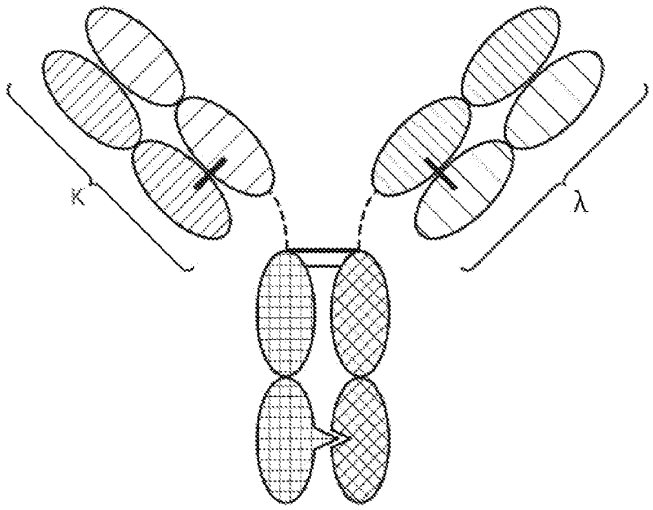


FIG. 14

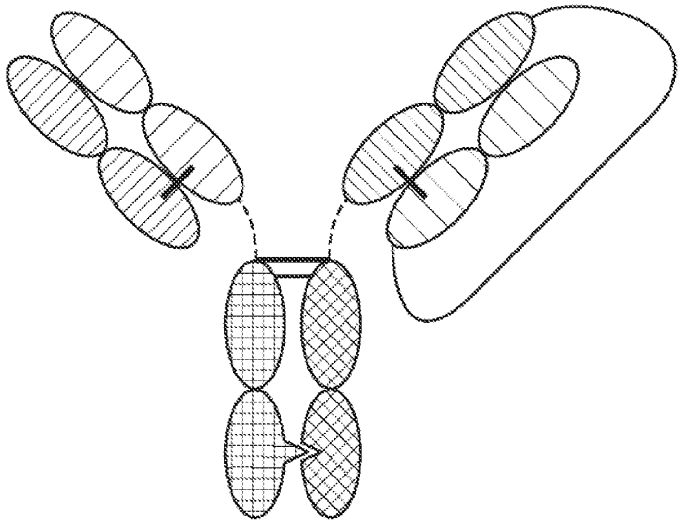


FIG. 15

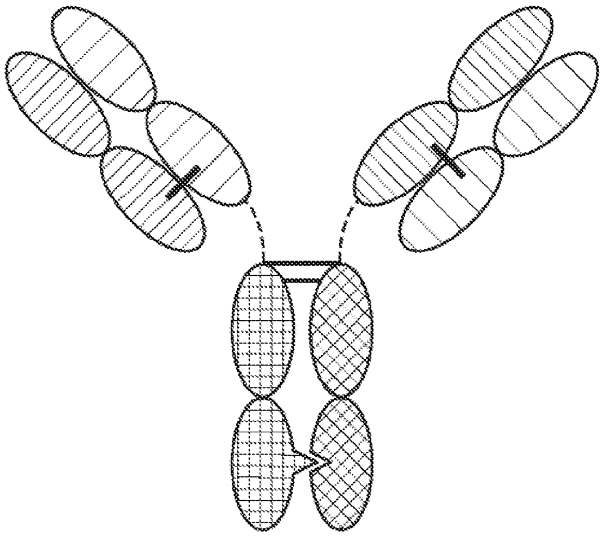


FIG. 16

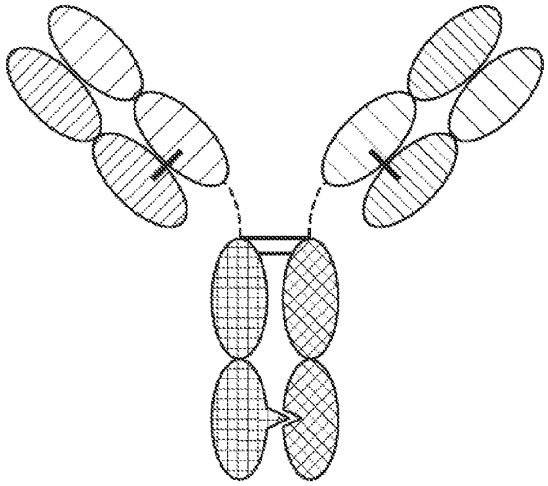
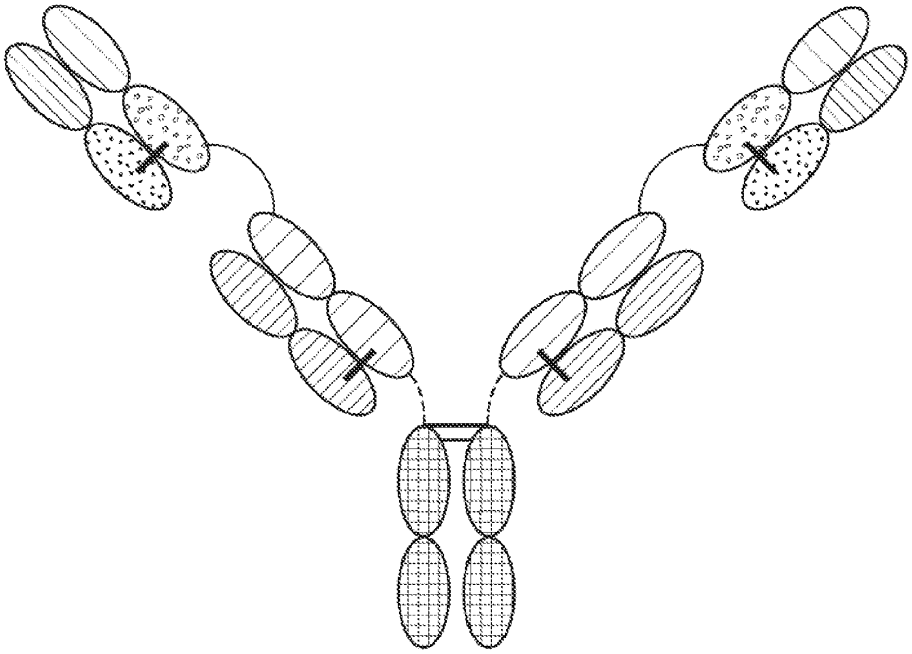


FIG. 17



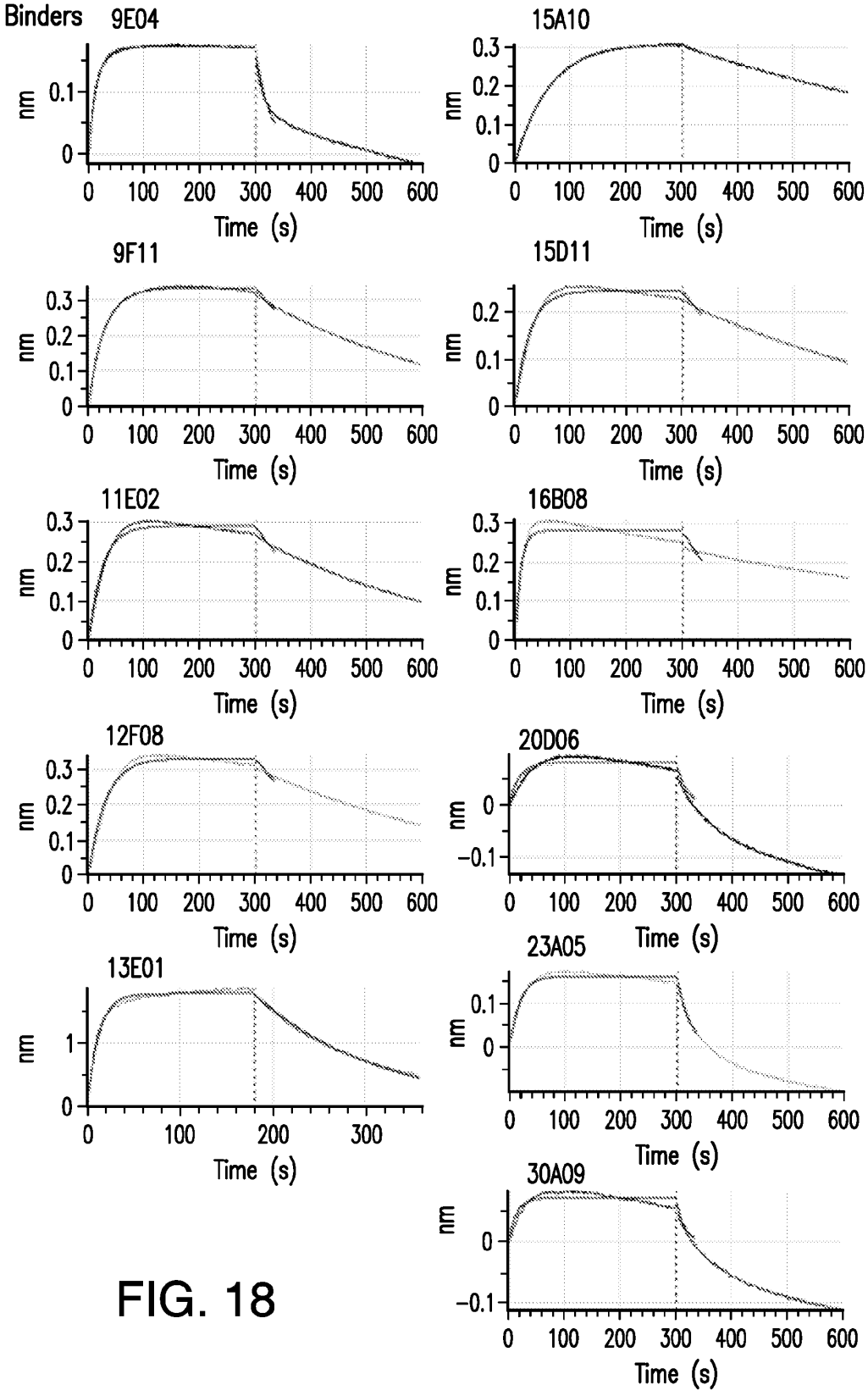

Binders
9E04
15A10
9F11
15D11
11E02
16B08
12F08
20D06
13E01
23A05
30A09
nm
Time (s)

FIG. 18

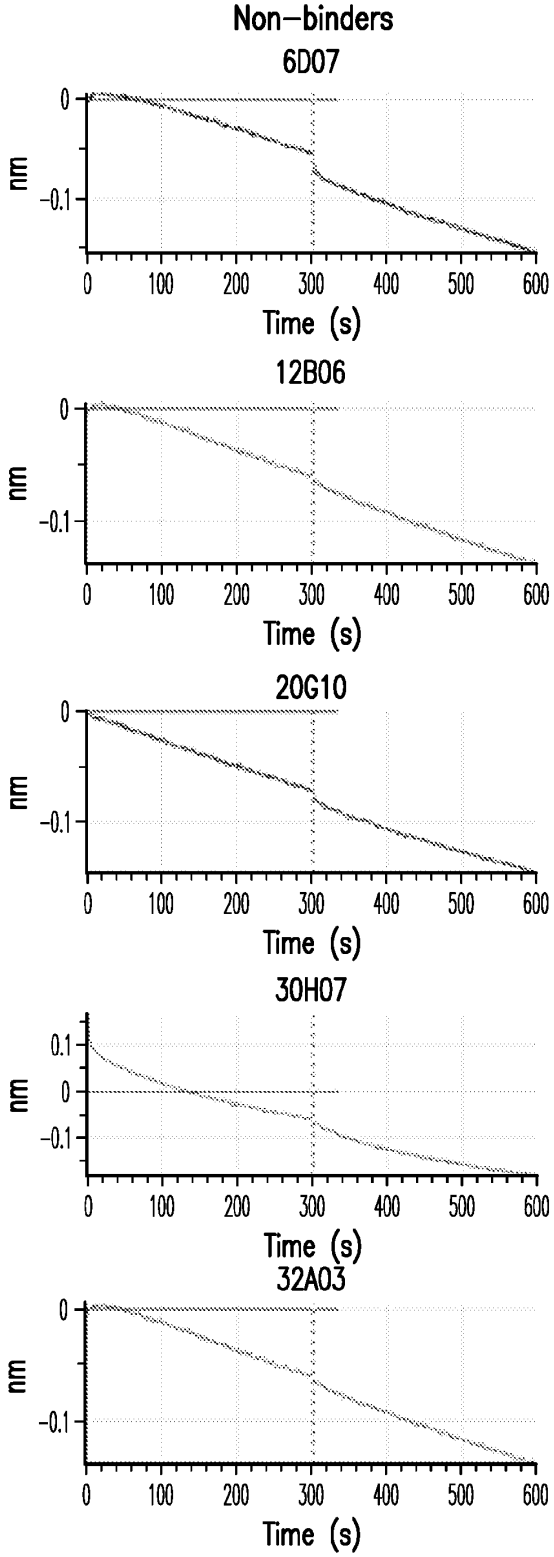


FIG. 18 Continued

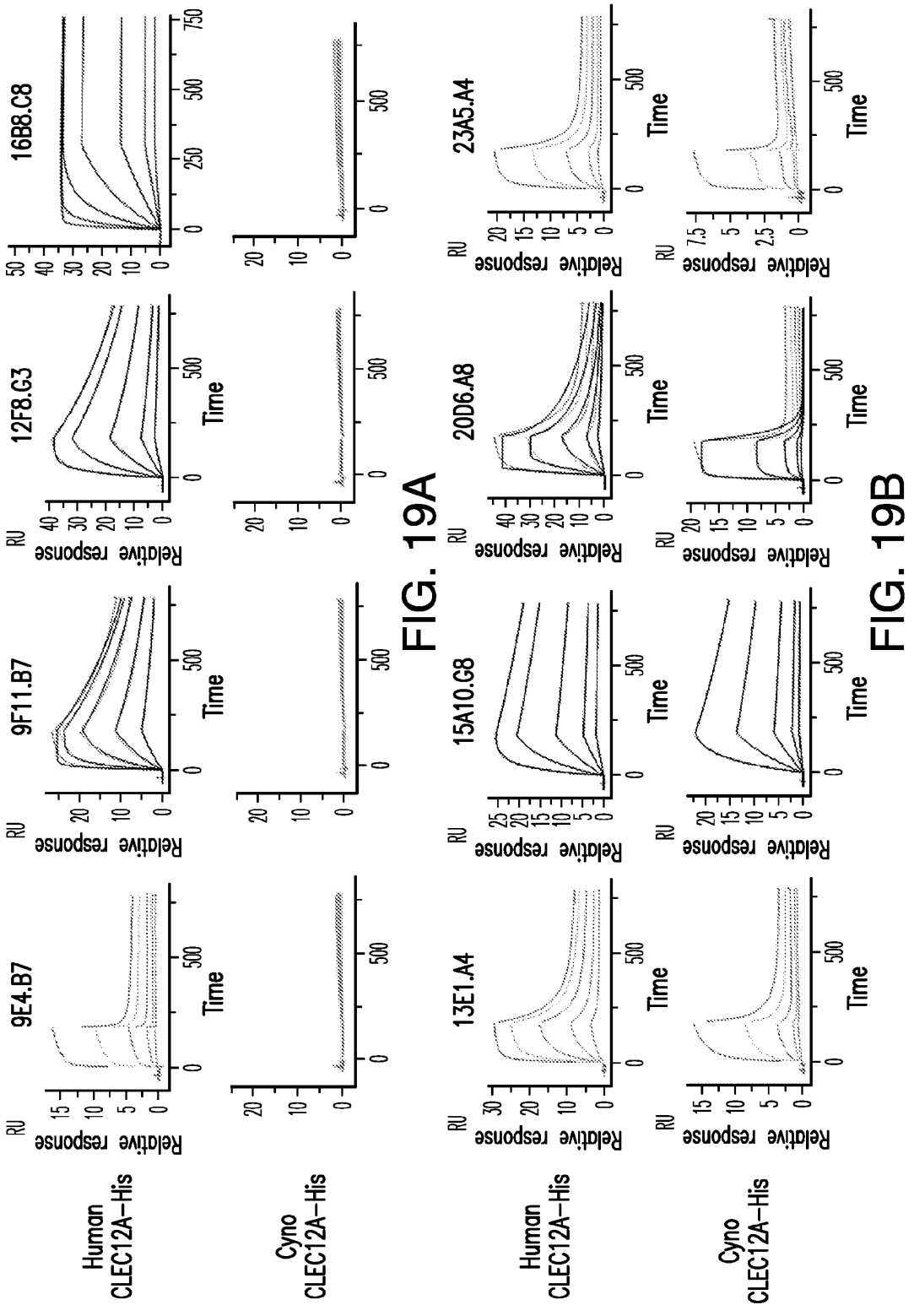
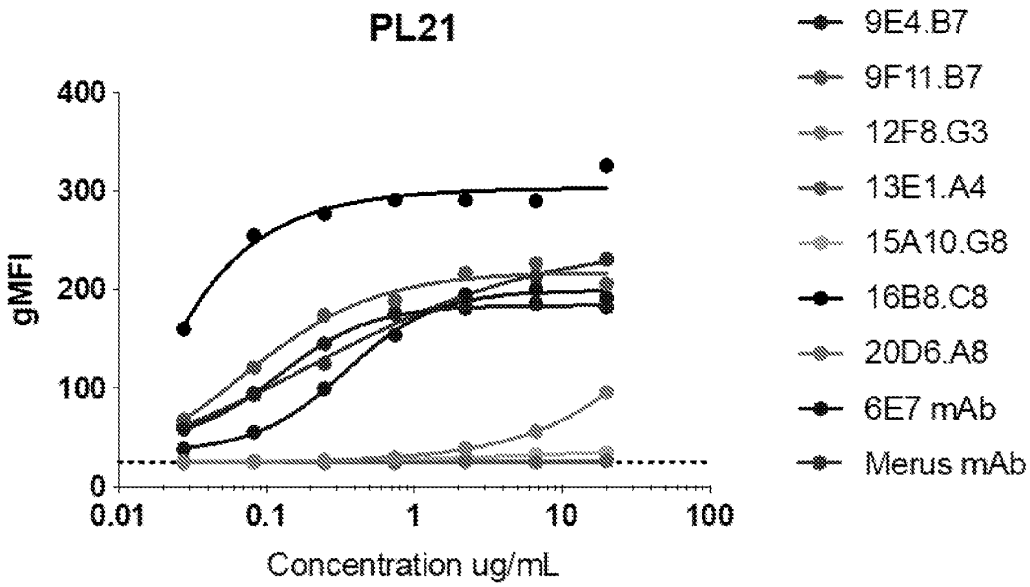


FIG. 19A

FIG. 19B

FIG. 20


PL21
gMFI
400
300
200
100
0
0.01
0.1
1
10
100
Concentration ug/mL
9E4.B7
9F11.B7
12F8.G3
13E1.A4
15A10.G8
16B8.C8
20D6.A8
6E7 mAb
Merus mAb

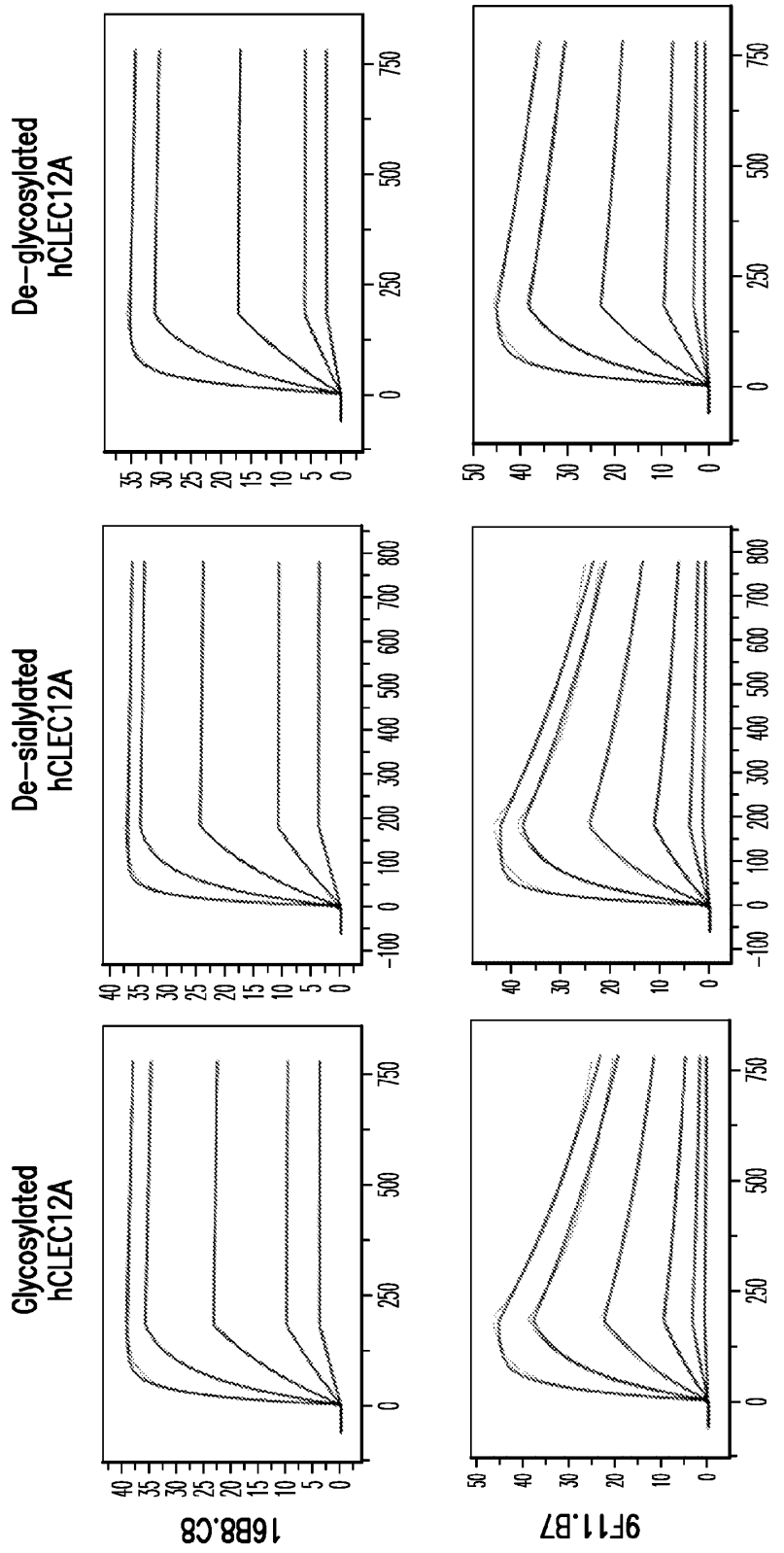

Glycosylated hCLEC12A
De−sialylated hCLEC12A
De−glycosylated hCLEC12A
16B8.C8
9F11.B7

FIG. 21

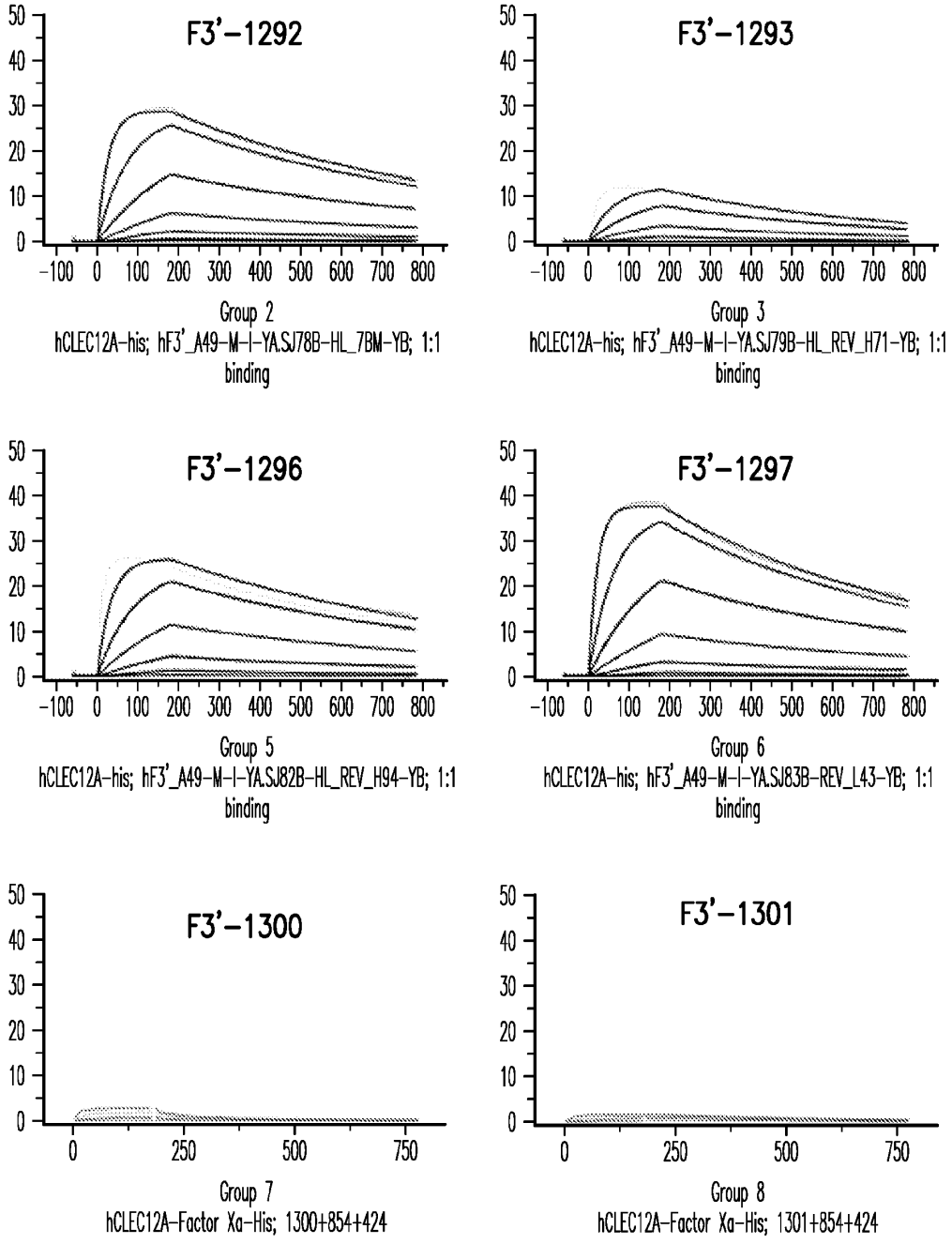

F3'-1292
Group 2
hCLEC12A-his; hF3'_A49-M-I-YA.SJ78B-HL_7BM-YB; 1:1 binding
F3'-1293
Group 3
hCLEC12A-his; hF3'_A49-M-I-YA.SJ79B-HL_REV_H71-YB; 1:1 binding
F3'-1296
Group 5
hCLEC12A-his; hF3'_A49-M-I-YA.SJ82B-HL_REV_H94-YB; 1:1 binding
F3'-1297
Group 6
hCLEC12A-his; hF3'_A49-M-I-YA.SJ83B-REV_L43-YB; 1:1 binding
F3'-1300
Group 7
hCLEC12A-Factor Xa-His; 1300+854+424
F3'-1301
Group 8
hCLEC12A-Factor Xa-His; 1301+854+424

FIG. 22

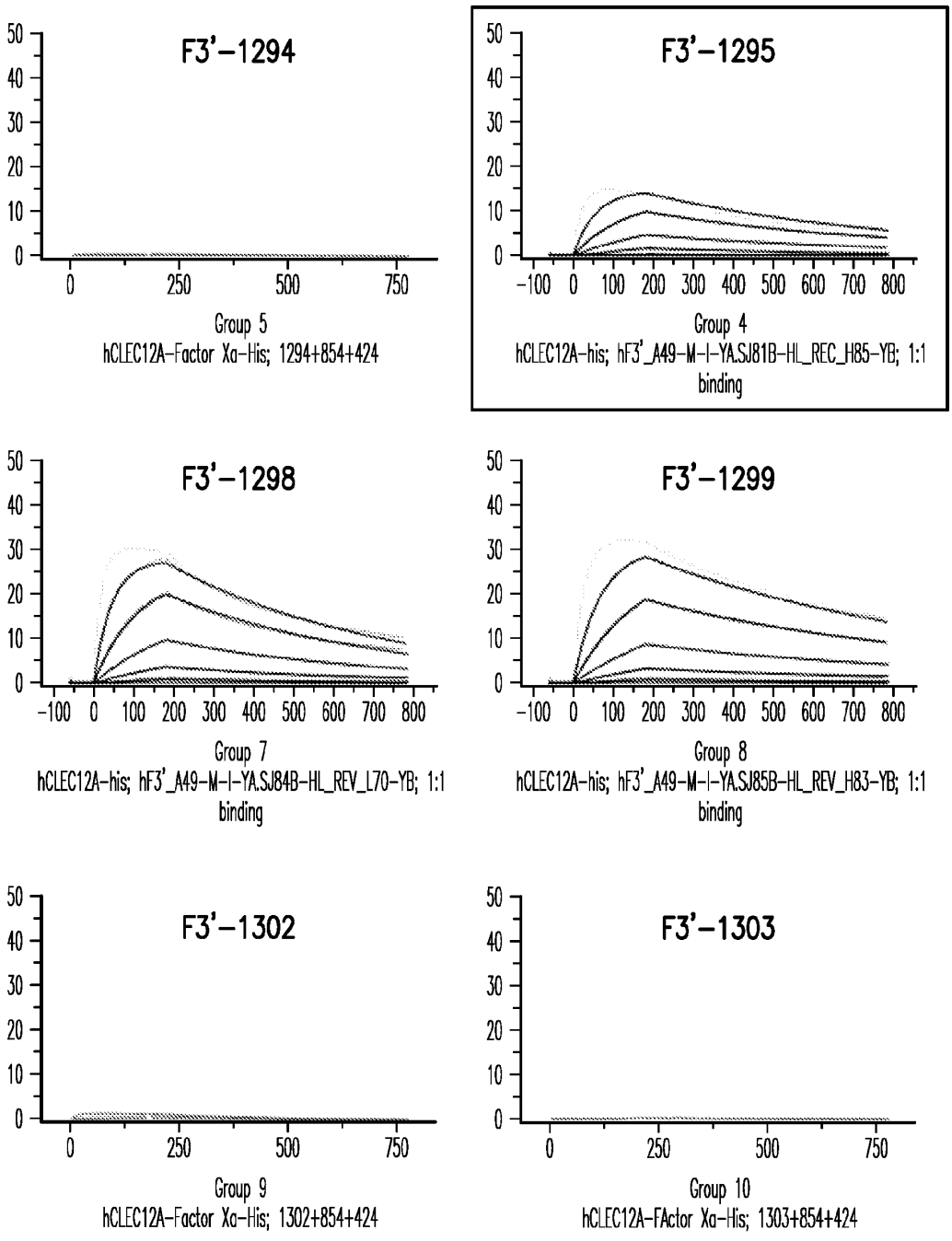


FIG. 22 Continued

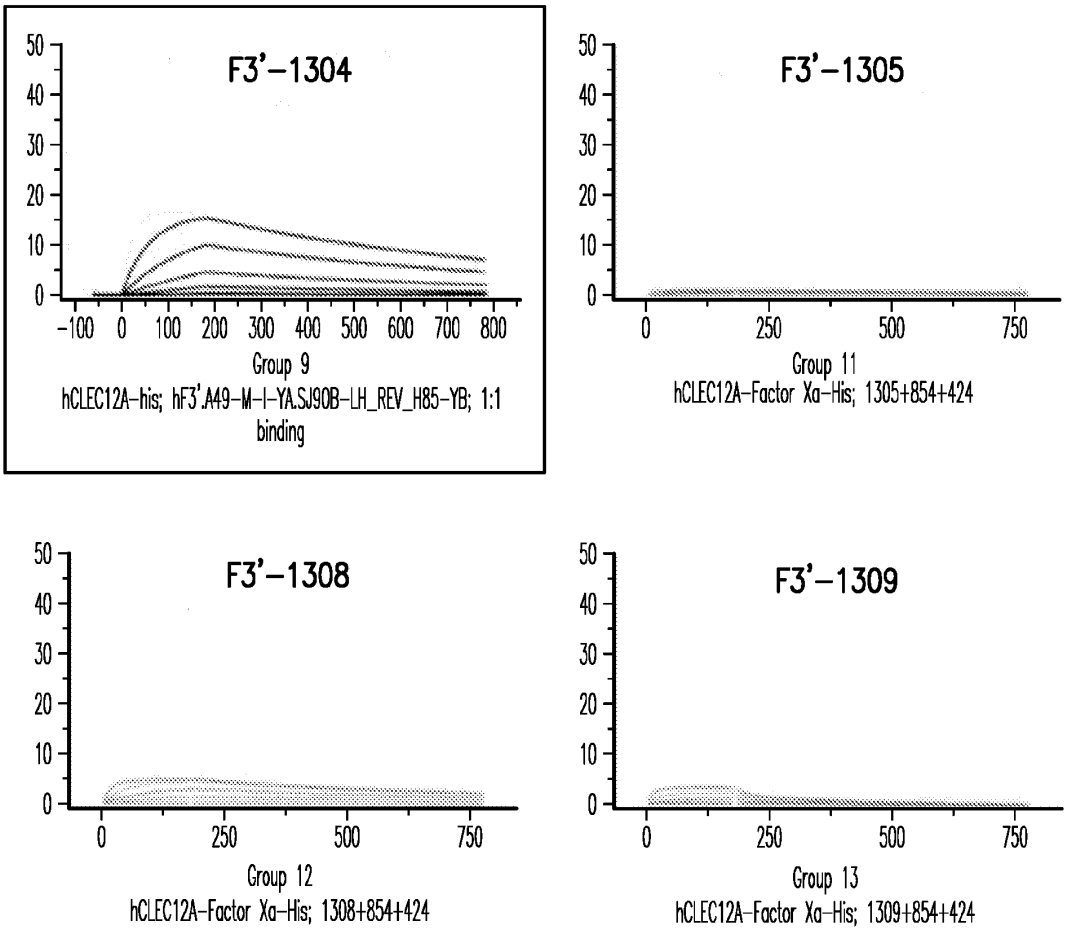


FIG. 22 Continued

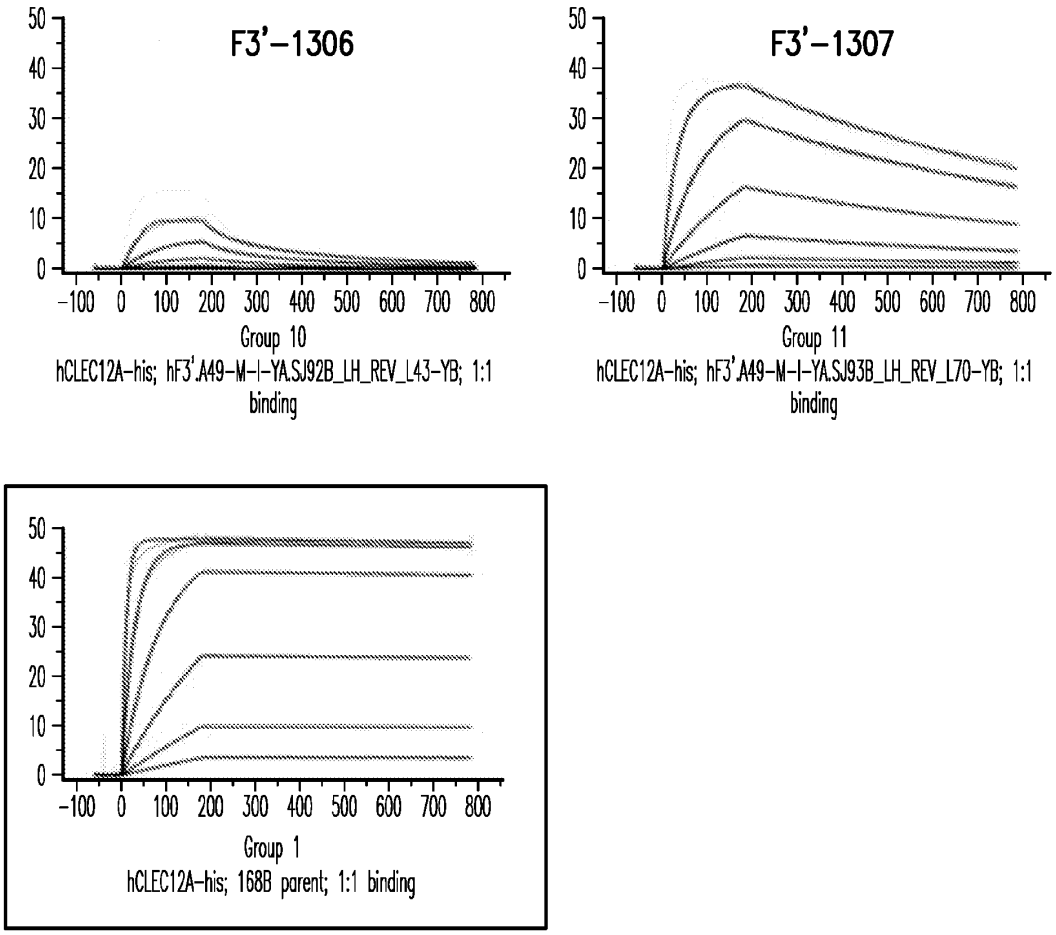


FIG. 22 Continued

FIG. 23

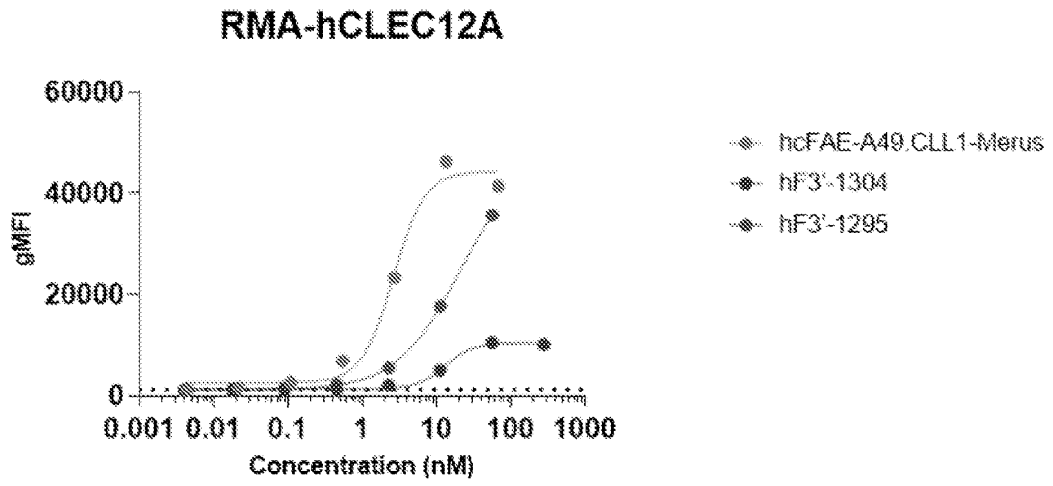
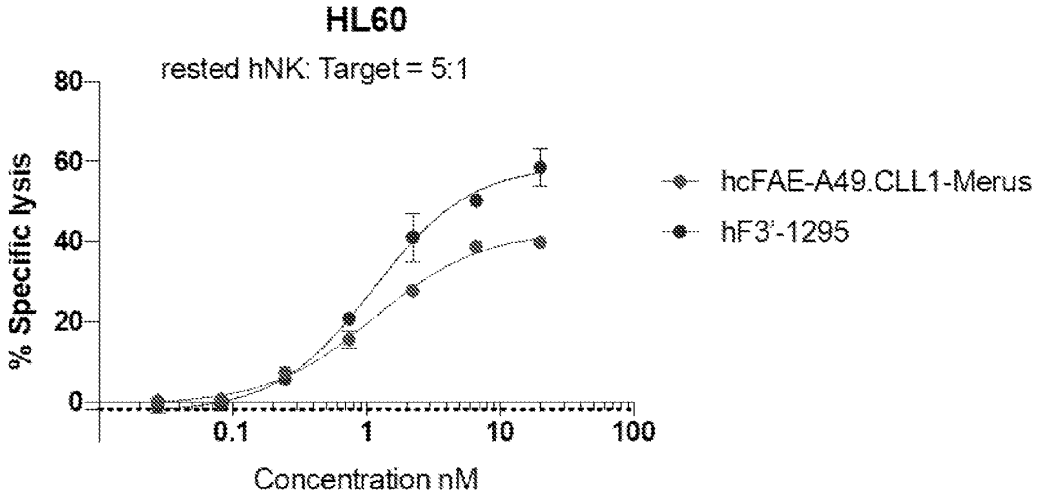

RMA-hCLEC12A
60000
40000
20000
0
gMFI
0.001 0.01 0.1 1 10 100 1000
Concentration (nM)
hcFAE-A49.CLL1-Merus
hF3'-1304
hF3'-1295

FIG. 24


HL60
rested hNK: Target = 5:1
80
60
40
20
0
% Specific lysis
0.1 1 10 100
Concentration nM
hcFAE-A49.CLL1-Merus
hF3'-1295

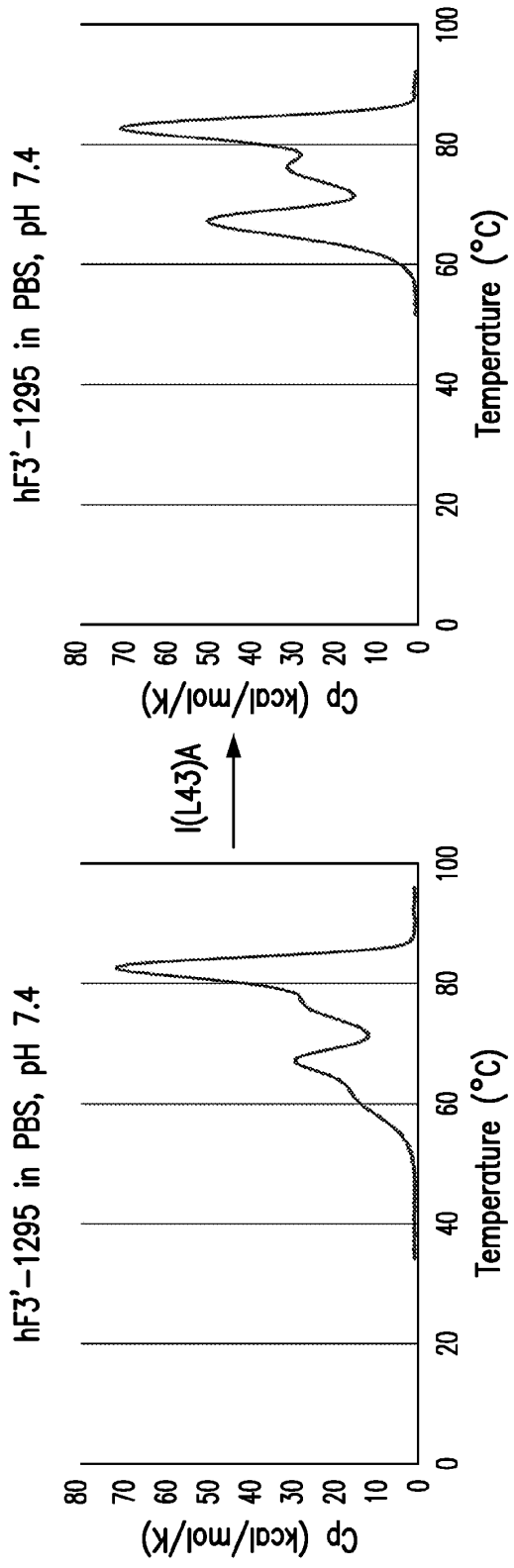

hF3'-1295 in PBS, pH 7.4
Cp (kcal/mol/K)
Temperature (°C)
I(L43)A
hF3'-1295 in PBS, pH 7.4
Cp (kcal/mol/K)
Temperature (°C)

FIG. 25

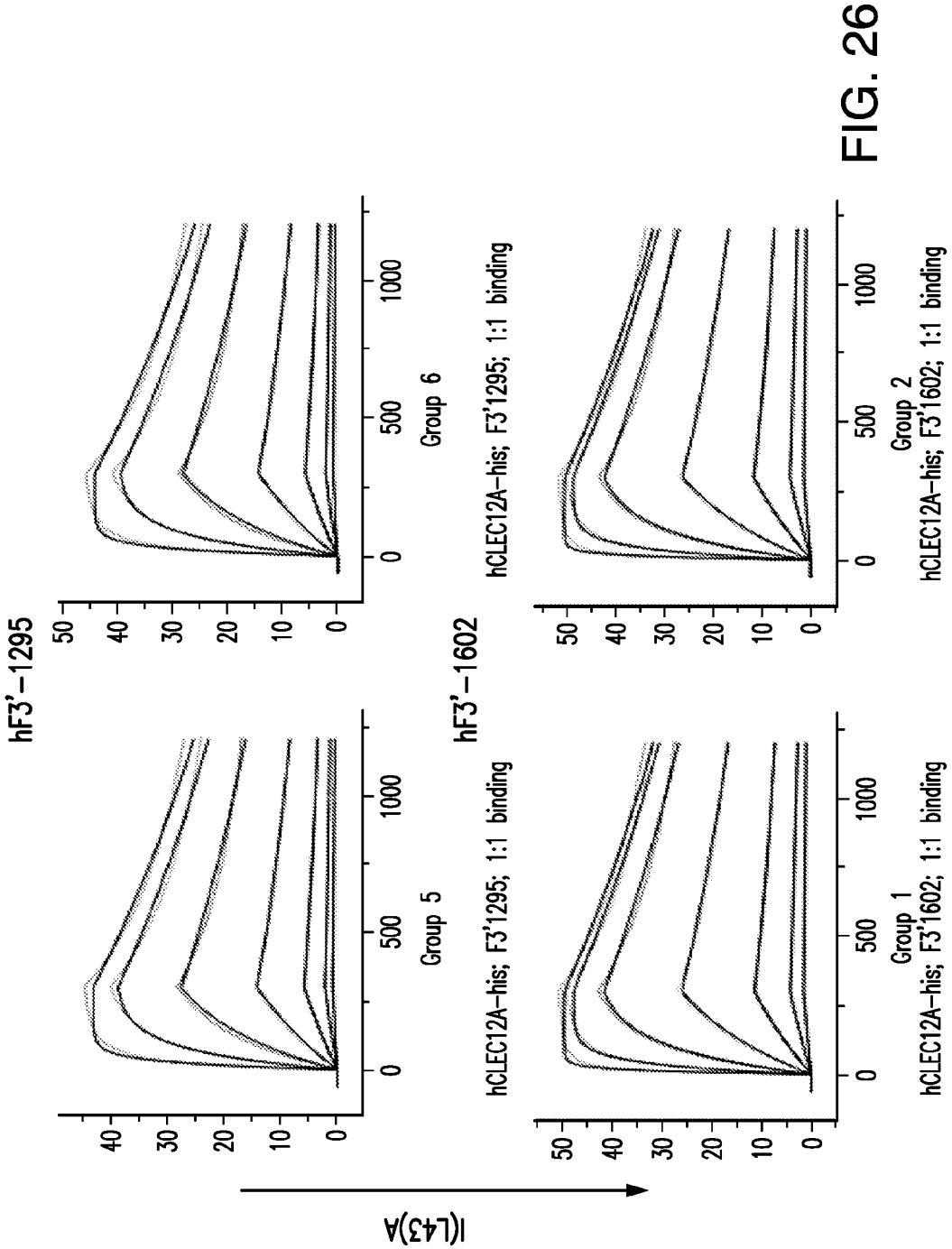

hF3'-1295
hF3'-1602
I(L43)A
hCLEC12A-his; F3'1295; 1:1 binding
hCLEC12A-his; F3'1602; 1:1 binding
Group 5
Group 6
Group 1
Group 2
0
10
20
30
40
50
500
1000

FIG. 26

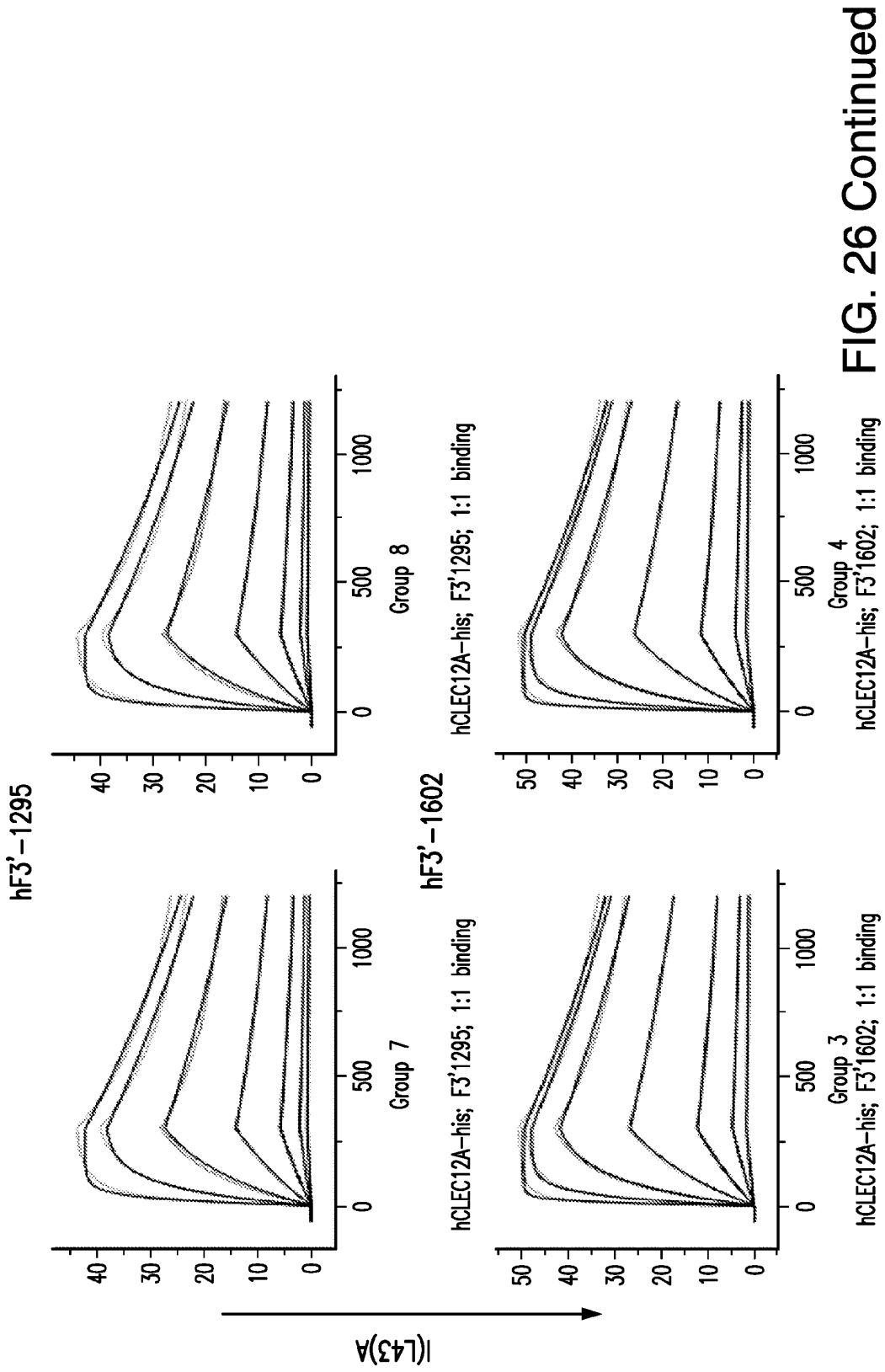


FIG. 26 Continued

FIG. 27A

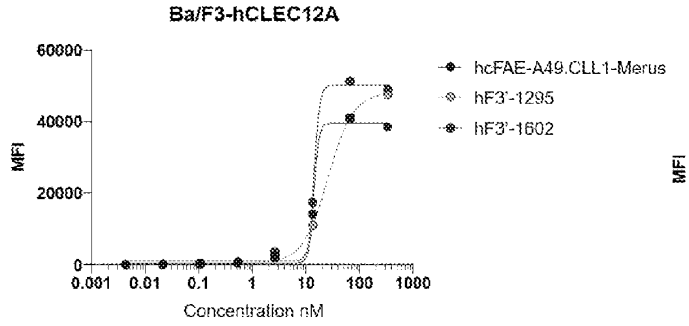


FIG. 27B

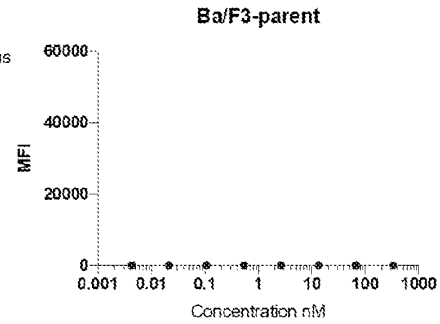


FIG. 27C

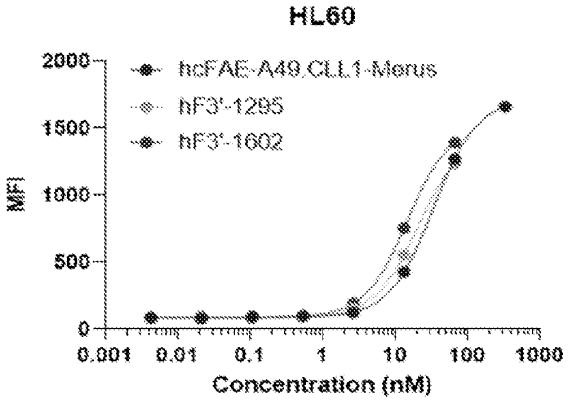


FIG. 27D

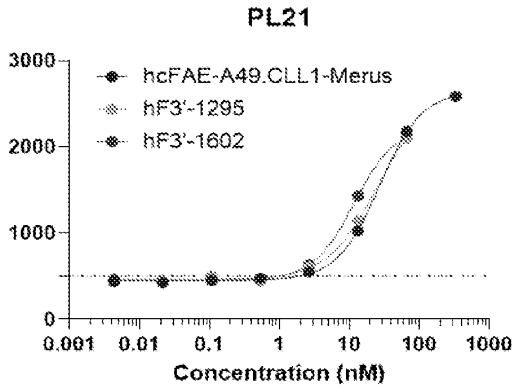


FIG. 28A

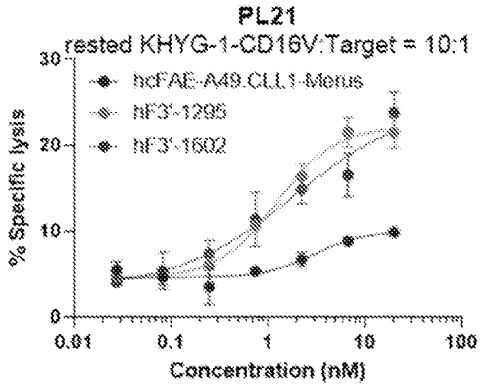
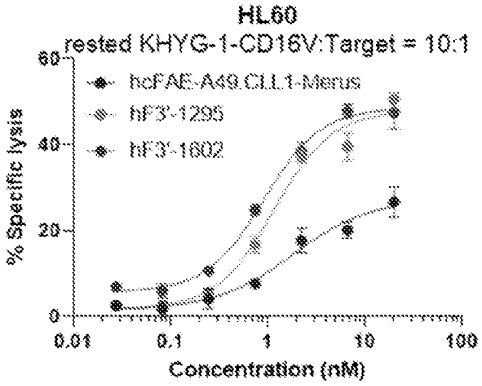


FIG. 28B



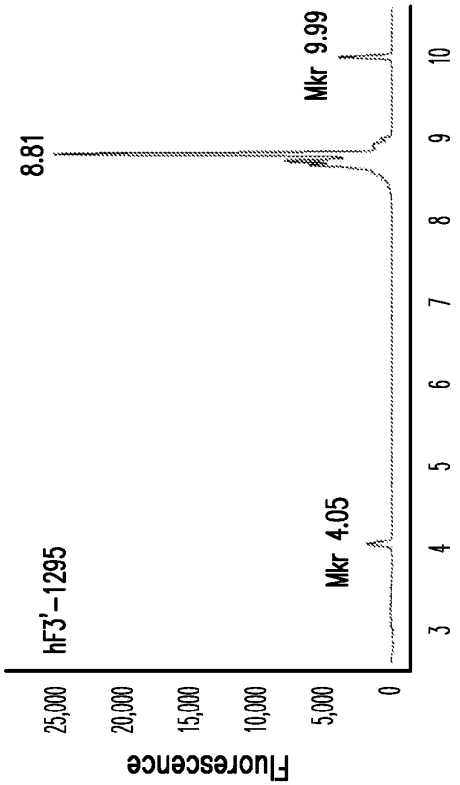


FIG. 29B

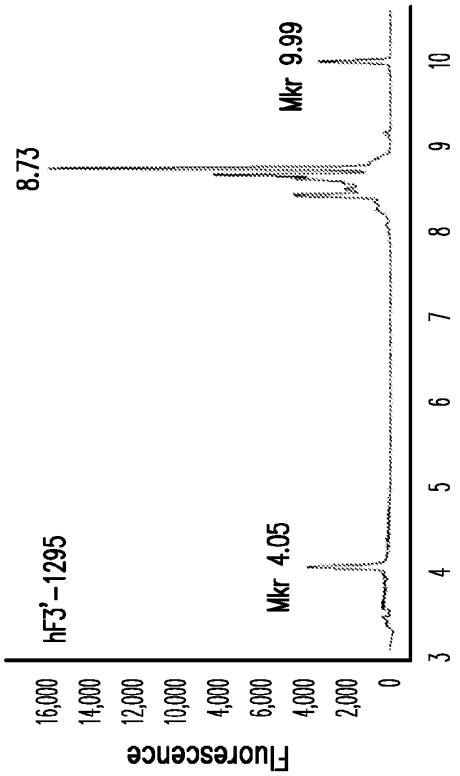


FIG. 29A

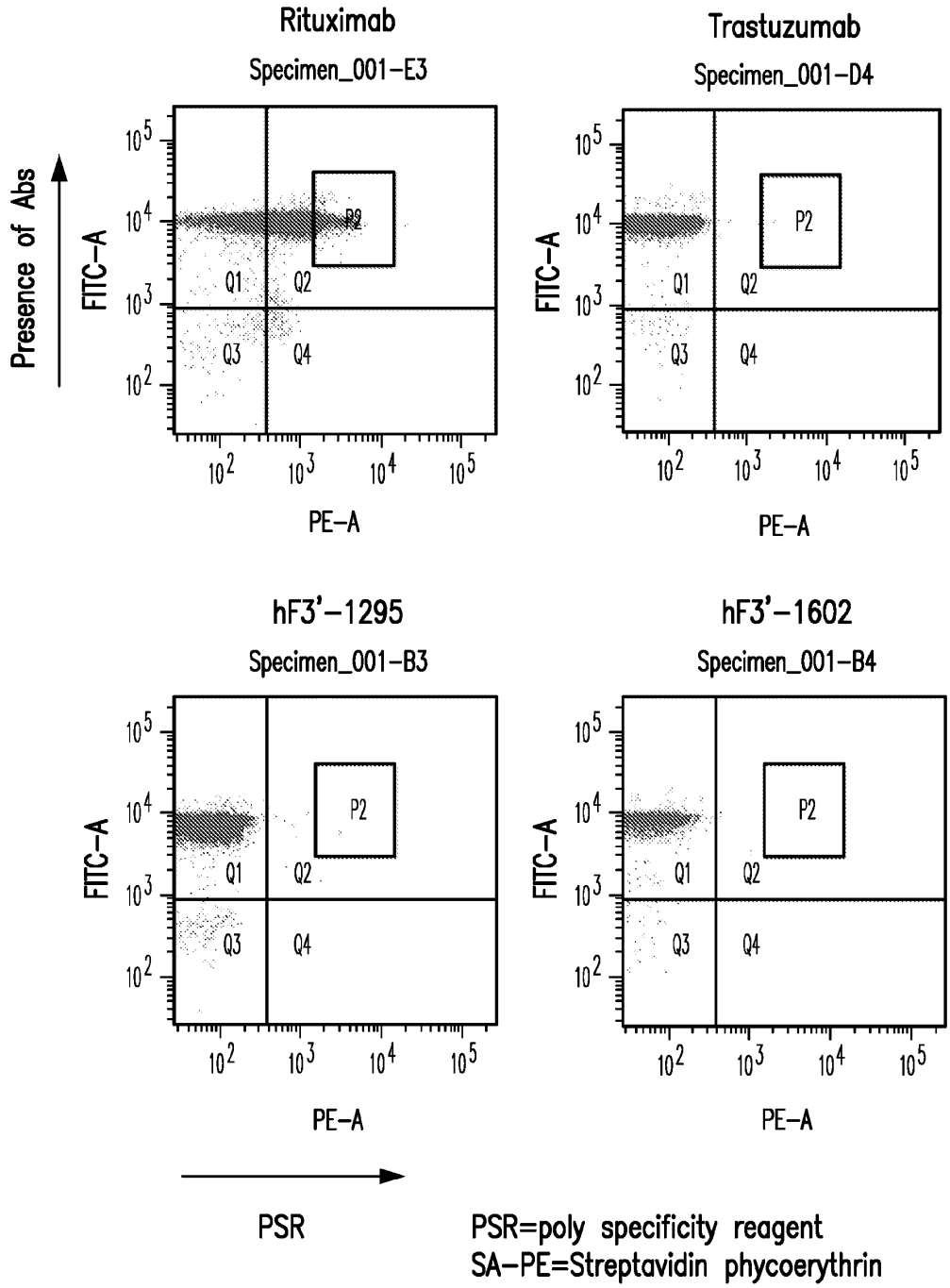

Rituximab
Specimen_001-E3
Trastuzumab
Specimen_001-D4
hF3'-1295
Specimen_001-B3
hF3'-1602
Specimen_001-B4
Presence of Abs
FITC-A
PE-A
Q1
Q2
Q3
Q4
P2
PSR
PSR=poly specificity reagent
SA-PE=Streptavidin phycoerythrin

FIG. 30

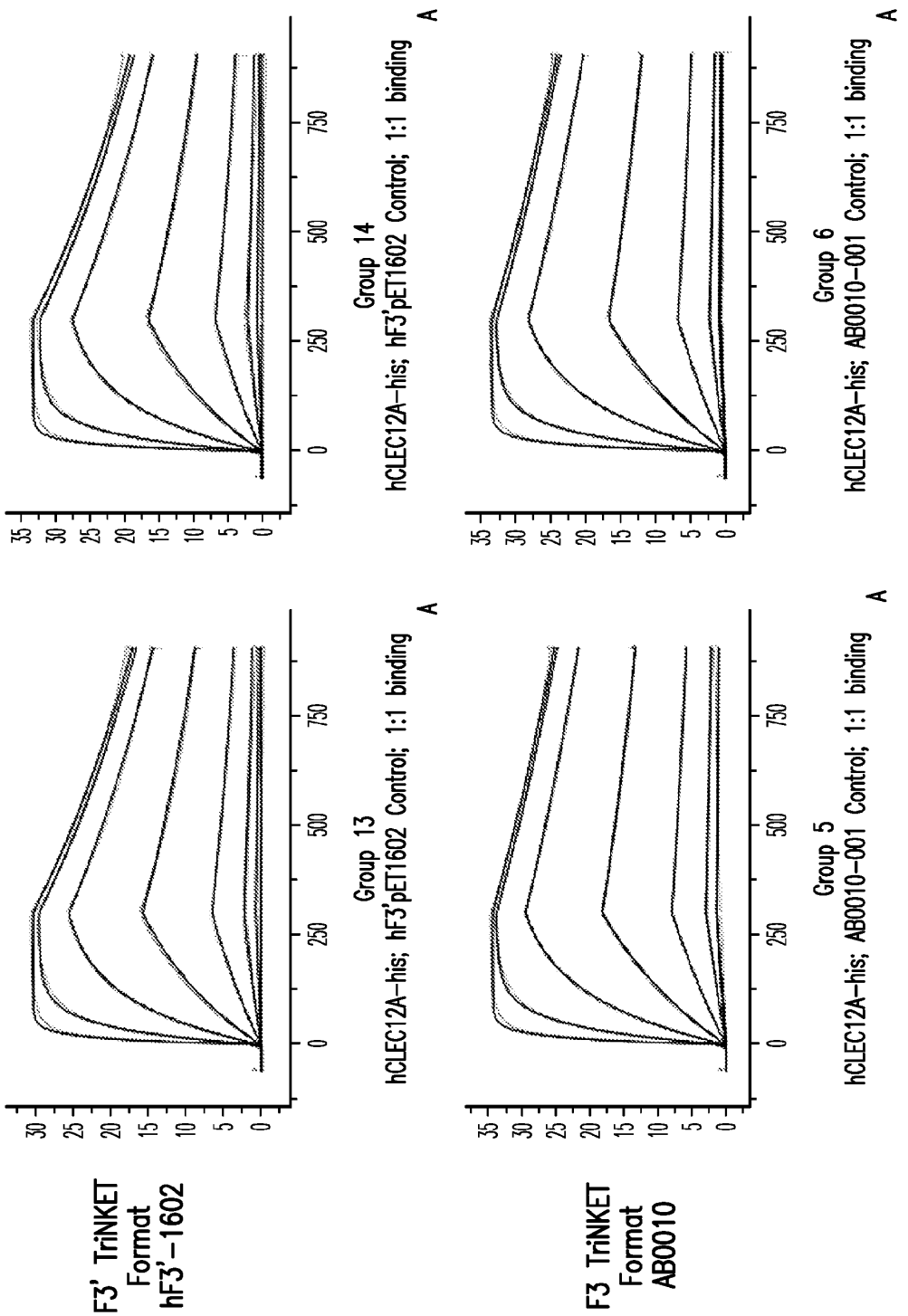

F3' TriNKET Format hF3'-1602
F3 TriNKET Format AB0010
Group 13
hCLEC12A-his; hF3'pET1602 Control; 1:1 binding
A
Group 14
hCLEC12A-his; hF3'pET1602 Control; 1:1 binding
A
Group 5
hCLEC12A-his; AB0010-001 Control; 1:1 binding
A
Group 6
hCLEC12A-his; AB0010-001 Control; 1:1 binding
A

FIG. 31

FIG. 32A

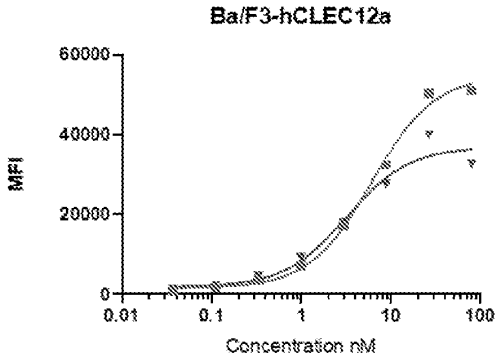


FIG. 32B

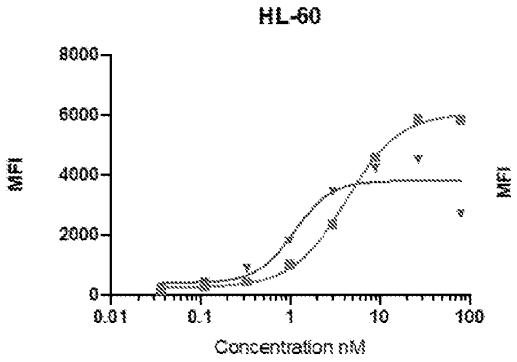


FIG. 32C

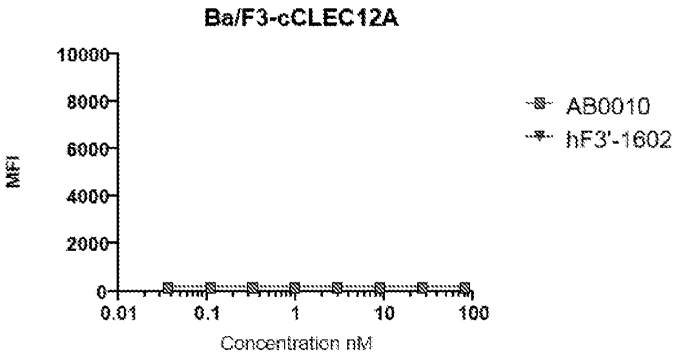


FIG. 33A

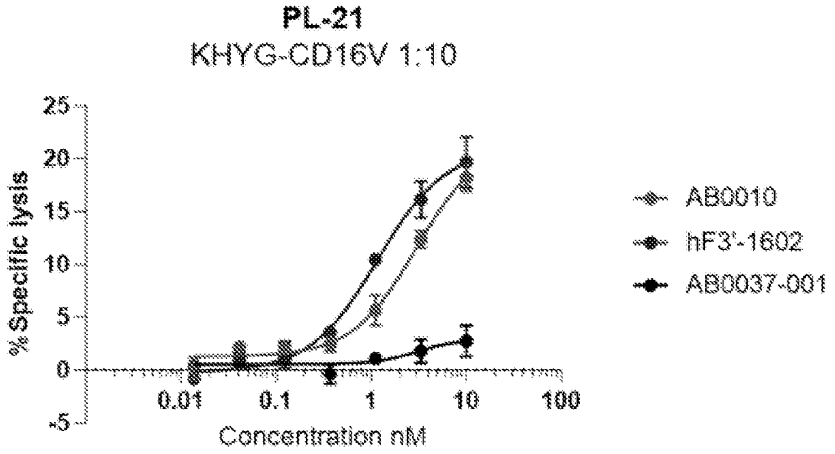


FIG. 33B

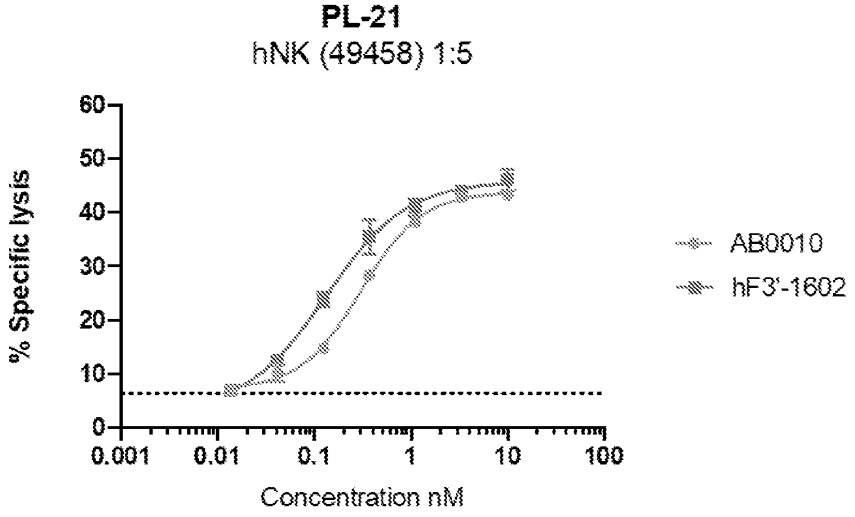


FIG. 34A

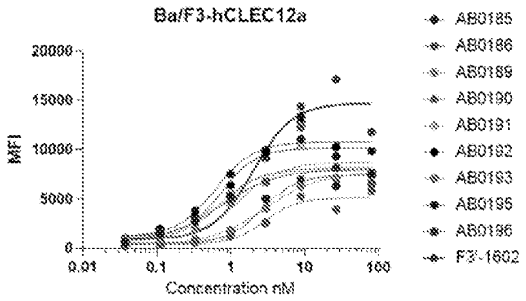


FIG. 34B

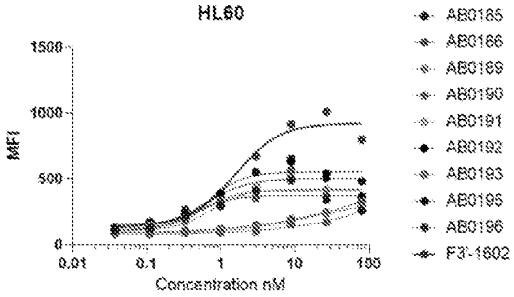


FIG. 35

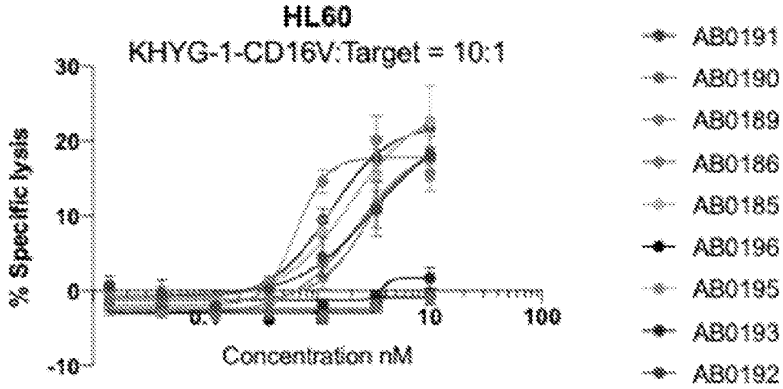


FIG. 36A

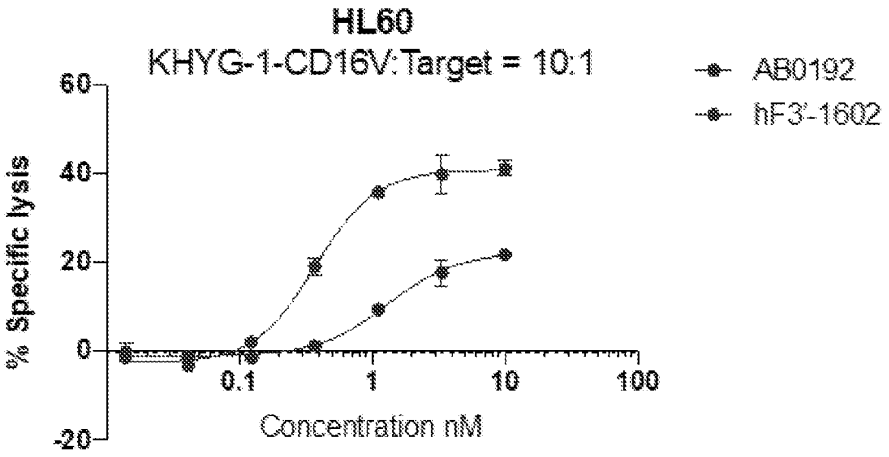


FIG. 36B

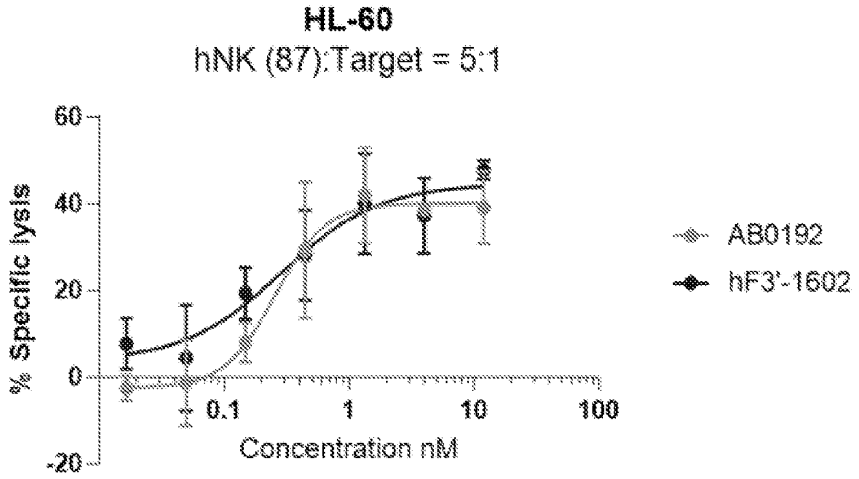


FIG. 37A

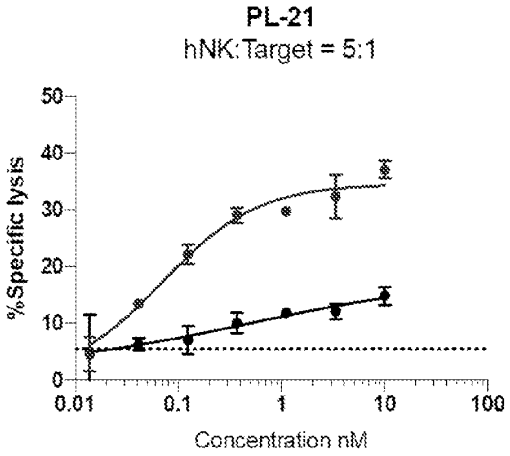


FIG. 37B

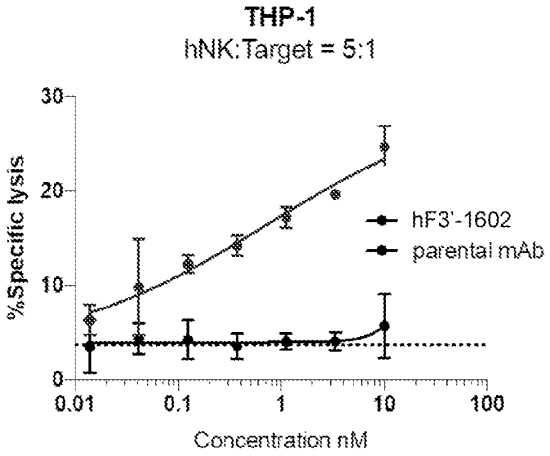


FIG. 38A

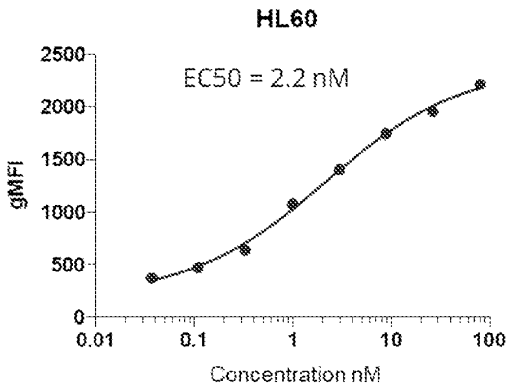


FIG. 38B

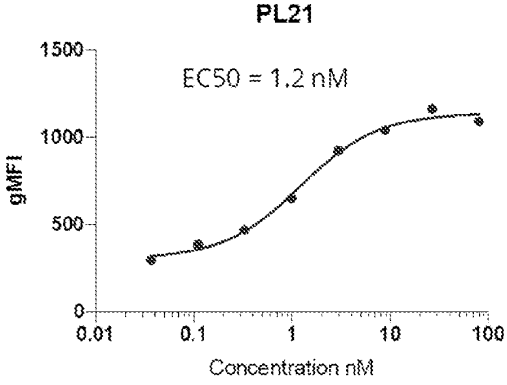


FIG. 39A

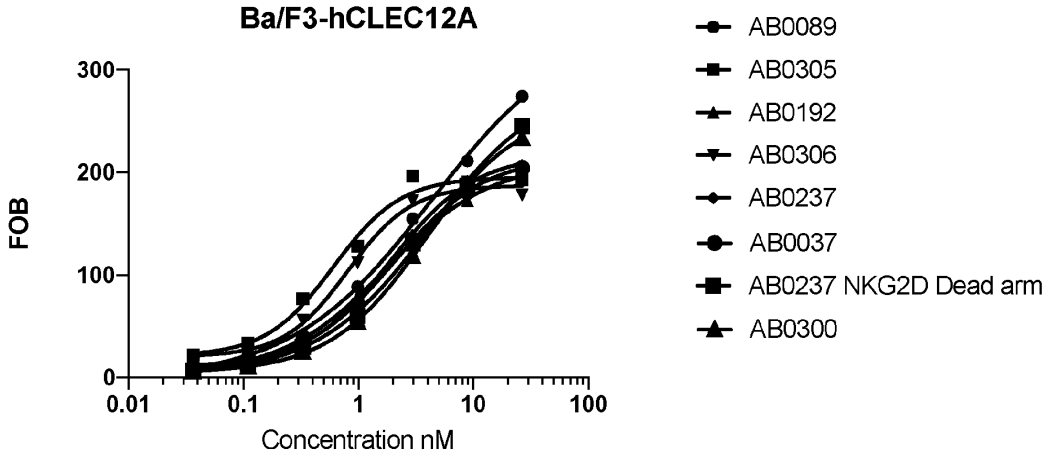


FIG. 39B

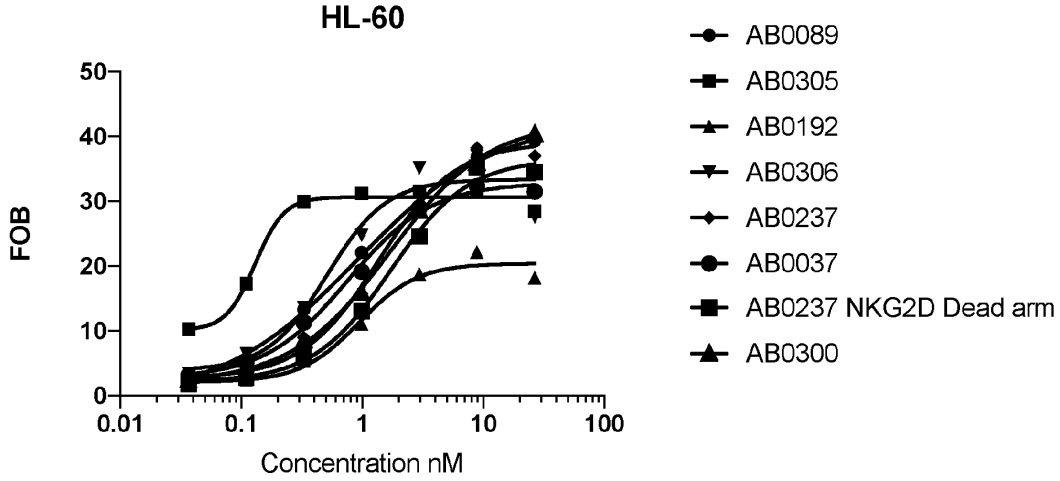


FIG. 39C

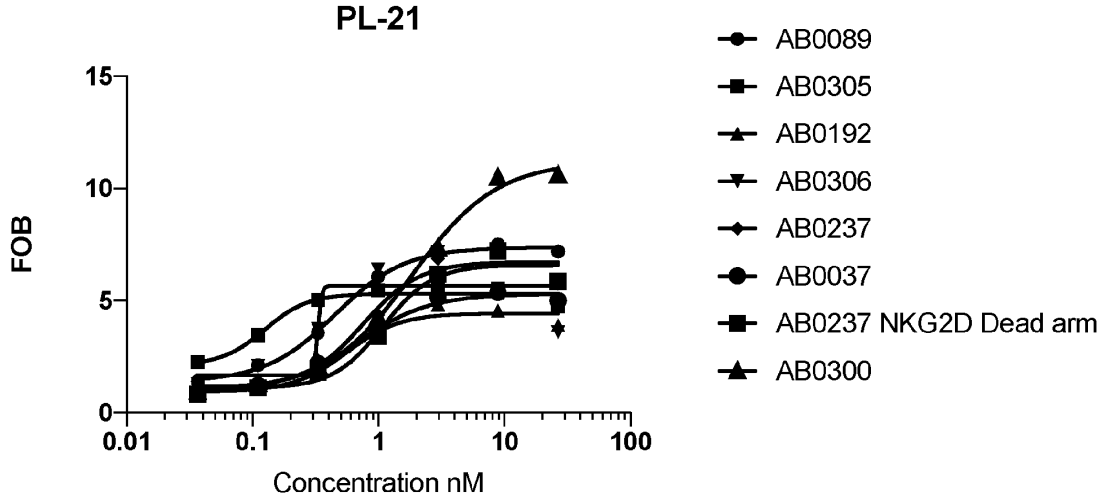

PL-21
FOB
15
10
5
0
0.01
0.1
1
10
100
Concentration nM
AB0089
AB0305
AB0192
AB0306
AB0237
AB0037
AB0237 NKG2D Dead arm
AB0300

FIG. 40A

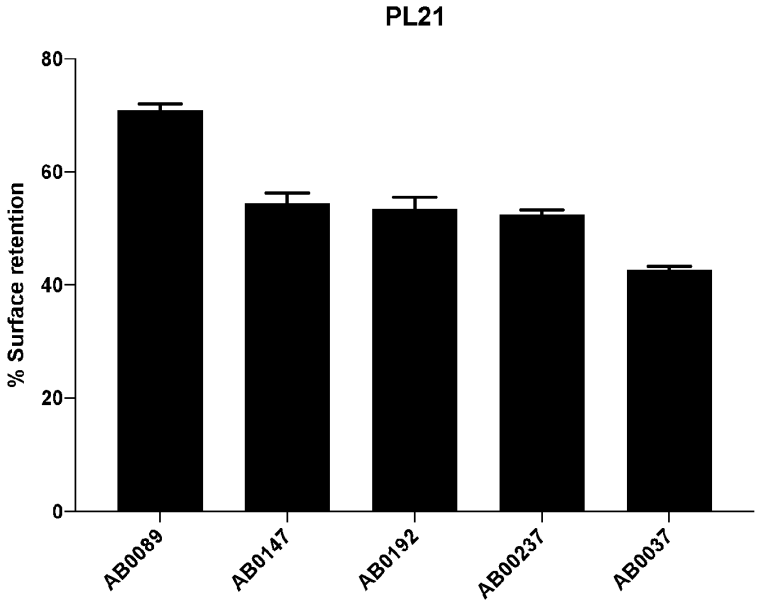


FIG. 40B

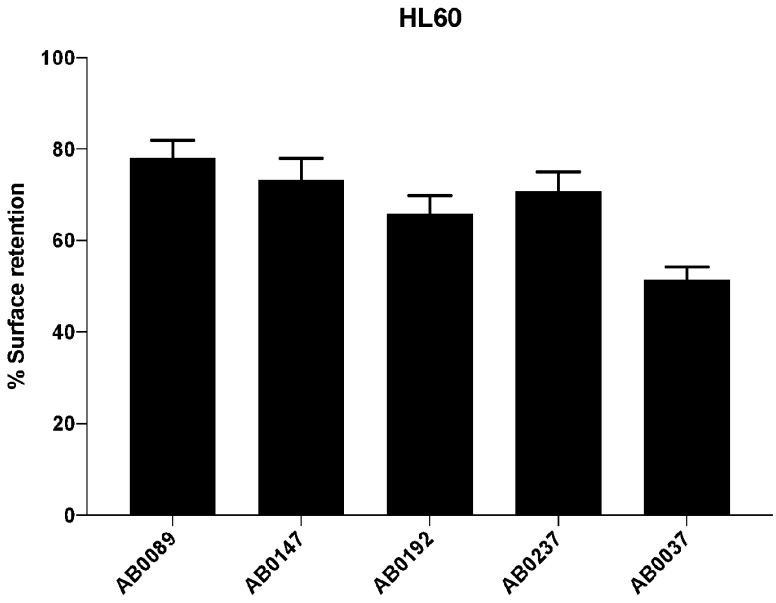


FIG. 41A

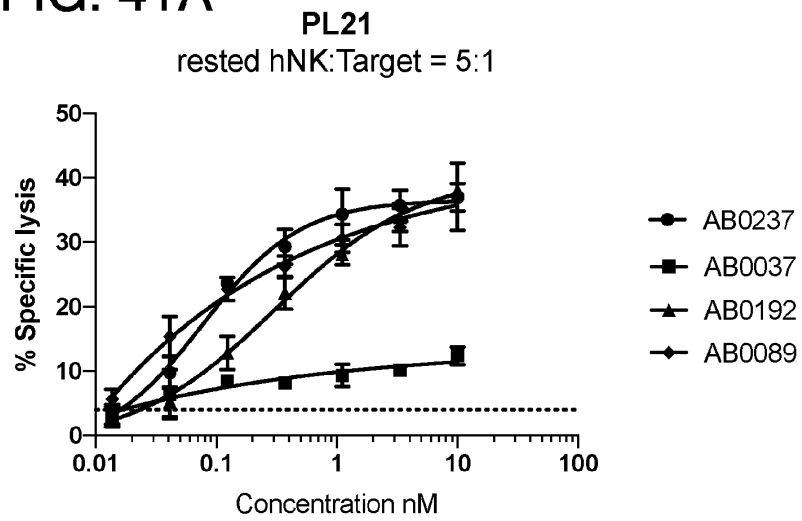


FIG. 41B

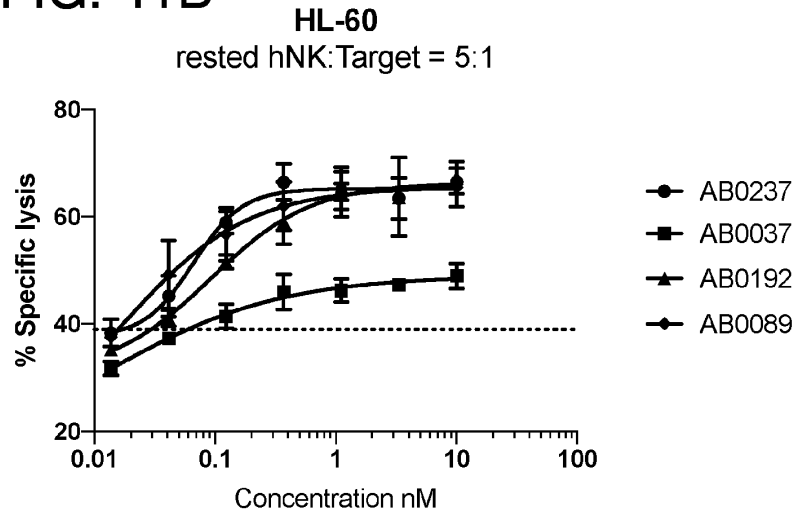


FIG. 42A

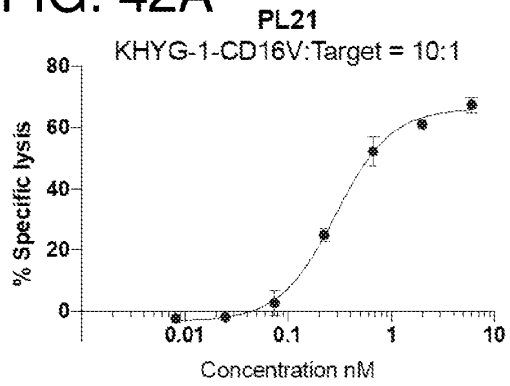


FIG. 42B

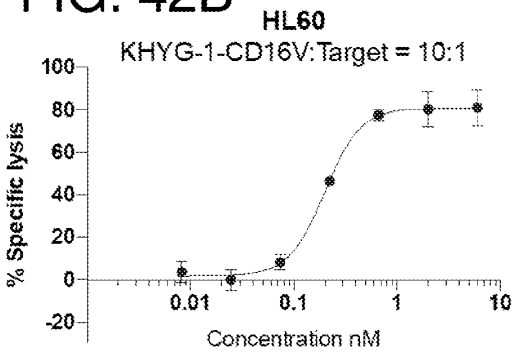


FIG. 43

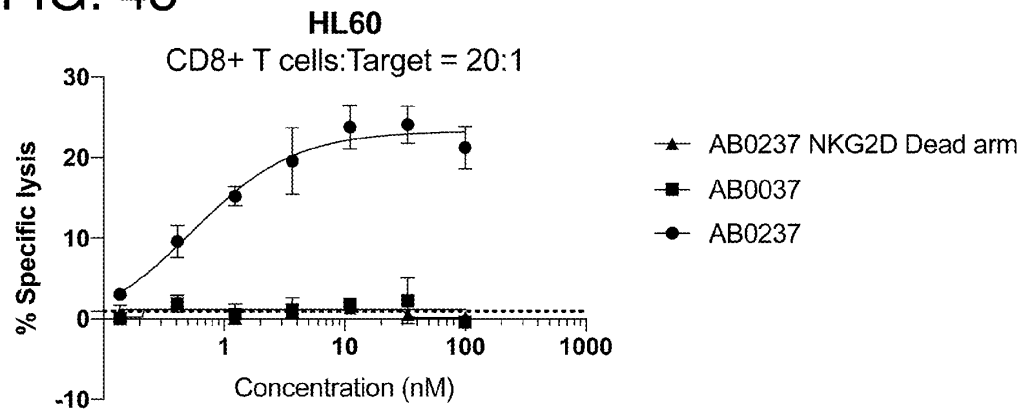
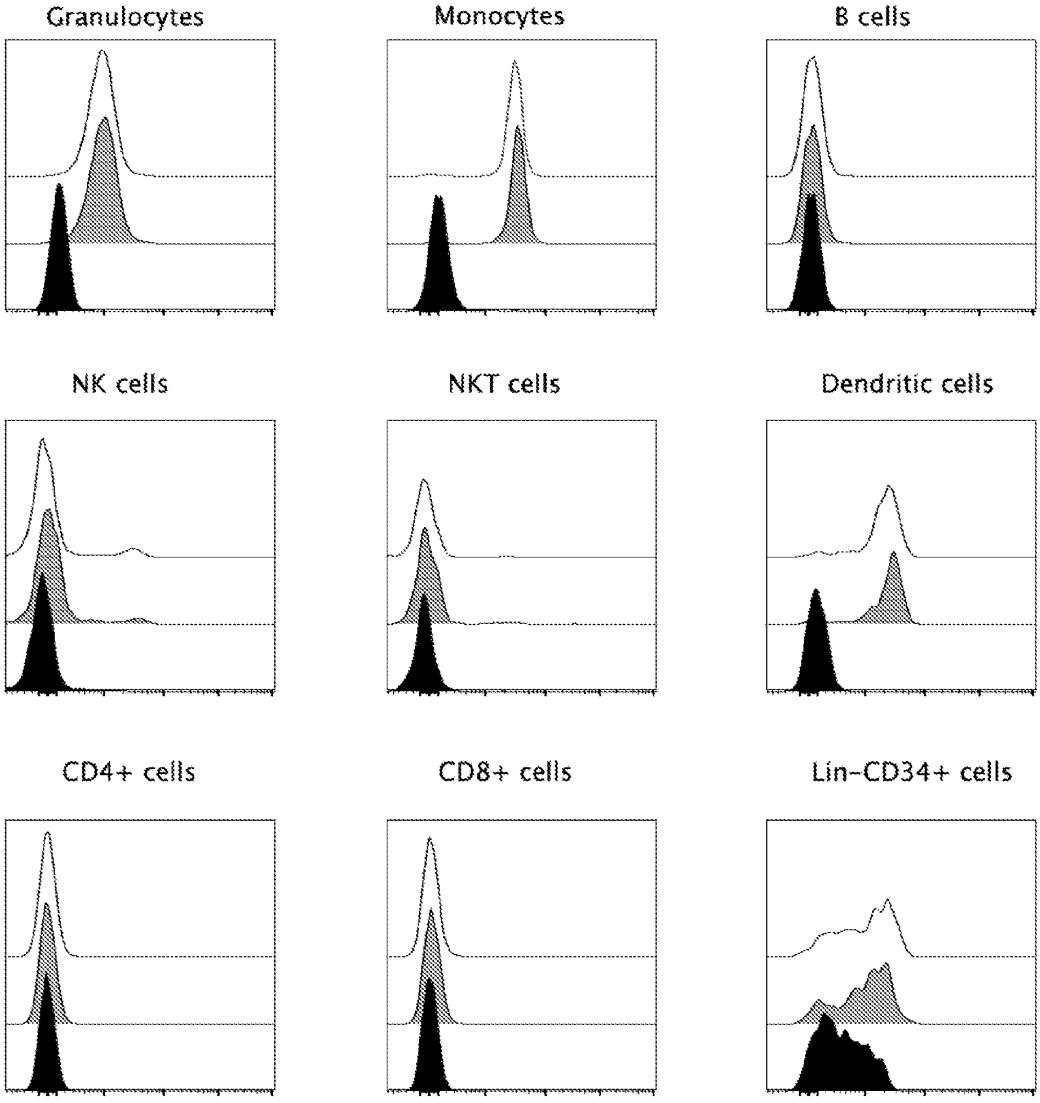


FIG. 44


Granulocytes
Monocytes
B cells
NK cells
NKT cells
Dendritic cells
CD4+ cells
CD8+ cells
Lin-CD34+ cells

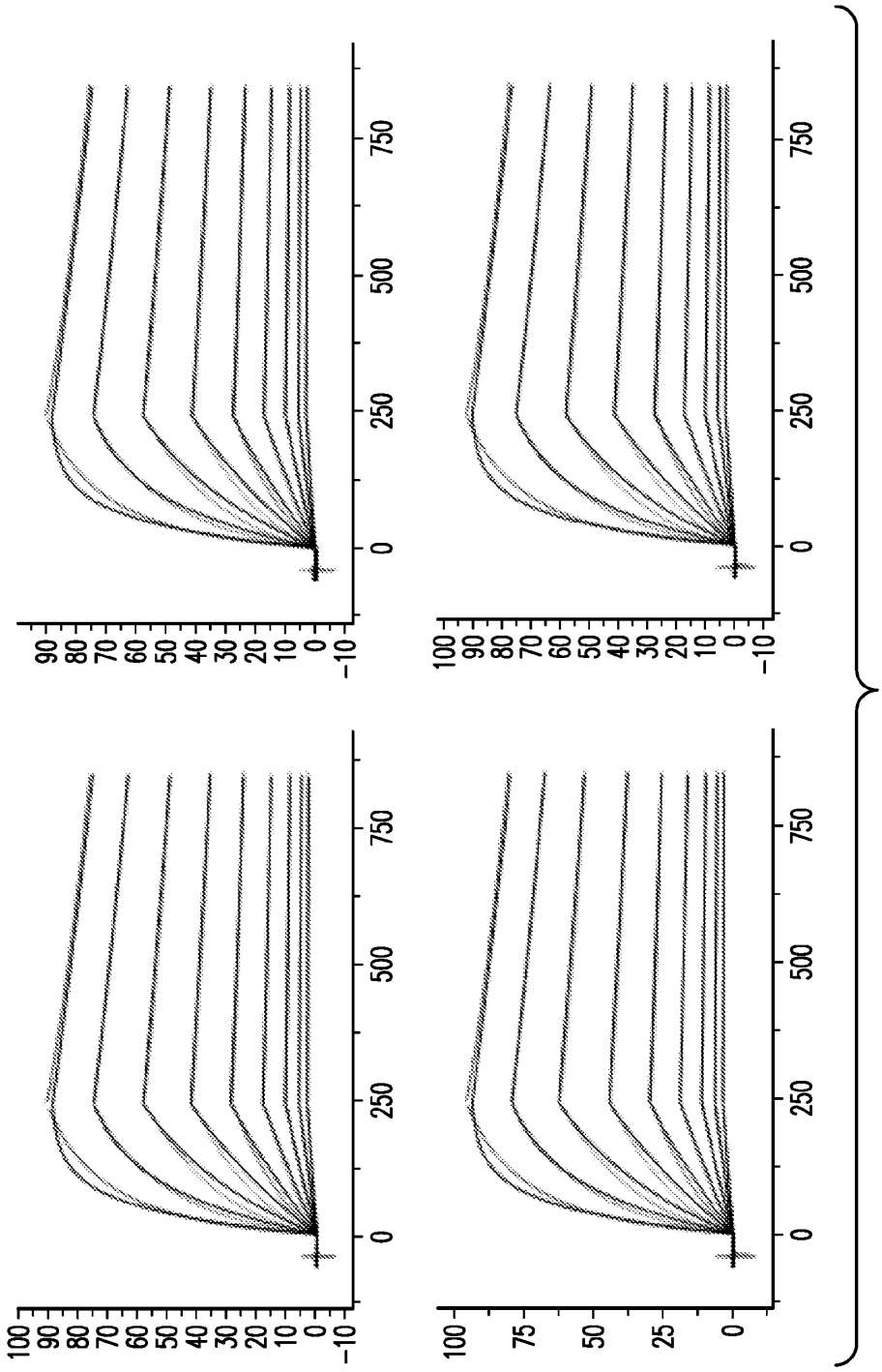


FIG. 45

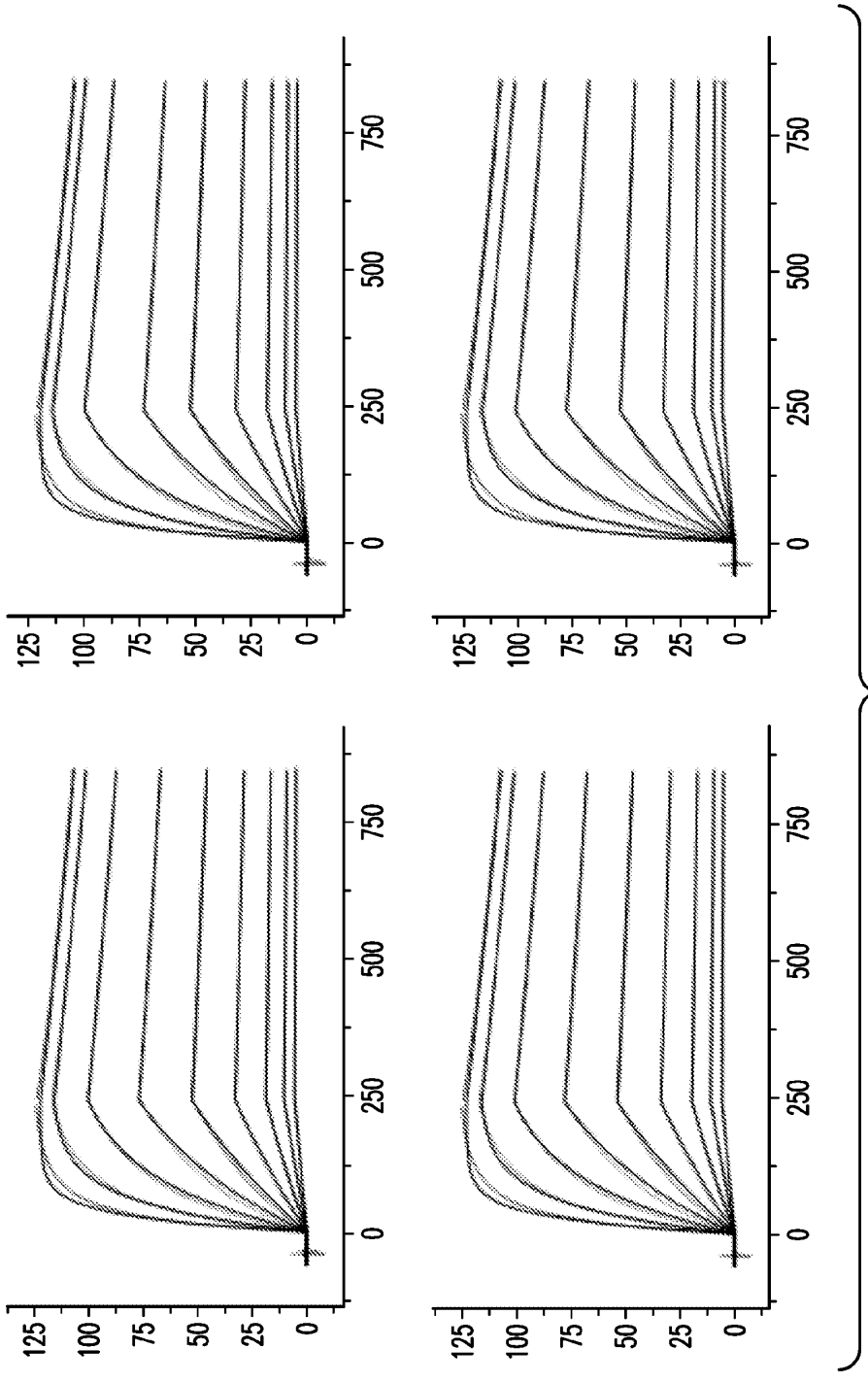


FIG. 45 Continued

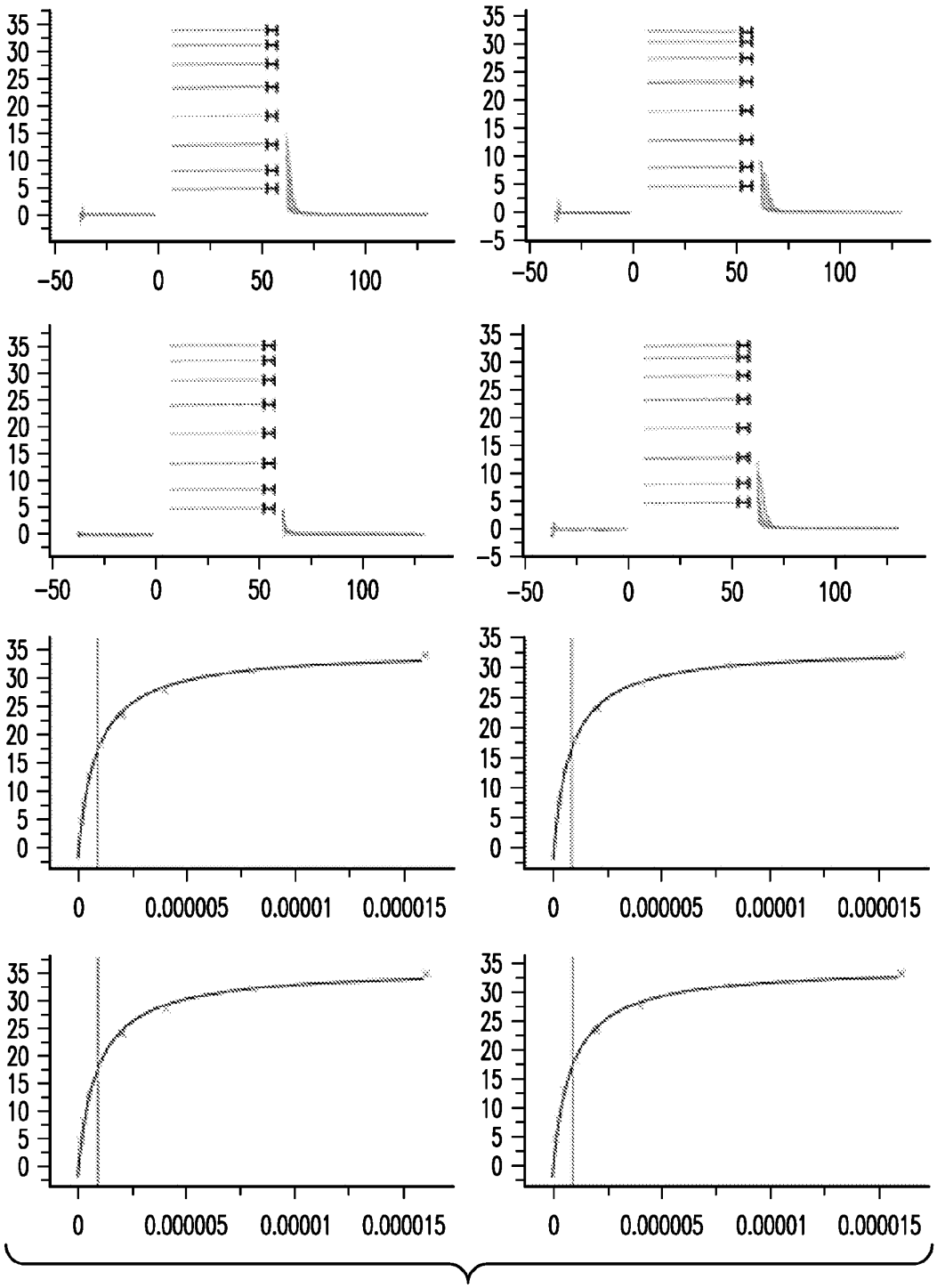


FIG. 46

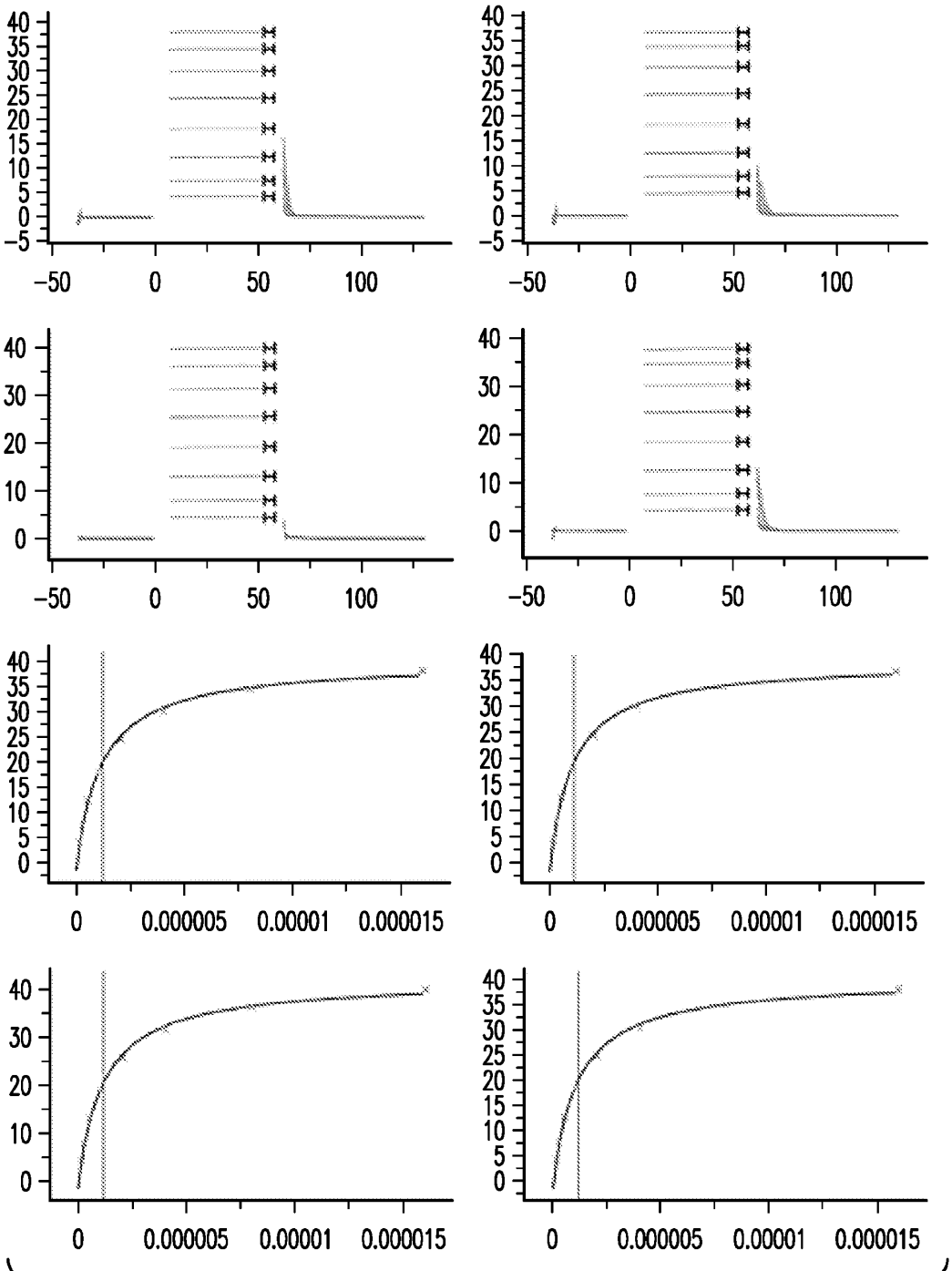


FIG. 46 Continued

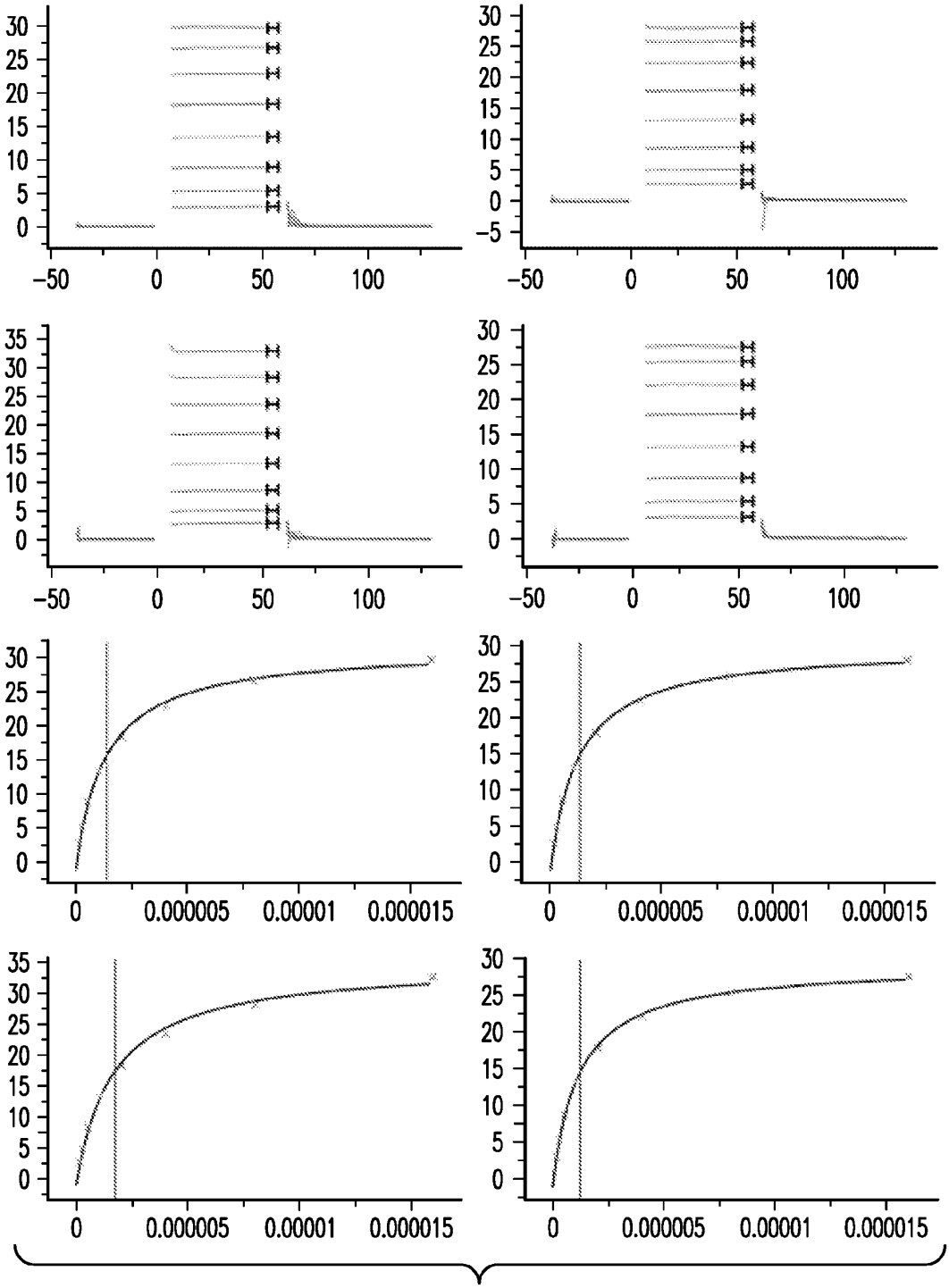


FIG. 47

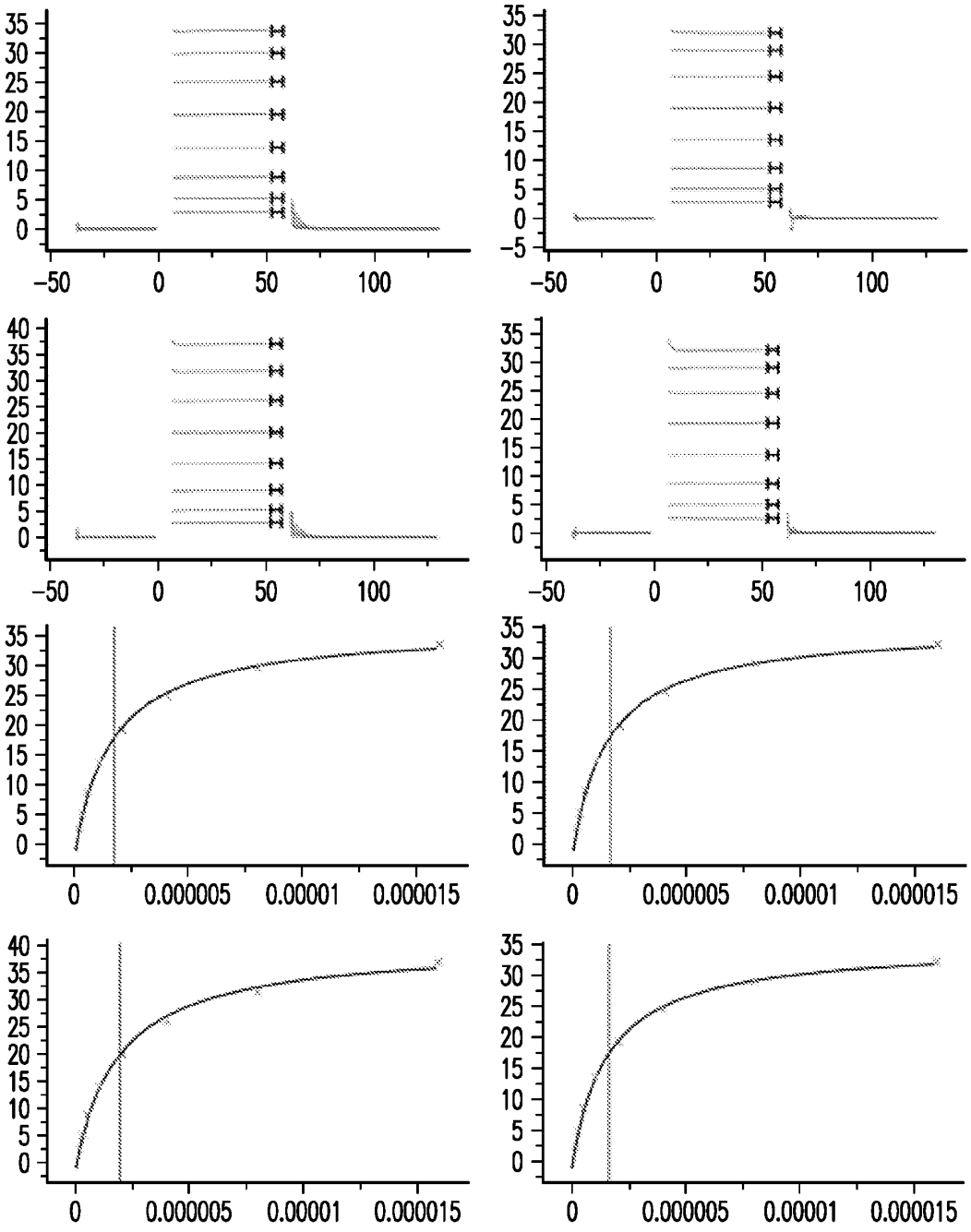


FIG. 47 Continued

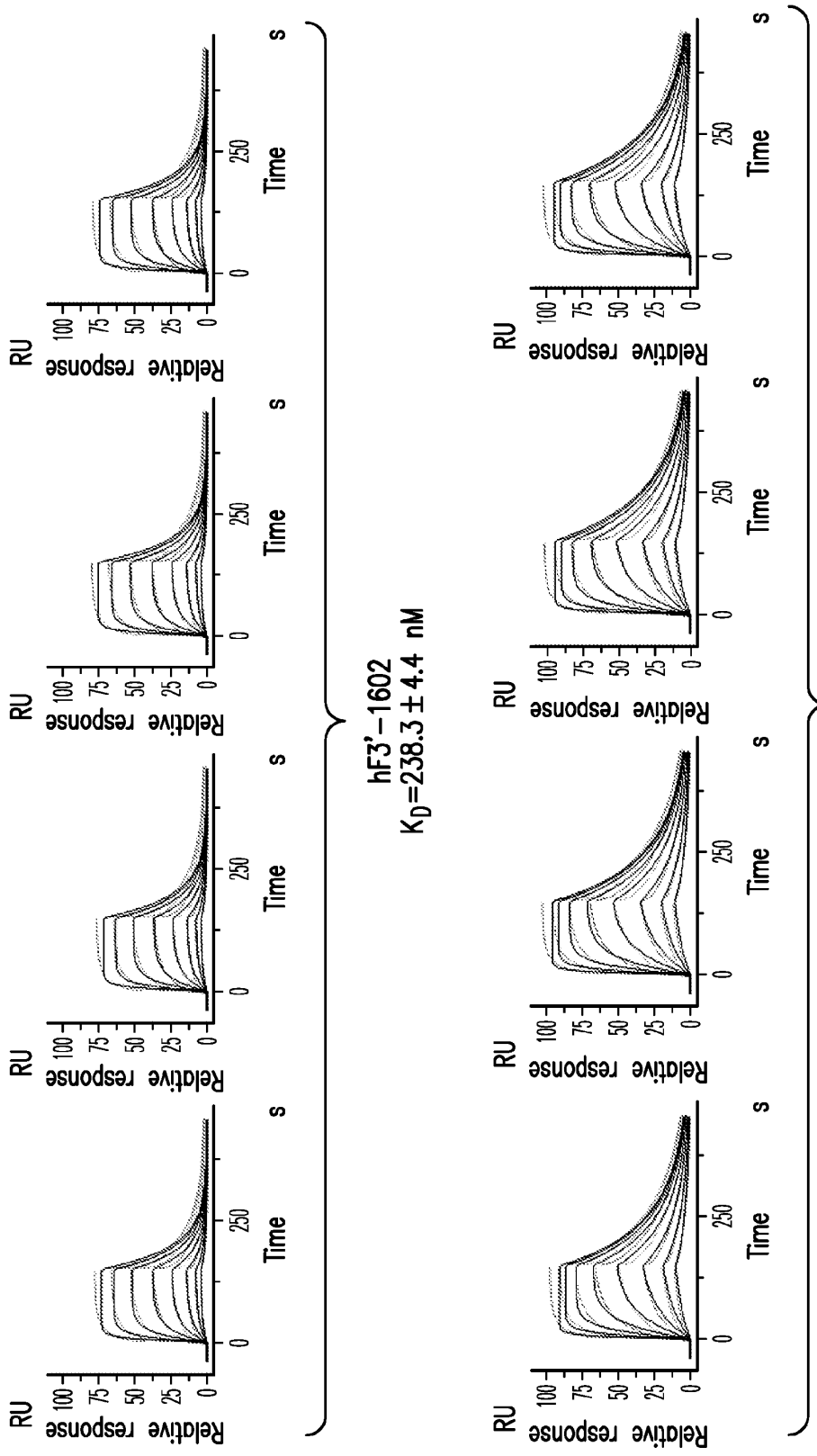
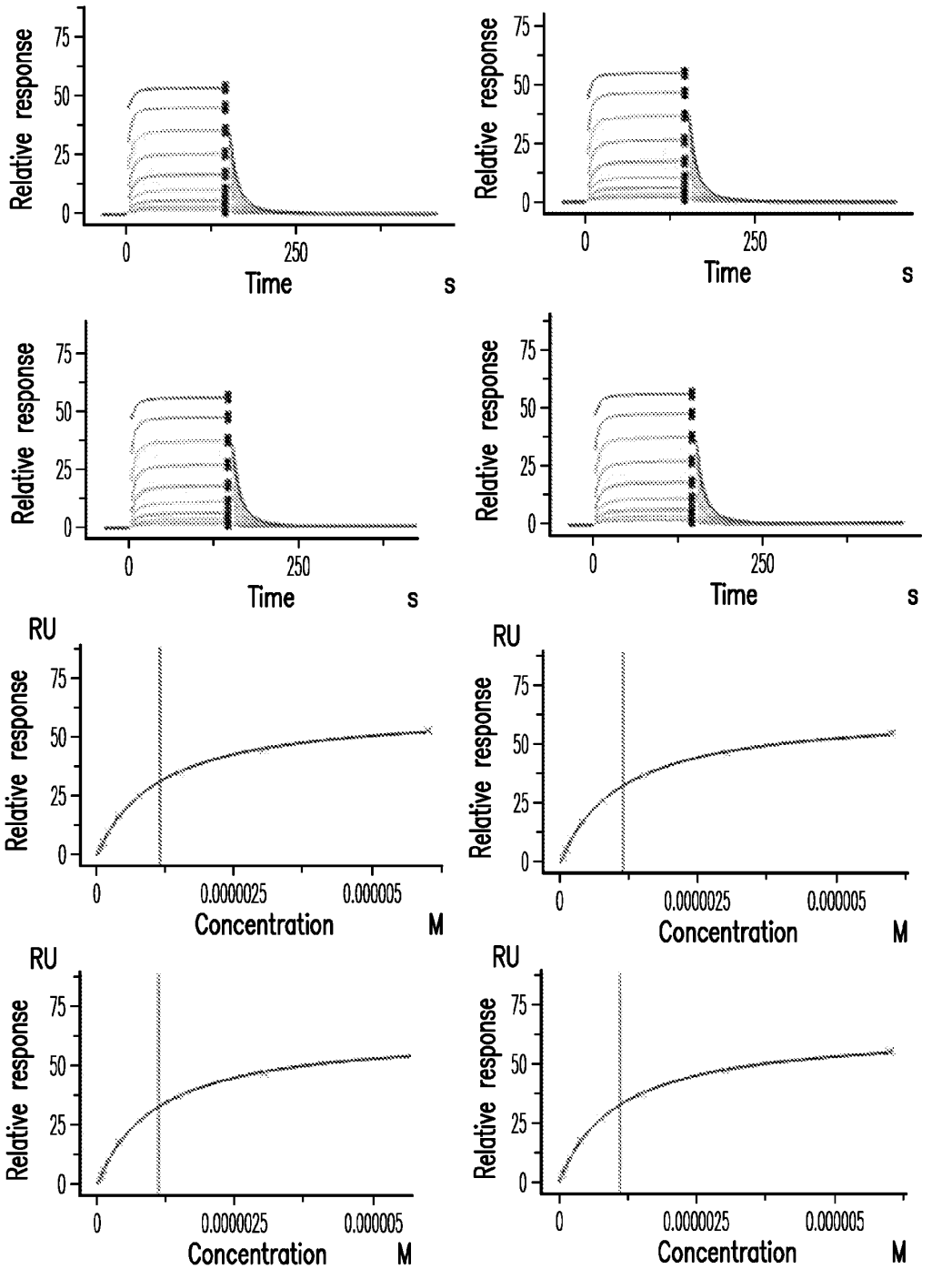
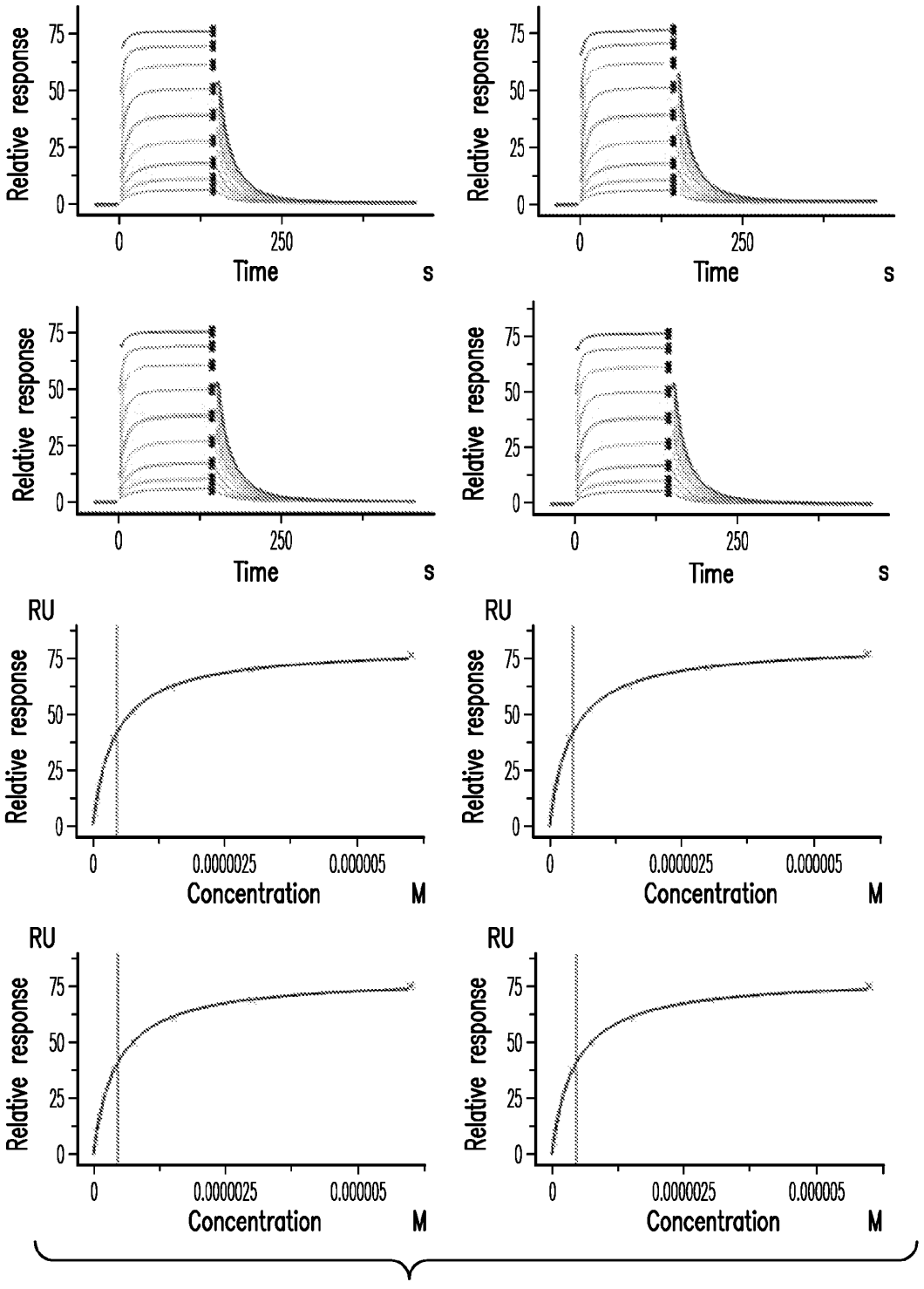

RU
Relative response
Time
s
hF3'-1602
K_D=238.3 ± 4.4 nM
trastuzumab
K_D=77.6 ± 1.9 nM

FIG. 48



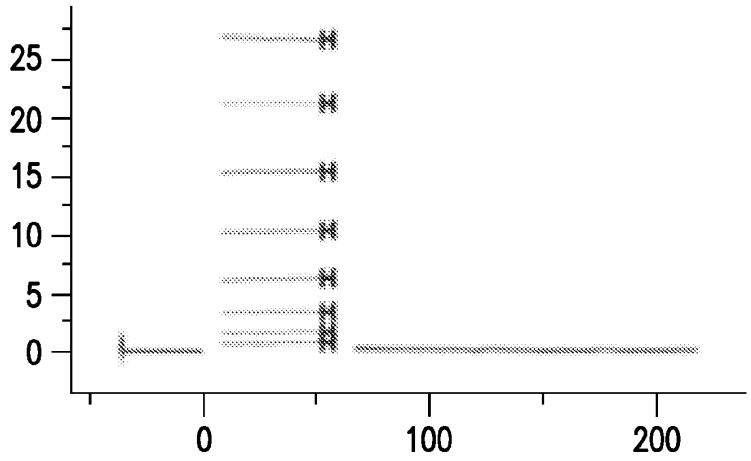
hF3'-1602
K_D=1107.5 ± 20.6 nM

FIG. 49



trastuzumab
K_D=439.8 ± 8.3 nM

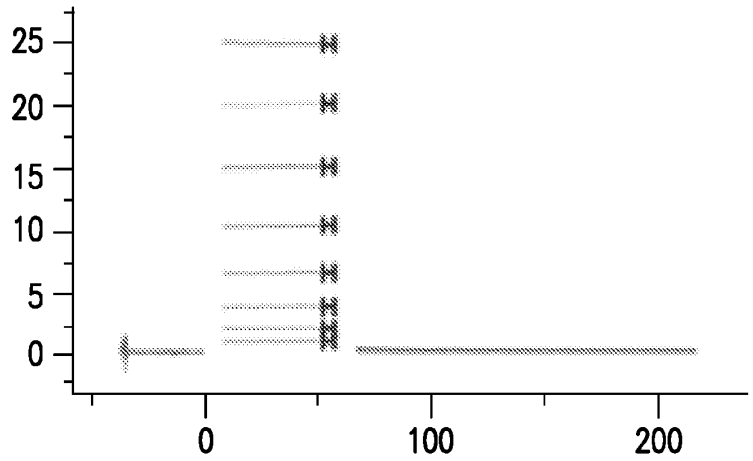
FIG. 49 Continued



hF3'-1602

K_D = 6560.0 ± 560.0 nM

(6.6 ± 0.6 μM)

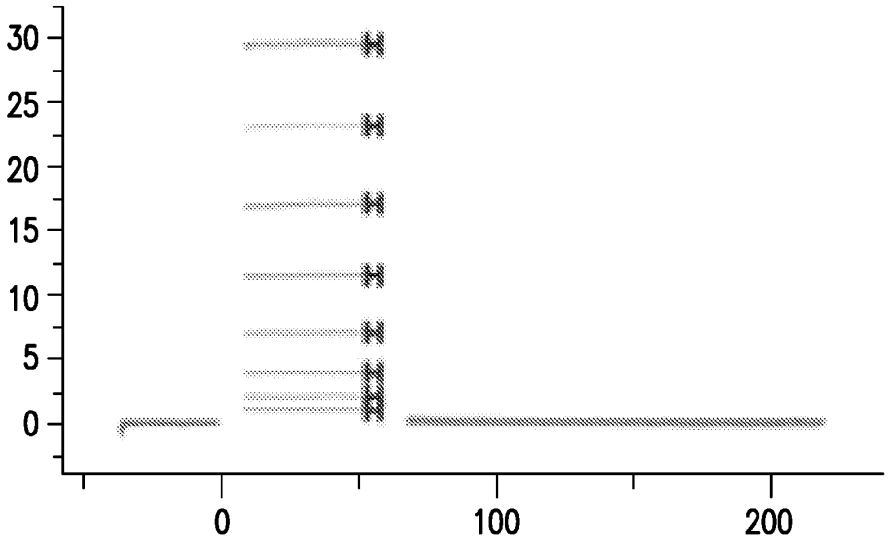


hF3'-1602

K_D = 6560.0 ± 560.0 nM

(6.6 ± 0.6 μM)

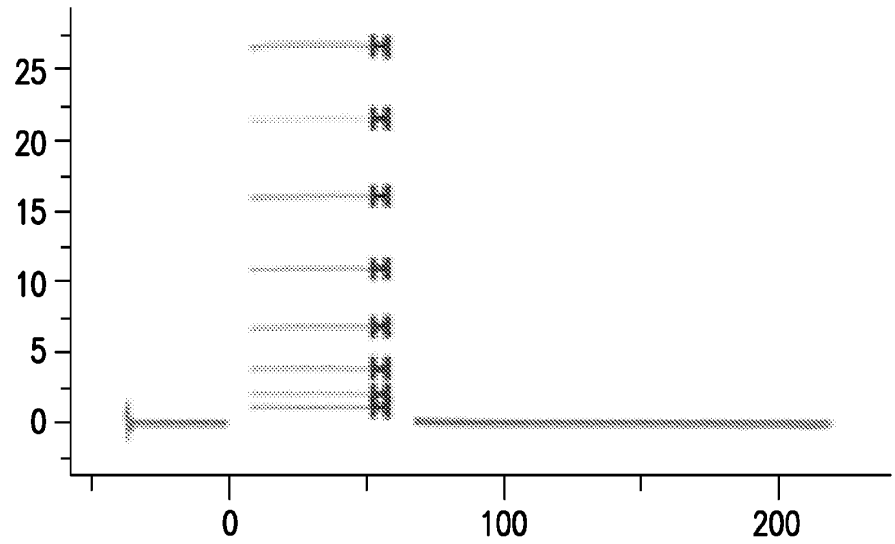
FIG. 50



hF3'-1602

K_D = 6560.0 ± 560.0 nM

(6.6 ± 0.6 μM)

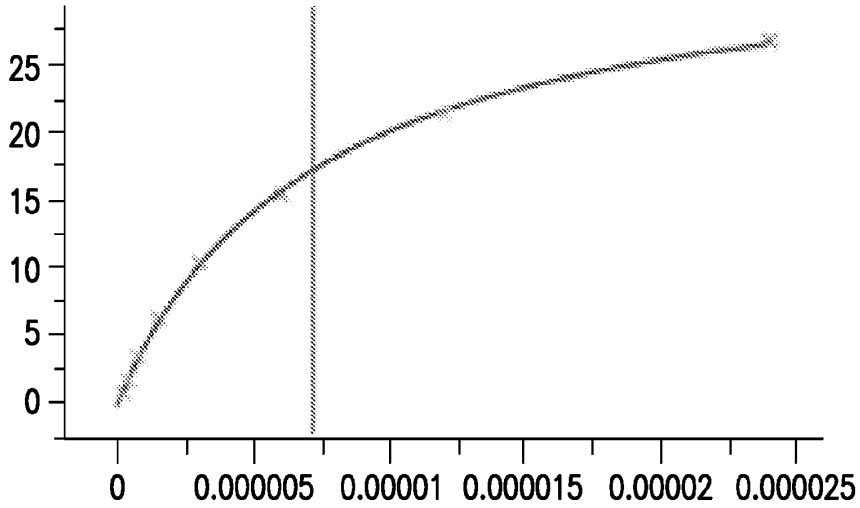


hF3'-1602

K_D = 6560.0 ± 560.0 nM

(6.6 ± 0.6 μM)

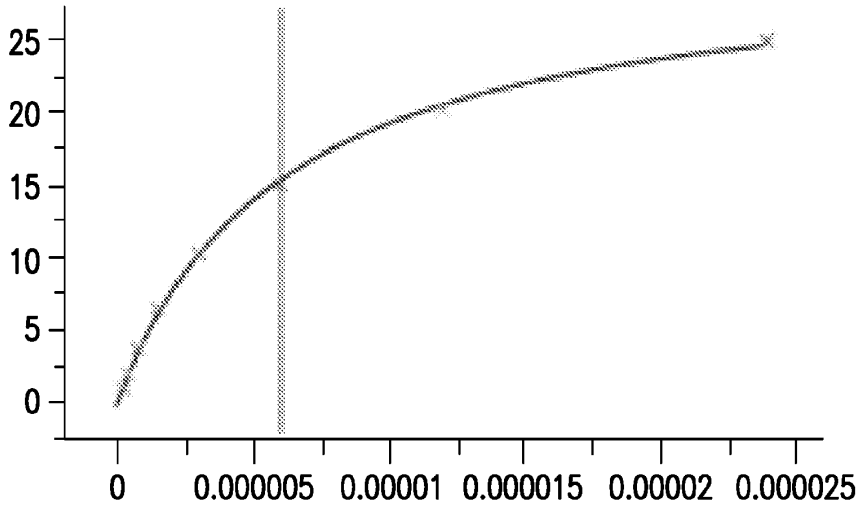
FIG. 50 Continued



hF3'-1602

K_D = 6560.0 ± 560.0 nM

(6.6 ± 0.6 µM)

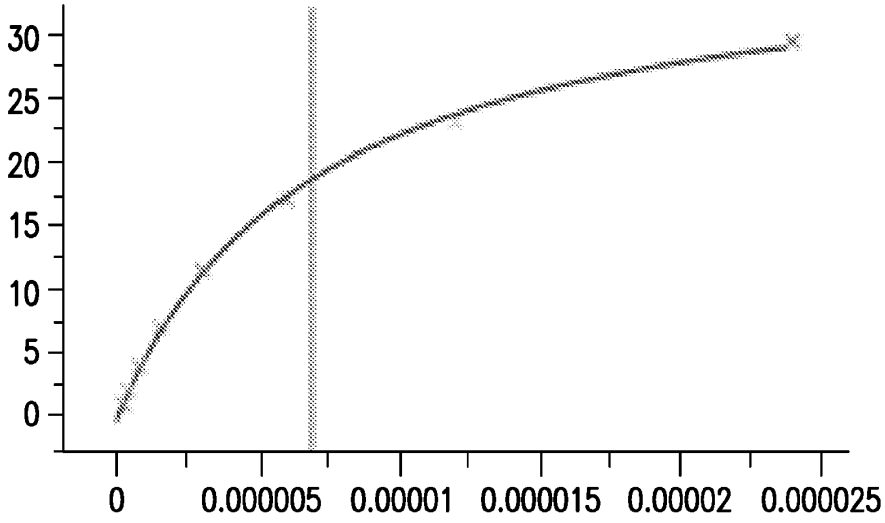


hF3'-1602

K_D = 6560.0 ± 560.0 nM

(6.6 ± 0.6 µM)

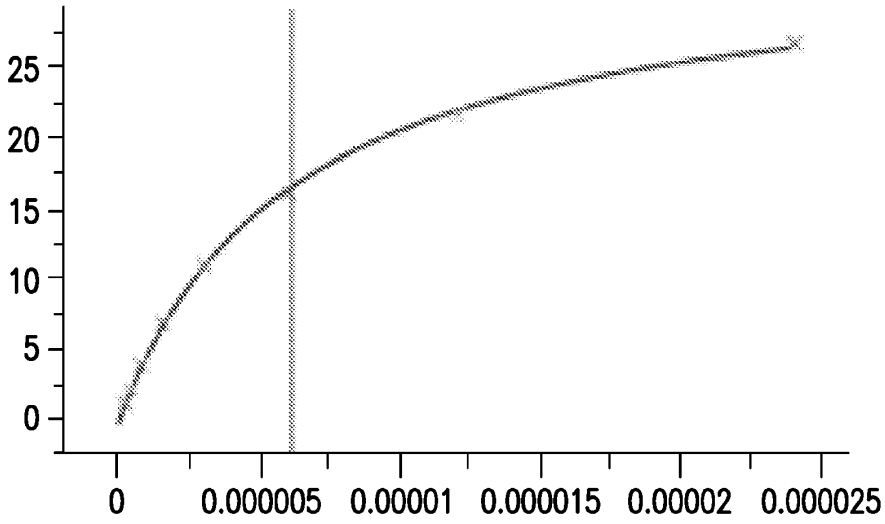
FIG. 50 Continued



hF3'-1602

K_D = 6560.0 ± 560.0 nM

(6.6 ± 0.6 µM)

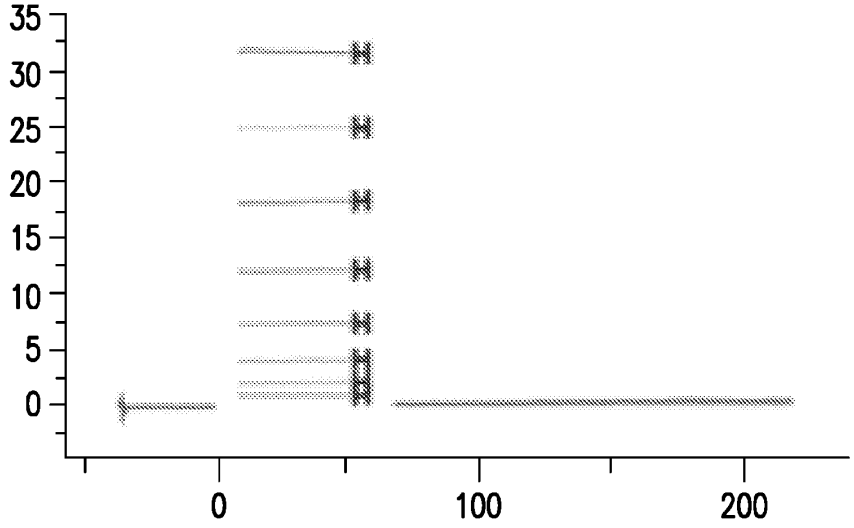


hF3'-1602

K_D = 6560.0 ± 560.0 nM

(6.6 ± 0.6 µM)

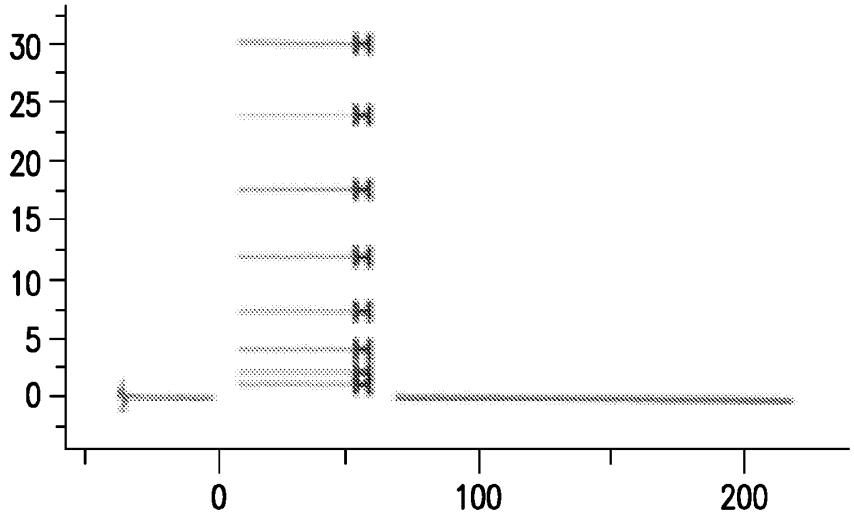
FIG. 50 Continued



trastuzumab

K_D = 6880.0 ± 270.0 nM

(6.9 ± 0.3 μM)

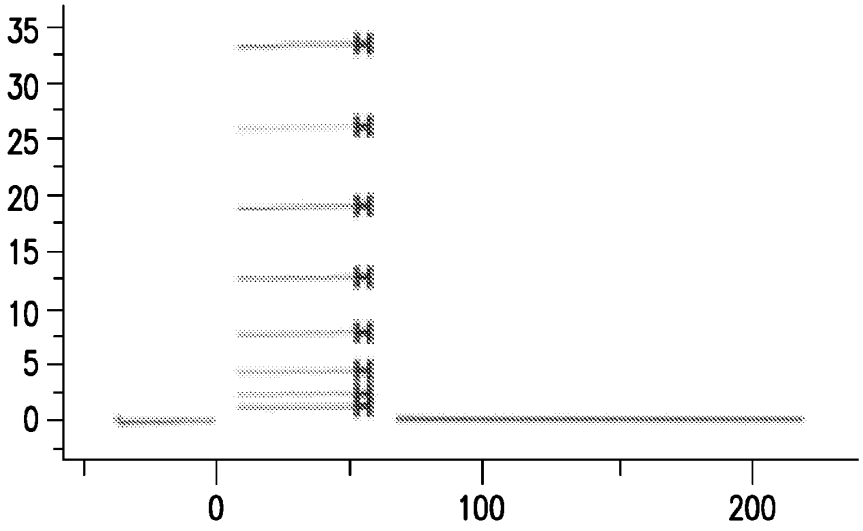


trastuzumab

K_D = 6880.0 ± 270.0 nM

(6.9 ± 0.3 μM)

FIG. 50 Continued



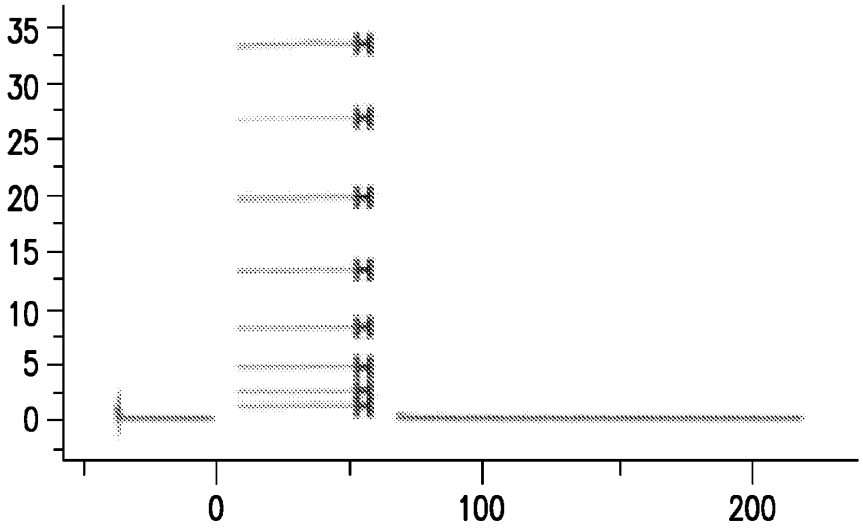
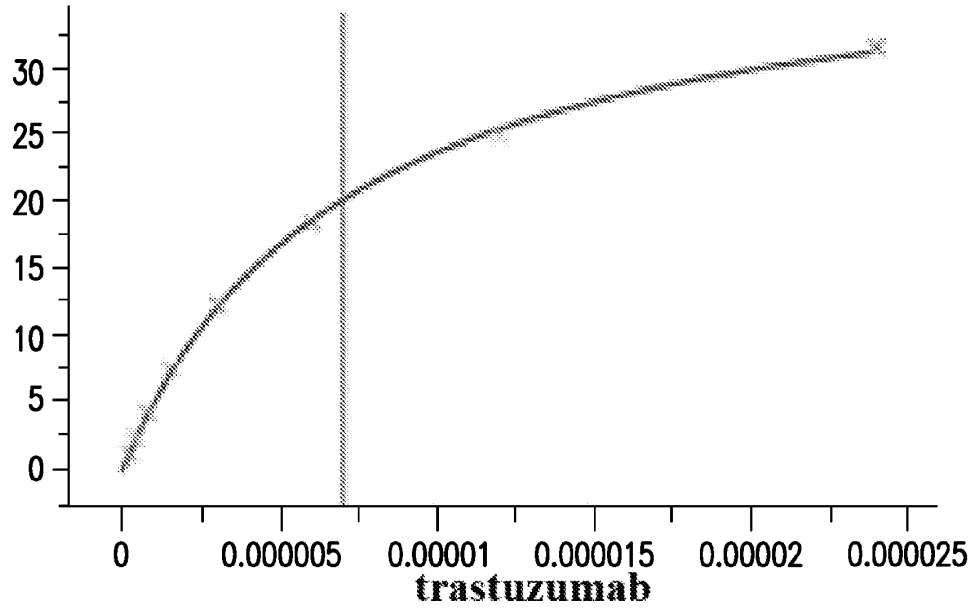
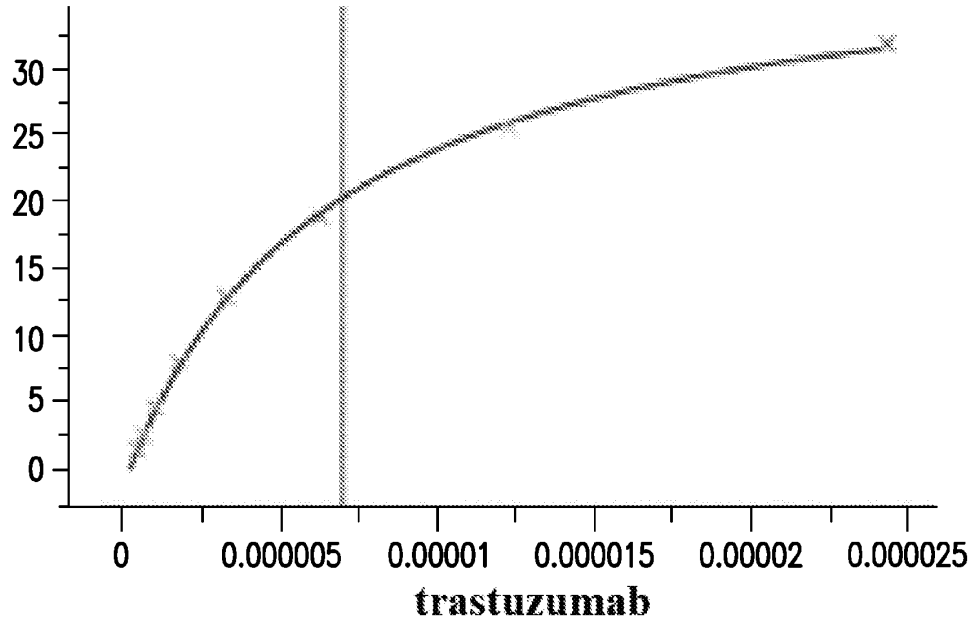


FIG. 50 Continued

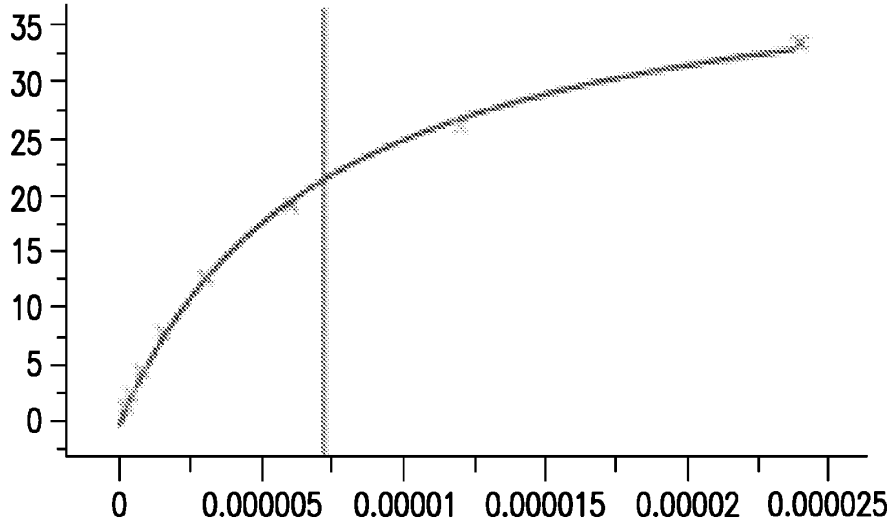


K_D = 6880.0 ± 270.0 nM
(6.9 ± 0.3 μM)



K_D = 6880.0 ± 270.0 nM
(6.9 ± 0.3 μM)

FIG. 50 Continued



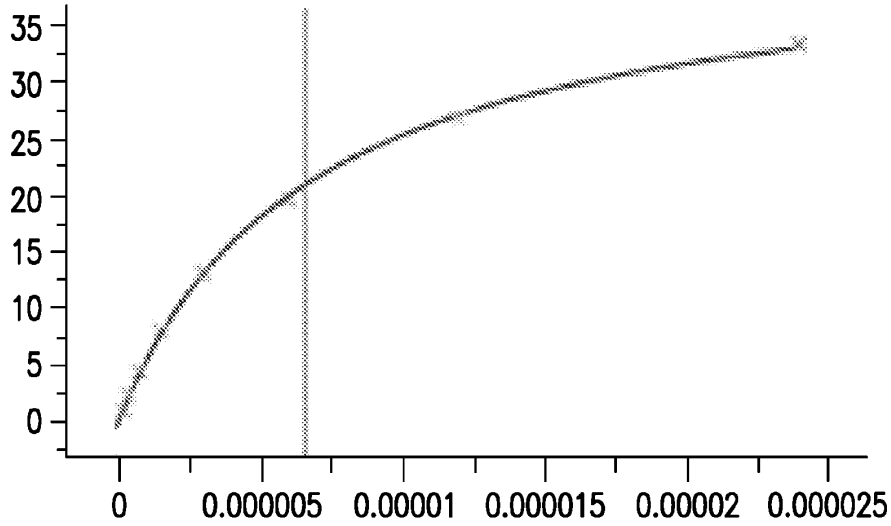
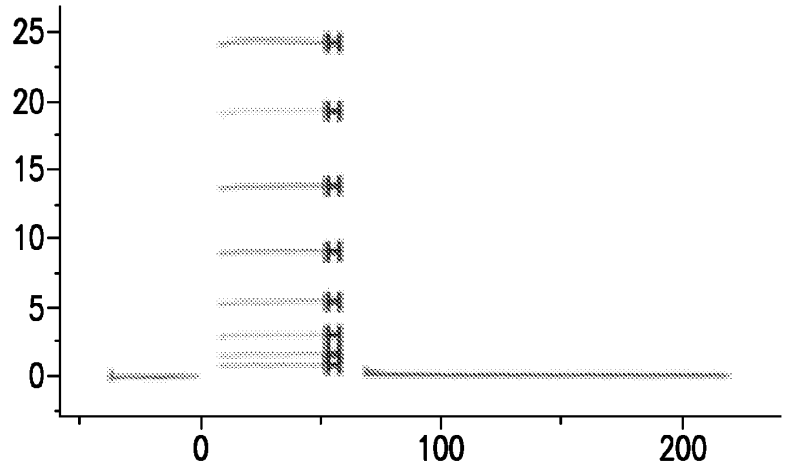


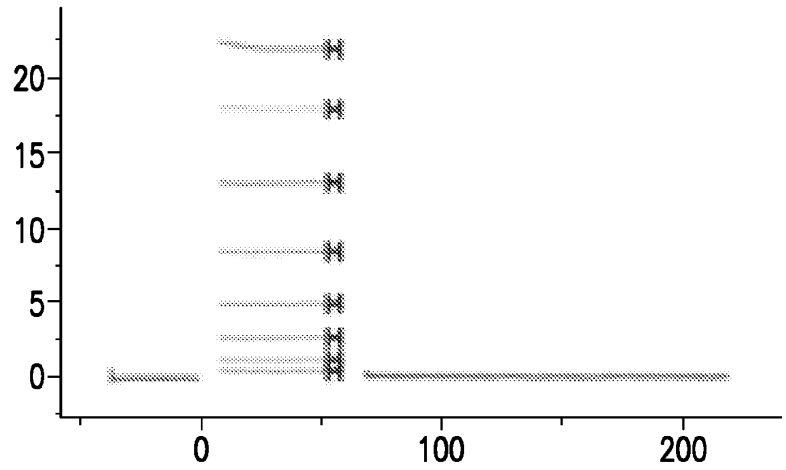
FIG. 50 Continued



hF3'-1602

K_D = 7460.0 ± 840.0 nM

(7.5 ± 0.8 μM)

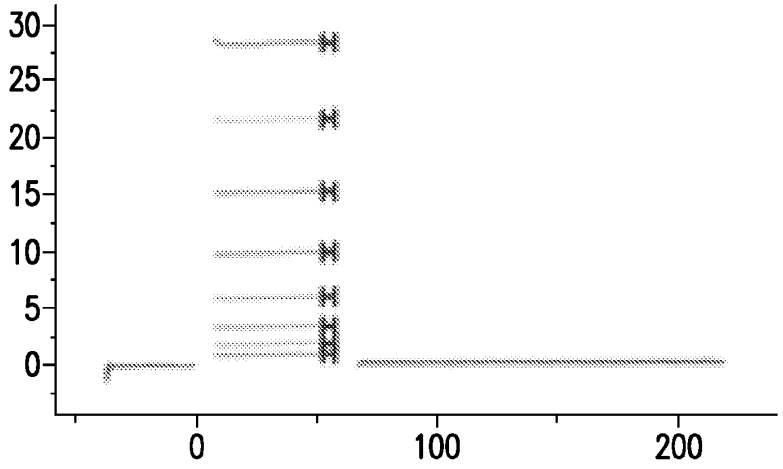


hF3'-1602

K_D = 7460.0 ± 840.0 nM

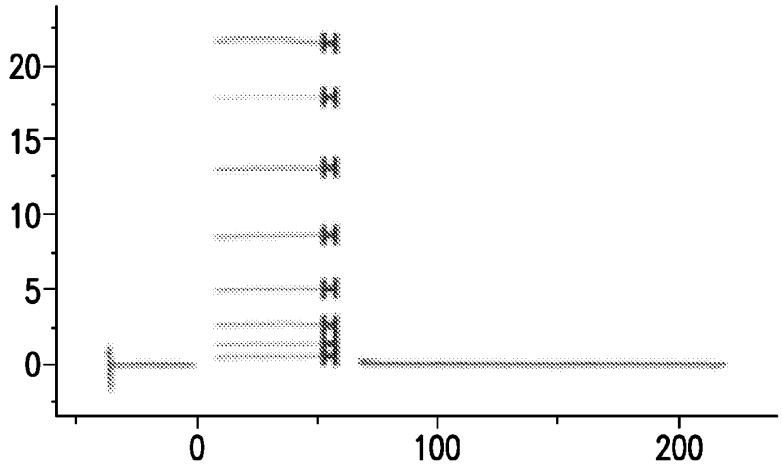
(7.5 ± 0.8 μM)

FIG. 51



hF3'-1602

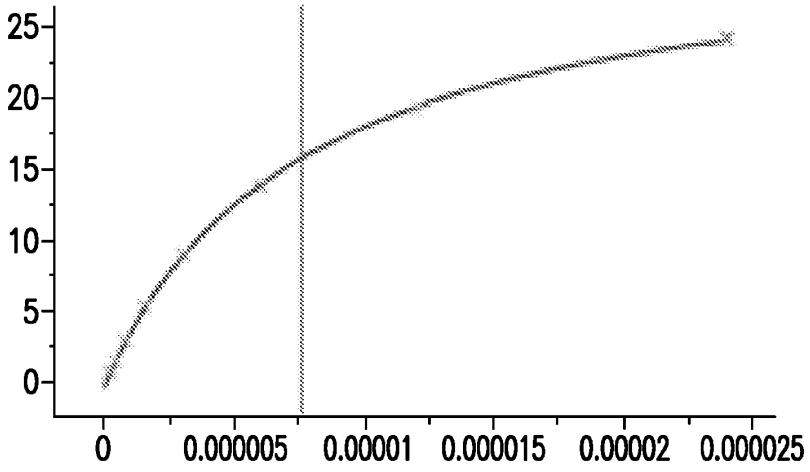
K_D = 7460.0 ± 840.0 nM
(7.5 ± 0.8 μM)



hF3'-1602

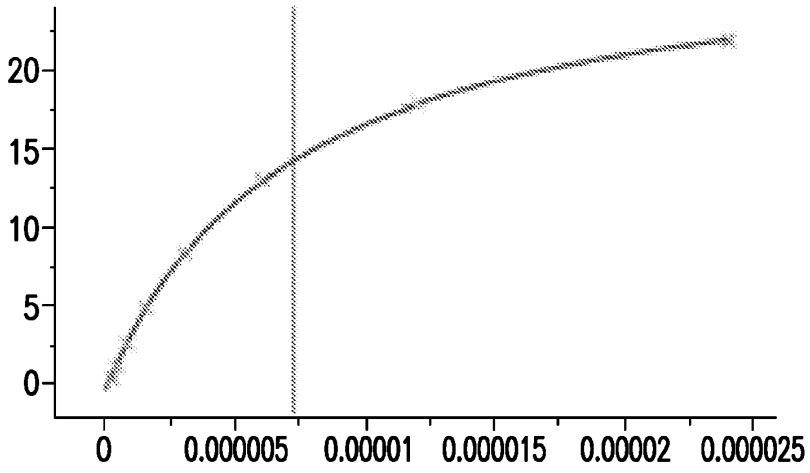
K_D = 7460.0 ± 840.0 nM
(7.5 ± 0.8 μM)

FIG. 51 Continued



hF3'-1602

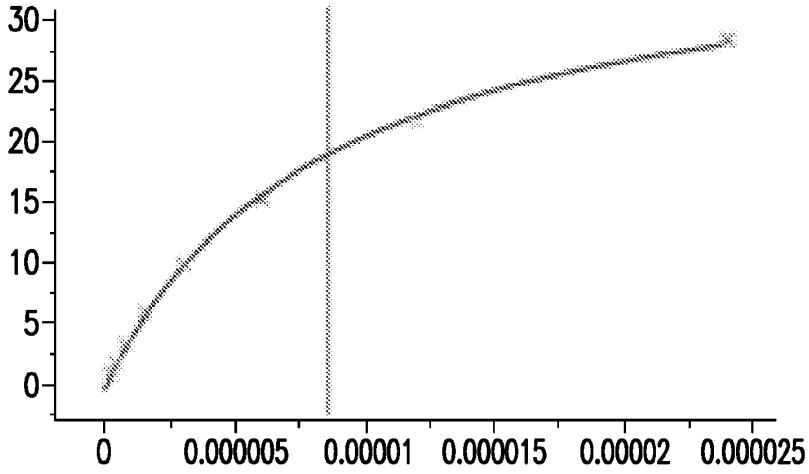
K_D = 7460.0 ± 840.0 nM
(7.5 ± 0.8 μM)



hF3'-1602

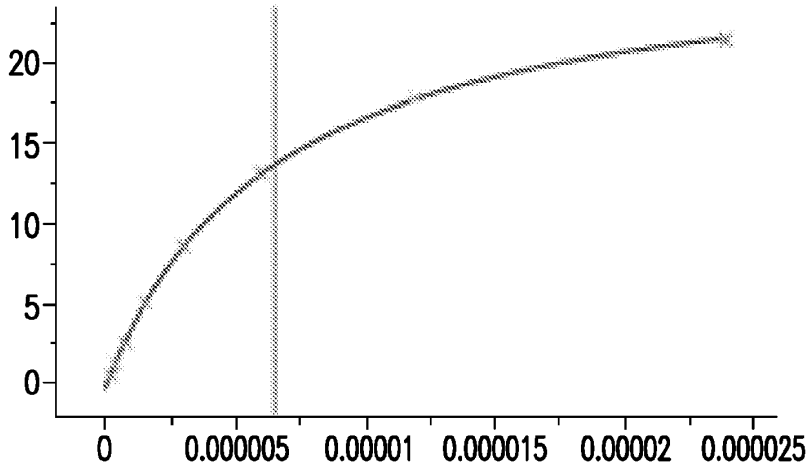
K_D = 7460.0 ± 840.0 nM
(7.5 ± 0.8 μM)

FIG. 51 Continued



hF3'-1602

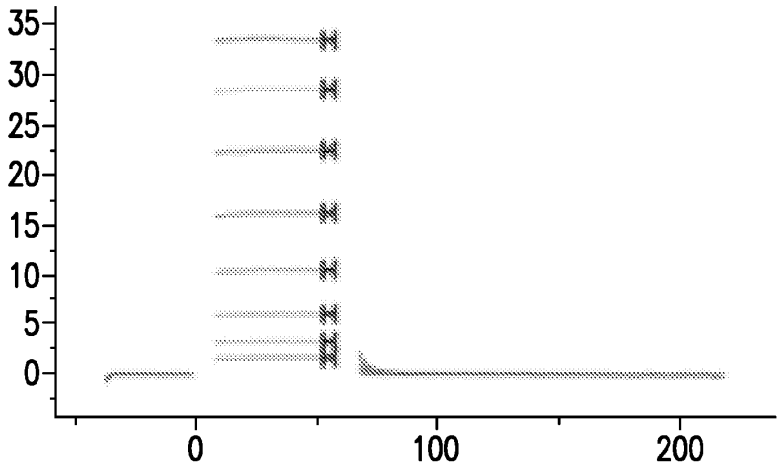
K_D = 7460.0 ± 840.0 nM
(7.5 ± 0.8 μM)



hF3'-1602

K_D = 7460.0 ± 840.0 nM
(7.5 ± 0.8 μM)

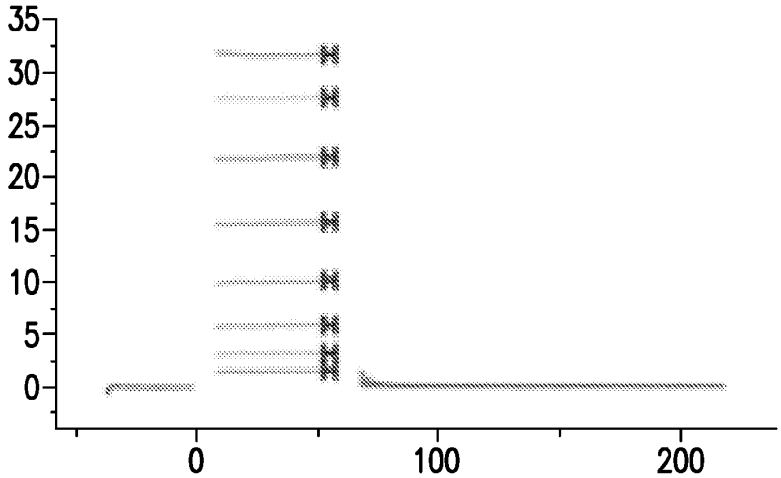
FIG. 51 Continued



trastuzumab

K_D = 4200.0 ± 430.0 nM

(4.2 ± 0.4 μM)

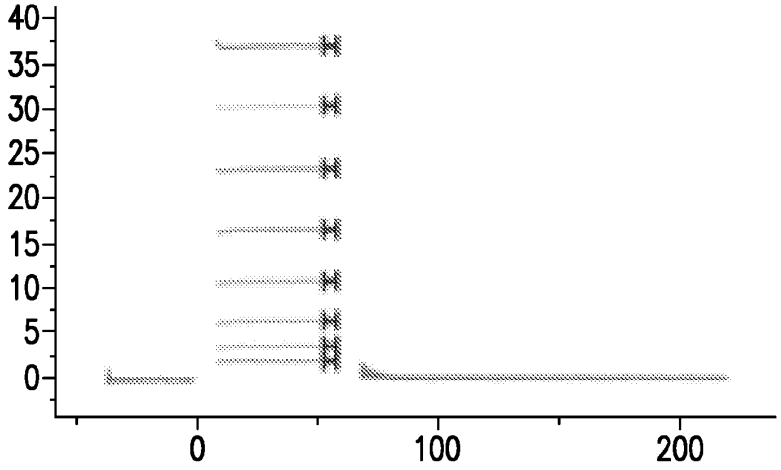


trastuzumab

K_D = 4200.0 ± 430.0 nM

(4.2 ± 0.4 μM)

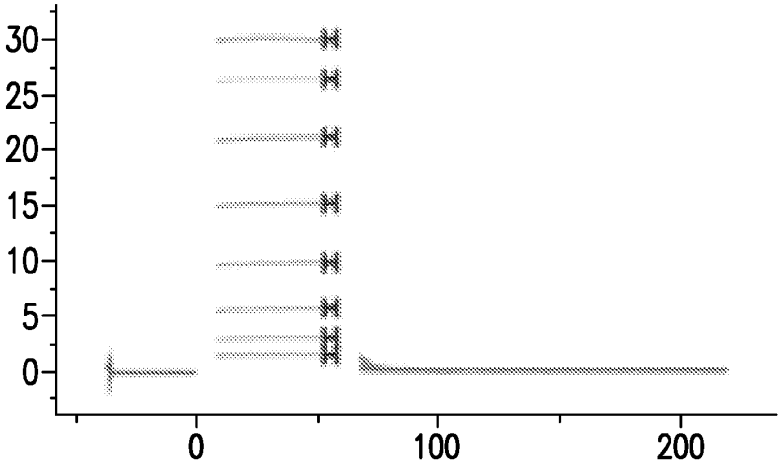
FIG. 51 Continued



trastuzumab

K_D = 4200.0 ± 430.0 nM

(4.2 ± 0.4 μM)

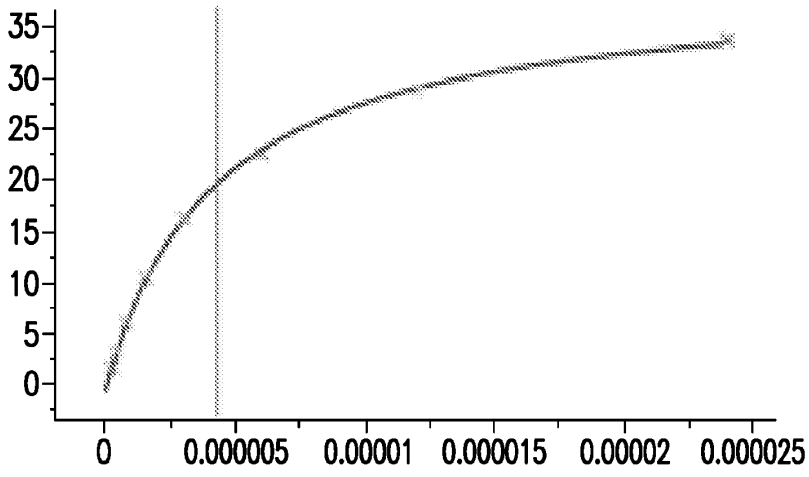


trastuzumab

K_D = 4200.0 ± 430.0 nM

(4.2 ± 0.4 μM)

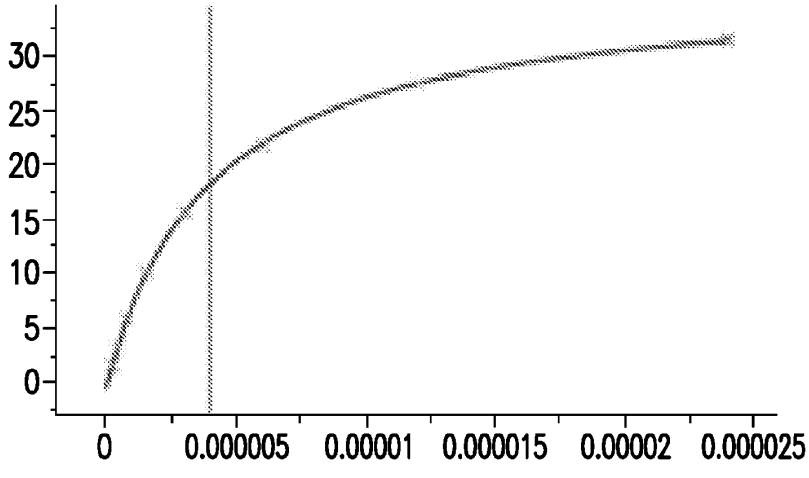
FIG. 51 Continued



trastuzumab

K_D = 4200.0 ± 430.0 nM

(4.2 ± 0.4 μM)

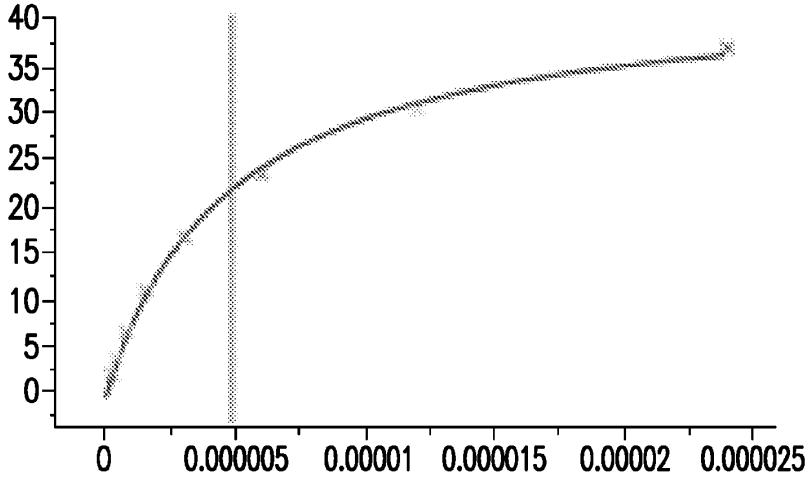


trastuzumab

K_D = 4200.0 ± 430.0 nM

(4.2 ± 0.4 μM)

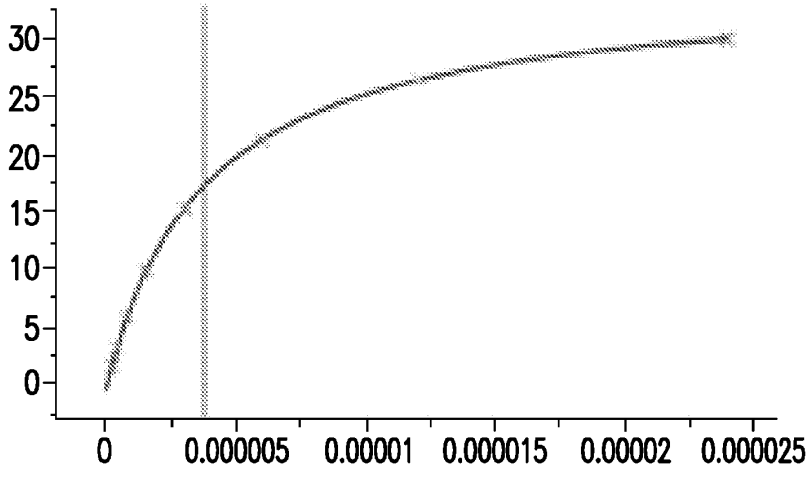
FIG. 51 Continued



trastuzumab

K_D = 4200.0 ± 430.0 nM

(4.2 ± 0.4 μM)

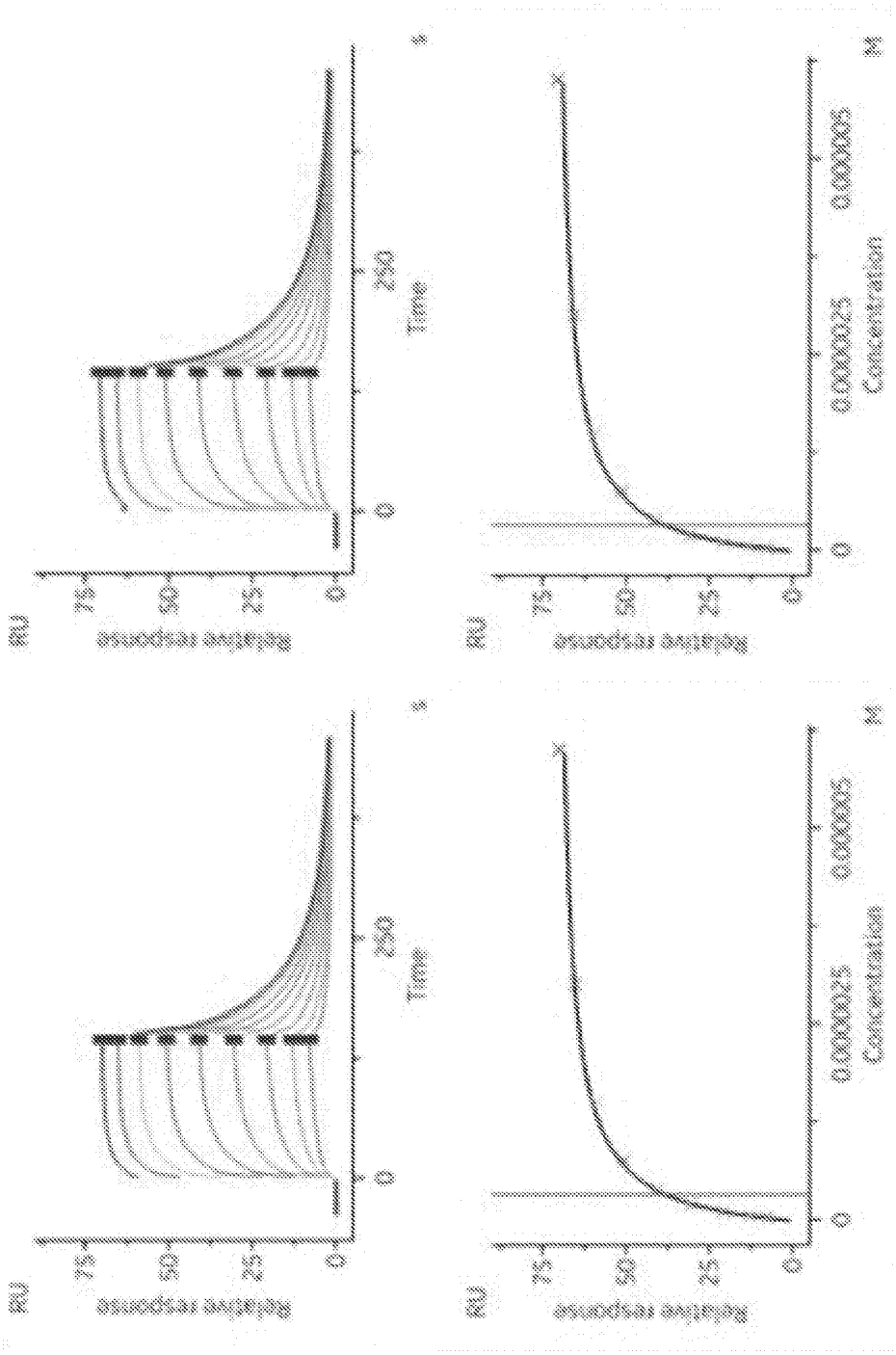


trastuzumab

K_D = 4200.0 ± 430.0 nM

(4.2 ± 0.4 μM)

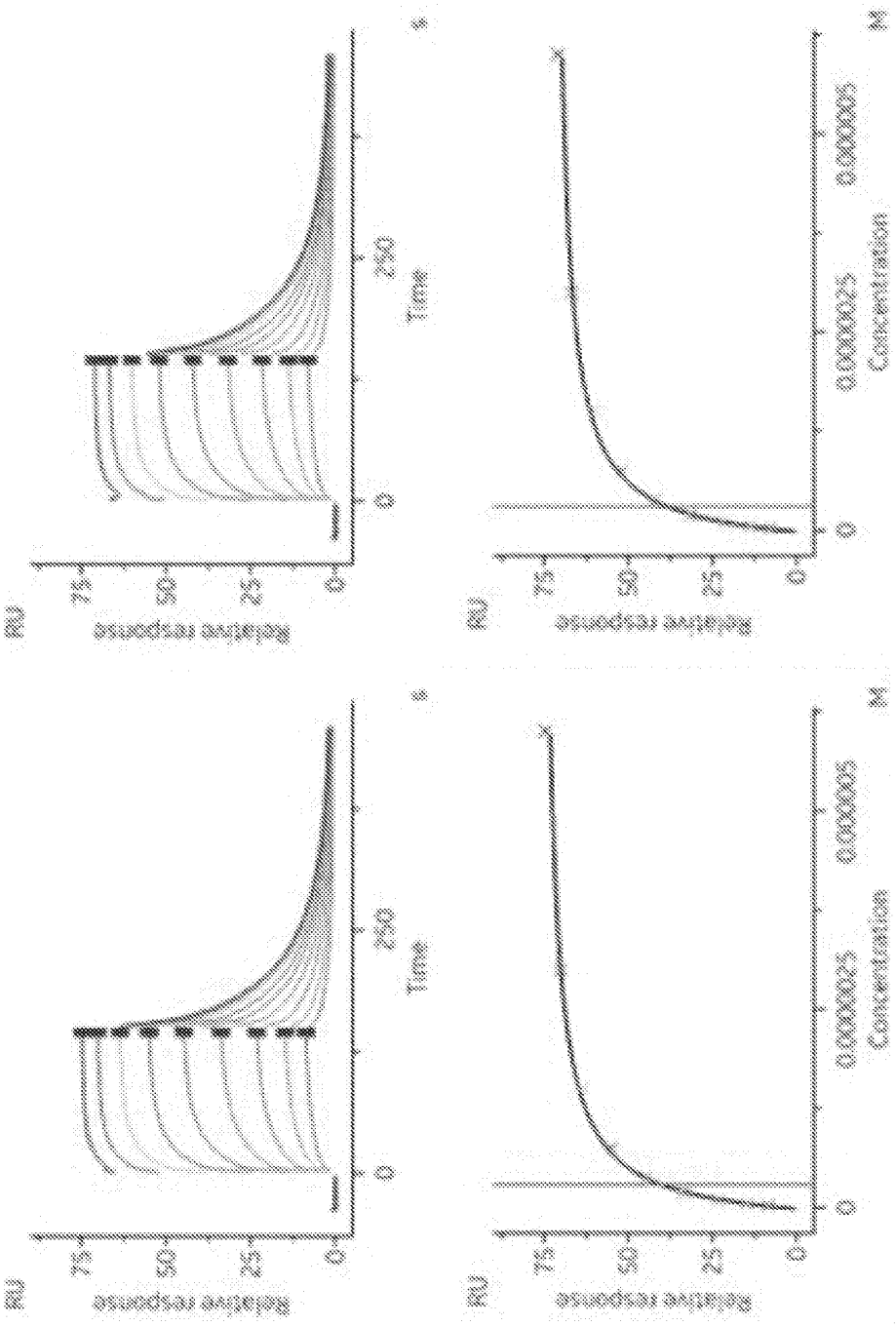
FIG. 51 Continued



hF3'-1602

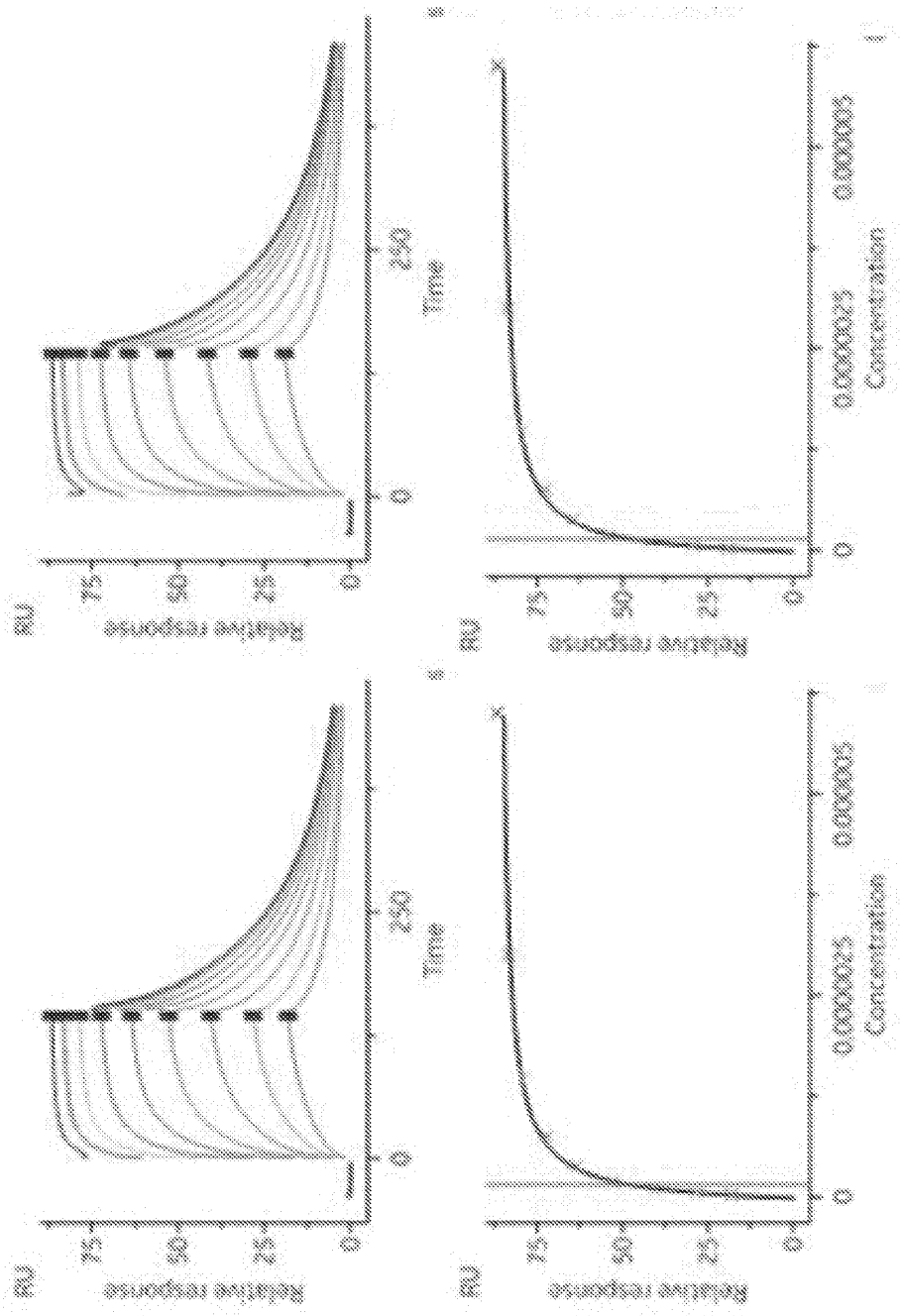
$K_D = 306.5 \pm 10.3$ nM

FIG. 52



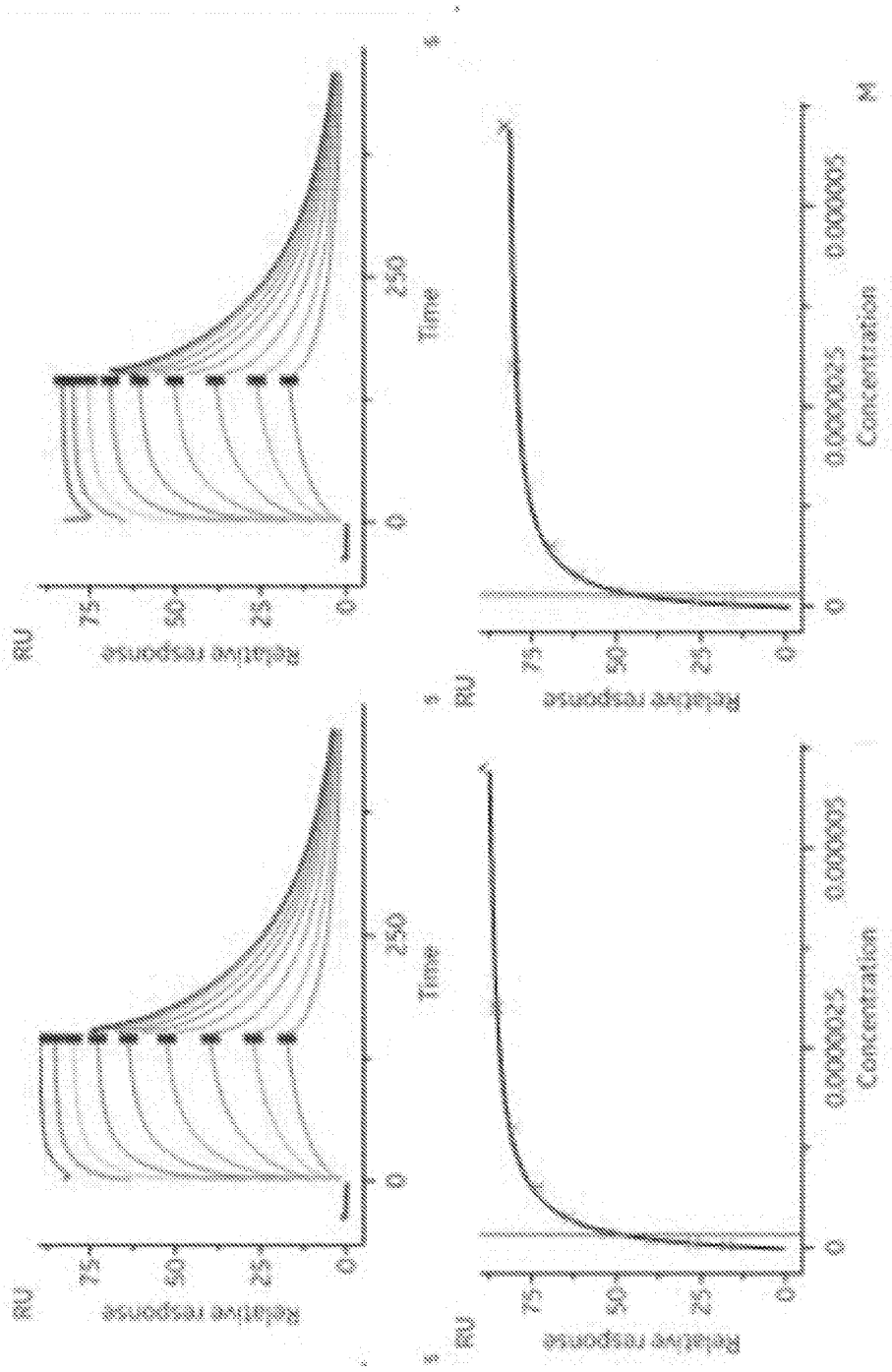
hF3'-1602

$K_D = 306.5 \pm 10.3$ nM FIG. 52 Continued



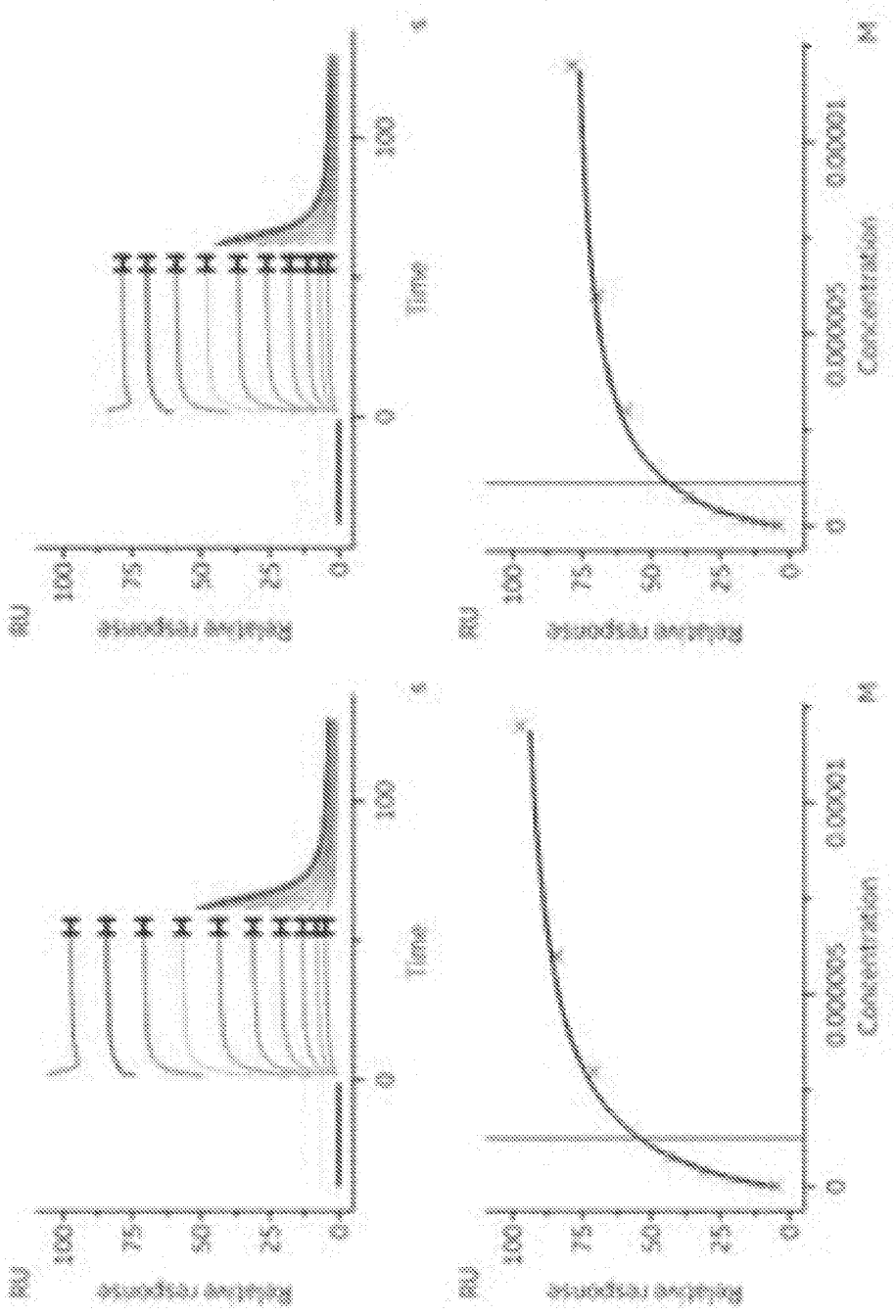
trastuzumab

$K_D = 150.5 \pm 9.7$ nM FIG. 52 Continued



trastuzumab

$K_D = 150.5 \pm 9.7$ nM FIG. 52 Continued

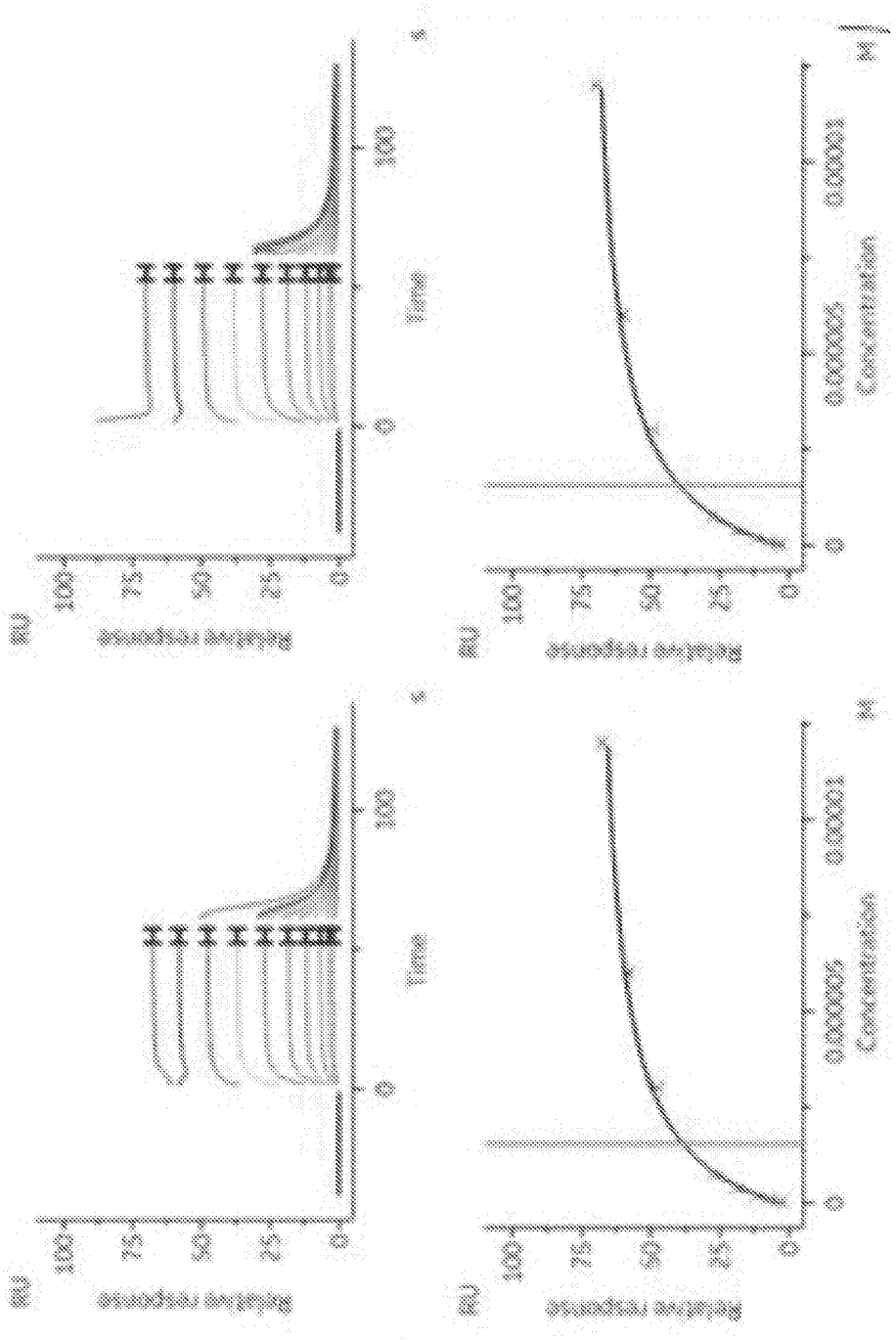


hF3'-1602

$K_D = 1367.5 \pm 219.6$ nM

$(1.4 \pm 0.2$ μM)

FIG. 53



hF3'-1602

$K_D = 1367.5 \pm 219.6$ nM

$(1.4 \pm 0.2\ \mu M)$

FIG. 53 Continued

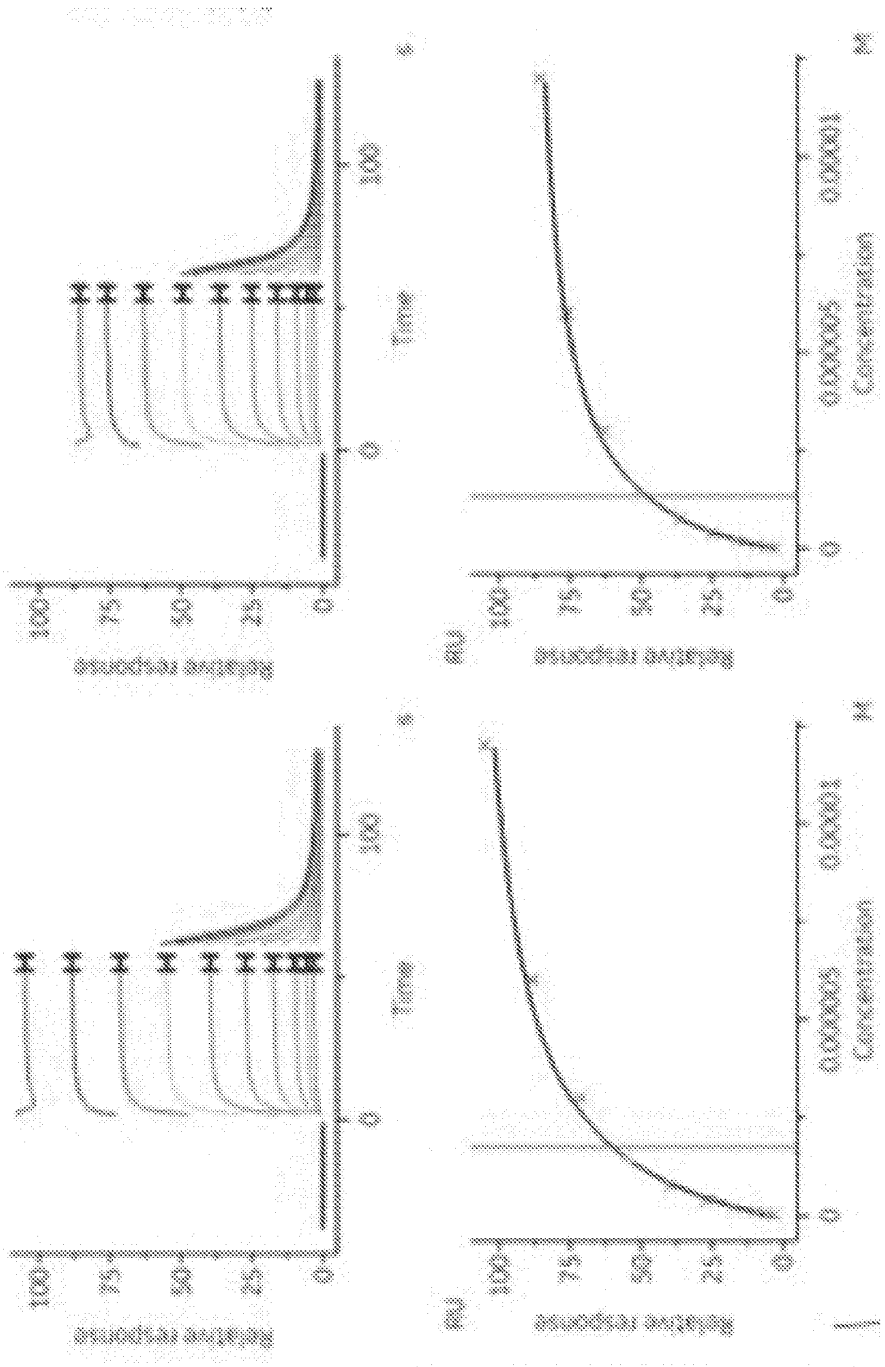


FIG. 53 Continued

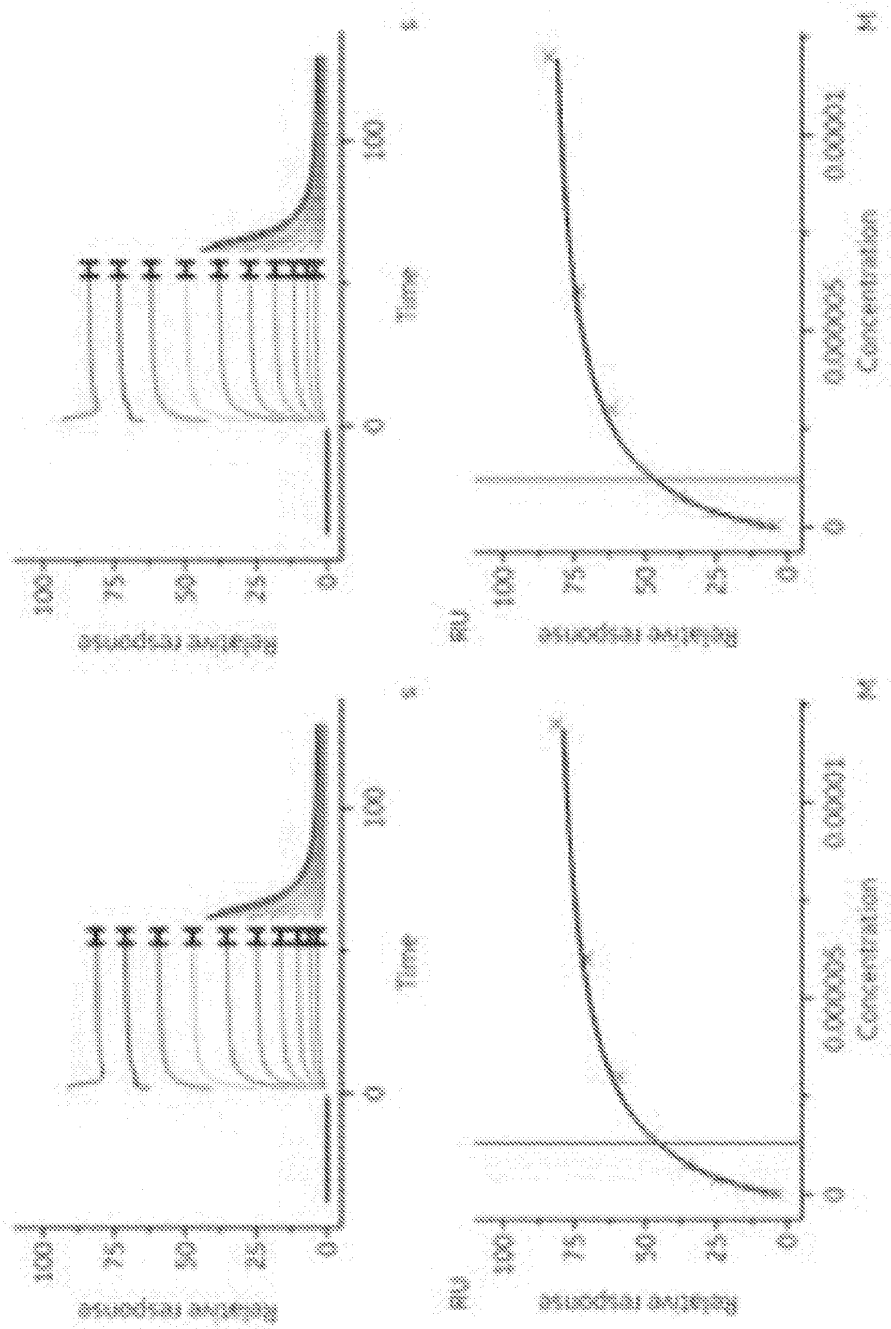
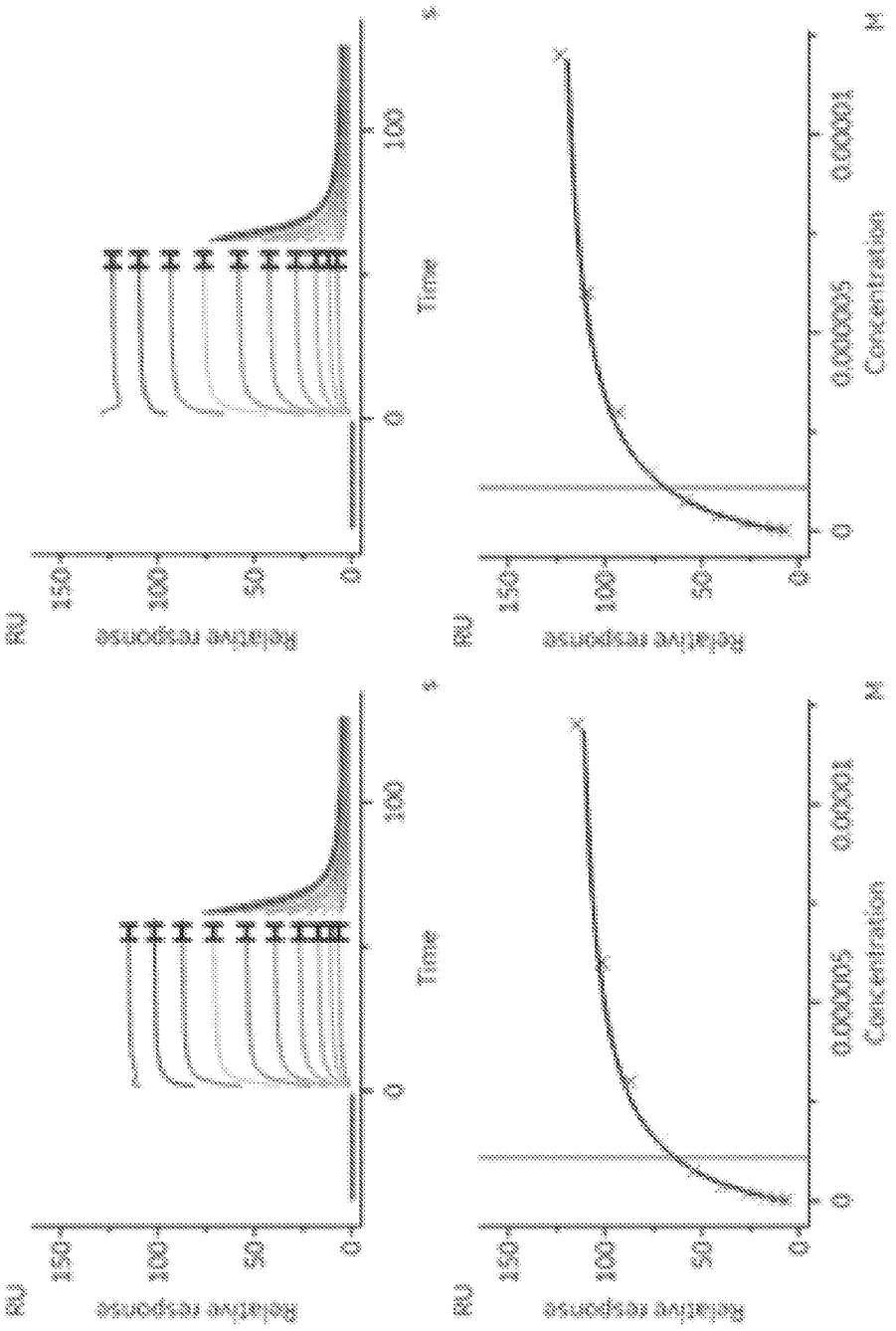


FIG. 53 Continued

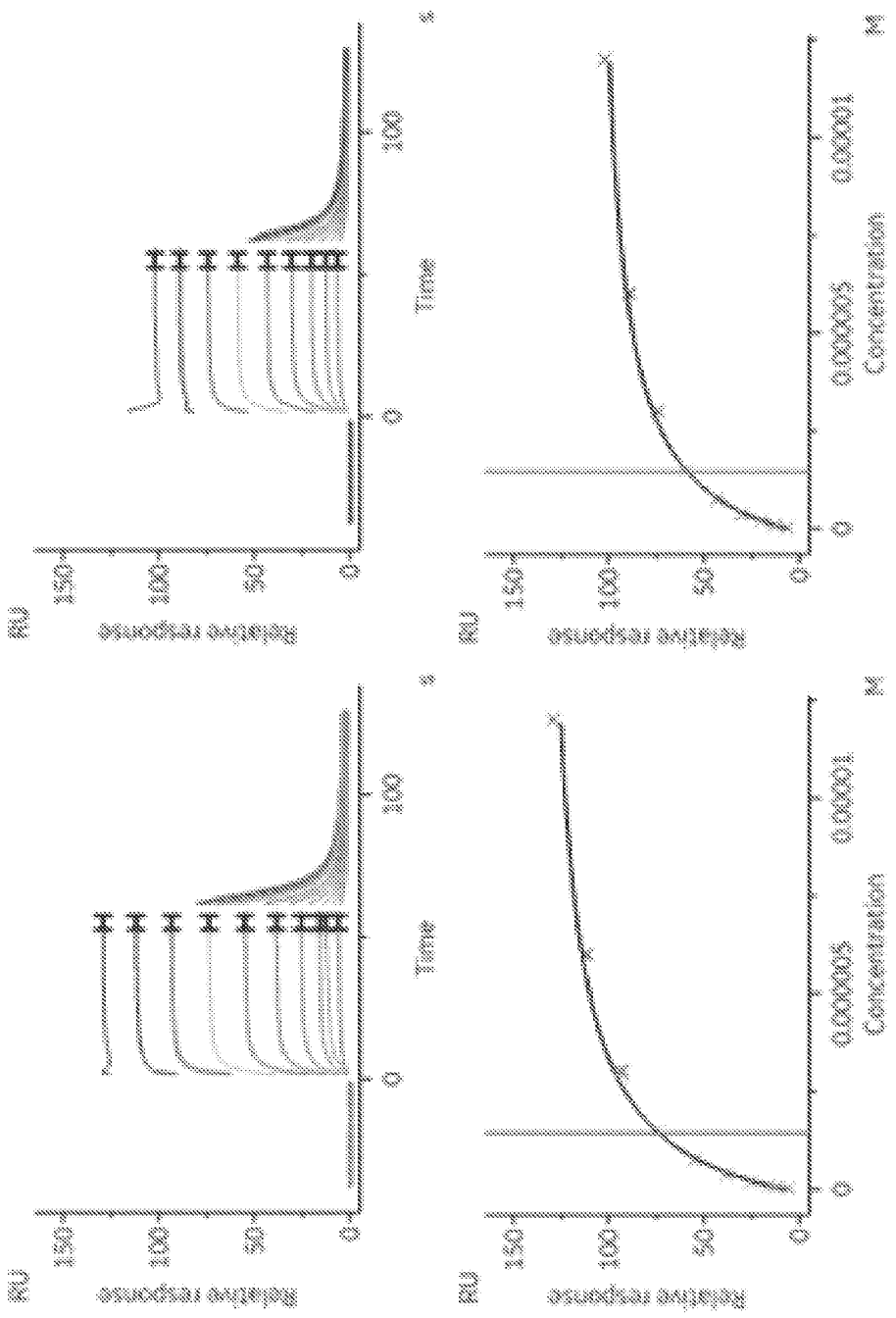


hF3'-1602

$K_D = 1270.0 \pm 214.0$ nM

$(1.3 \pm 0.2$ μM)

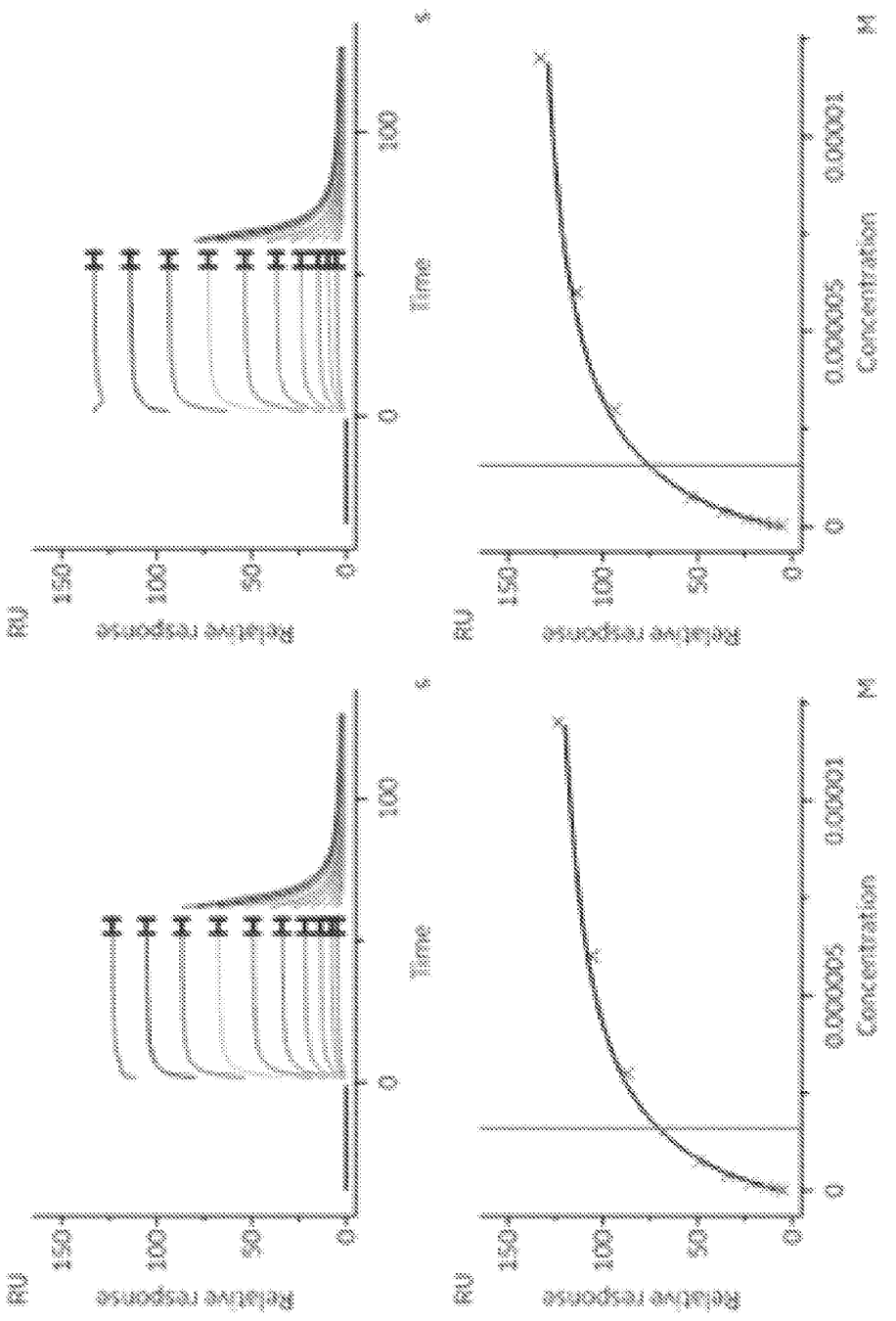
FIG. 54



hF3'-1602

$K_D = 1270.0 \pm 214.0$ nM
$(1.3 \pm 0.2$ μM)

FIG. 54 Continued

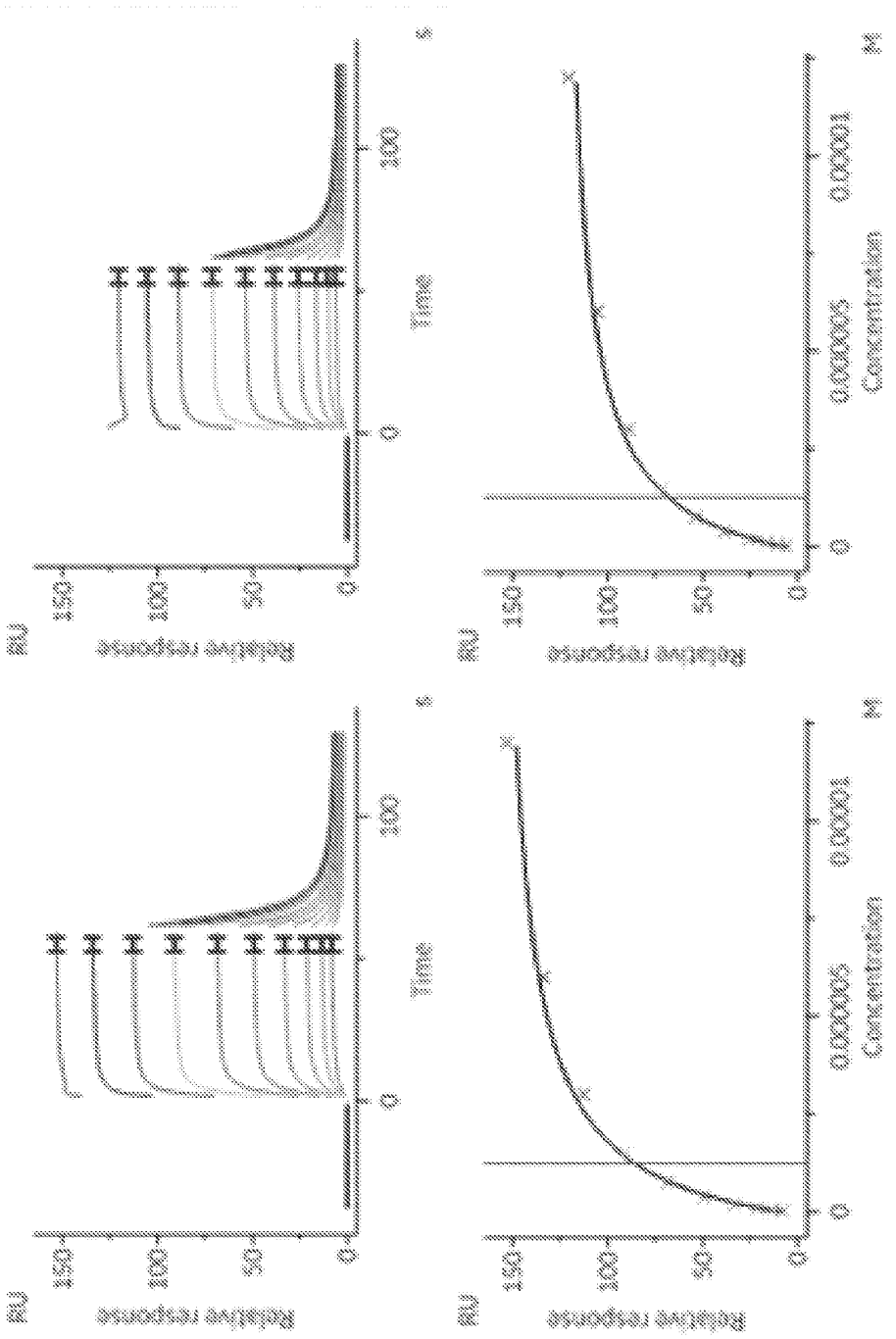


trastuzumab

$K_D = 1415.0 \pm 196.4$ nM

$(1.4 \pm 0.2$ μM)

FIG. 54 Continued

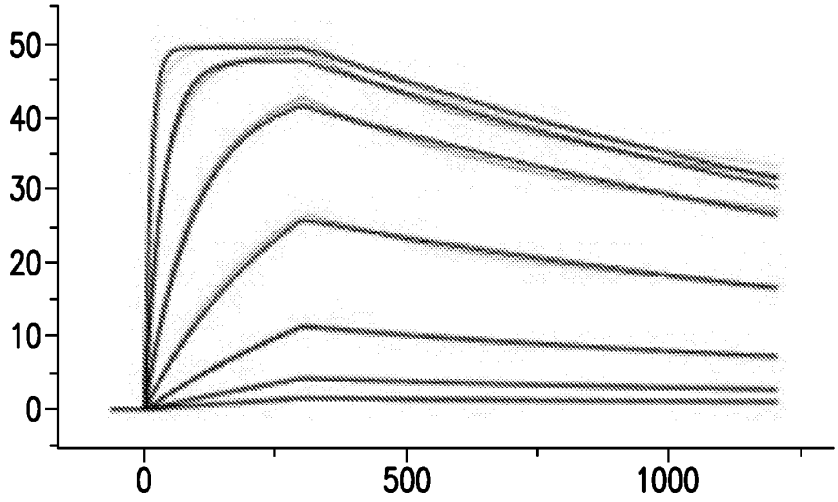


trastuzumab

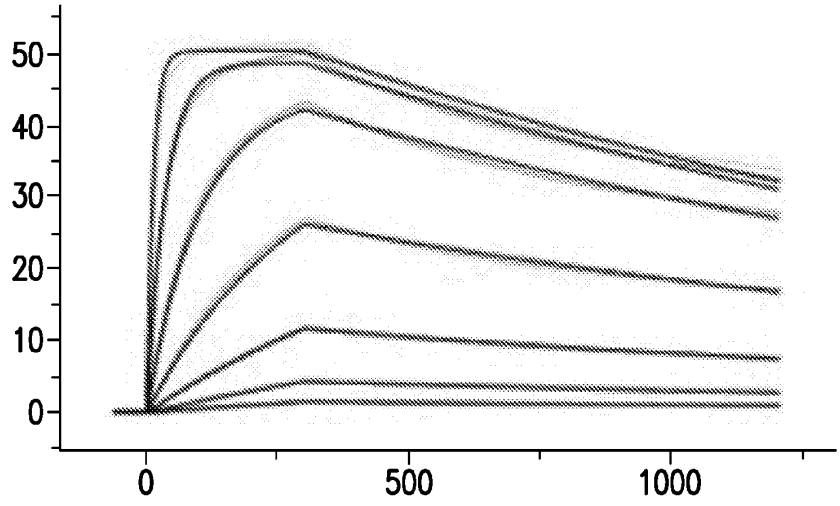
$K_D = 1415.0 \pm 196.4$ nM

$(1.4 \pm 0.2$ μM)

FIG. 54 Continued

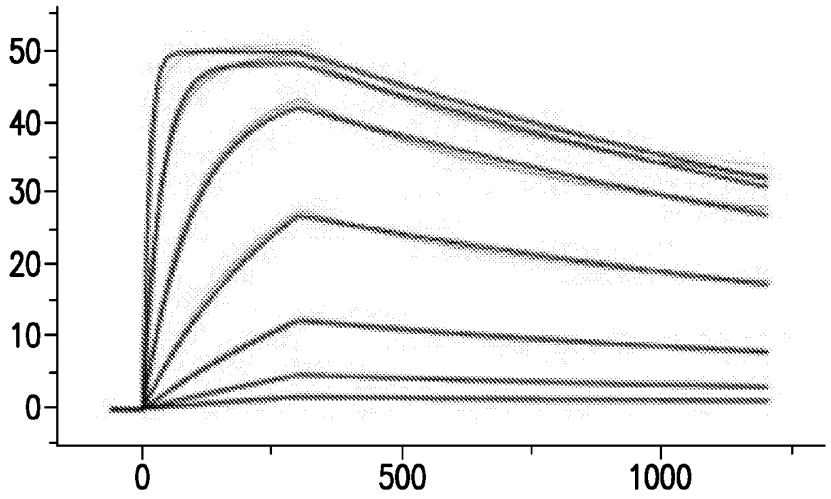


Group 1
hCLEC12A–his; F3'1602; 1:1 binding

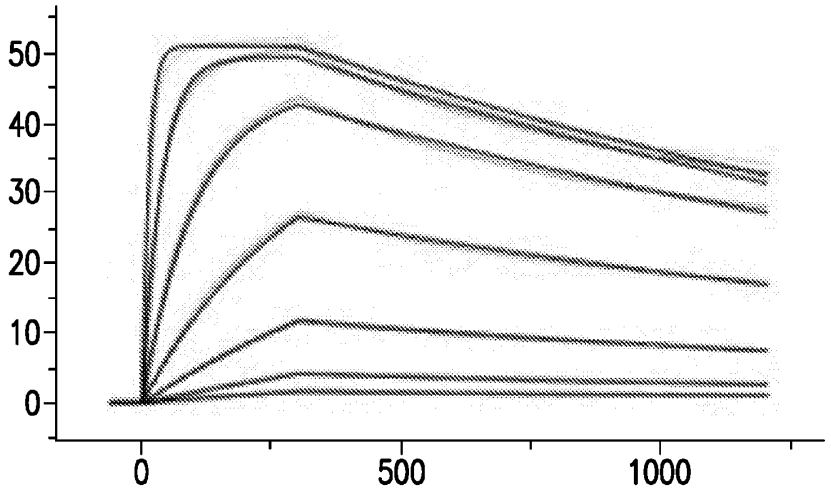


Group 2
hCLEC12A–his; F3'1602; 1:1 binding

FIG. 55



Group 3
hCLEC12A–his; F3'1602; 1:1 binding



Group 4
hCLEC12A–his; F3'1602; 1:1 binding

FIG. 55 Continued

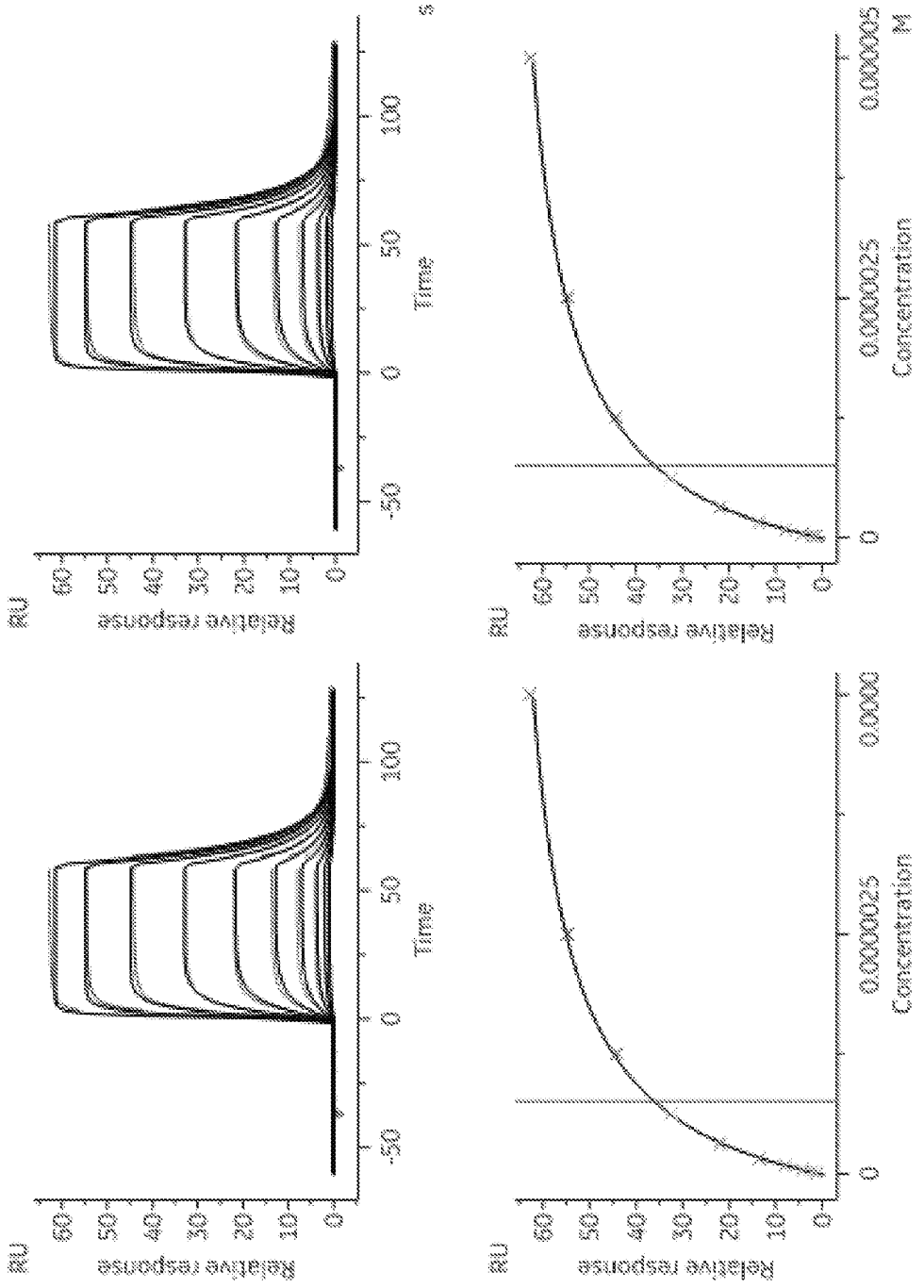


FIG. 56

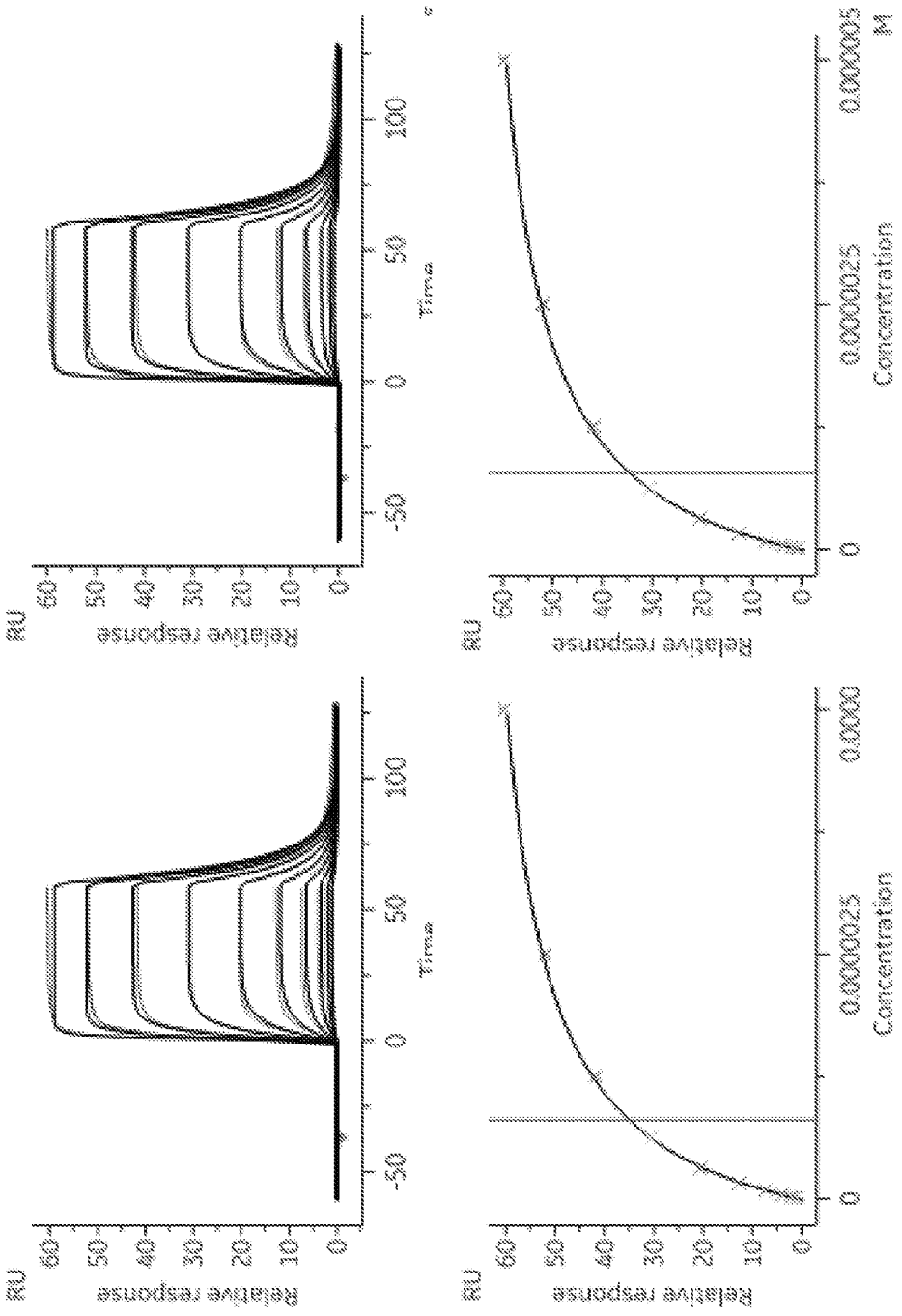


FIG. 56 Continued

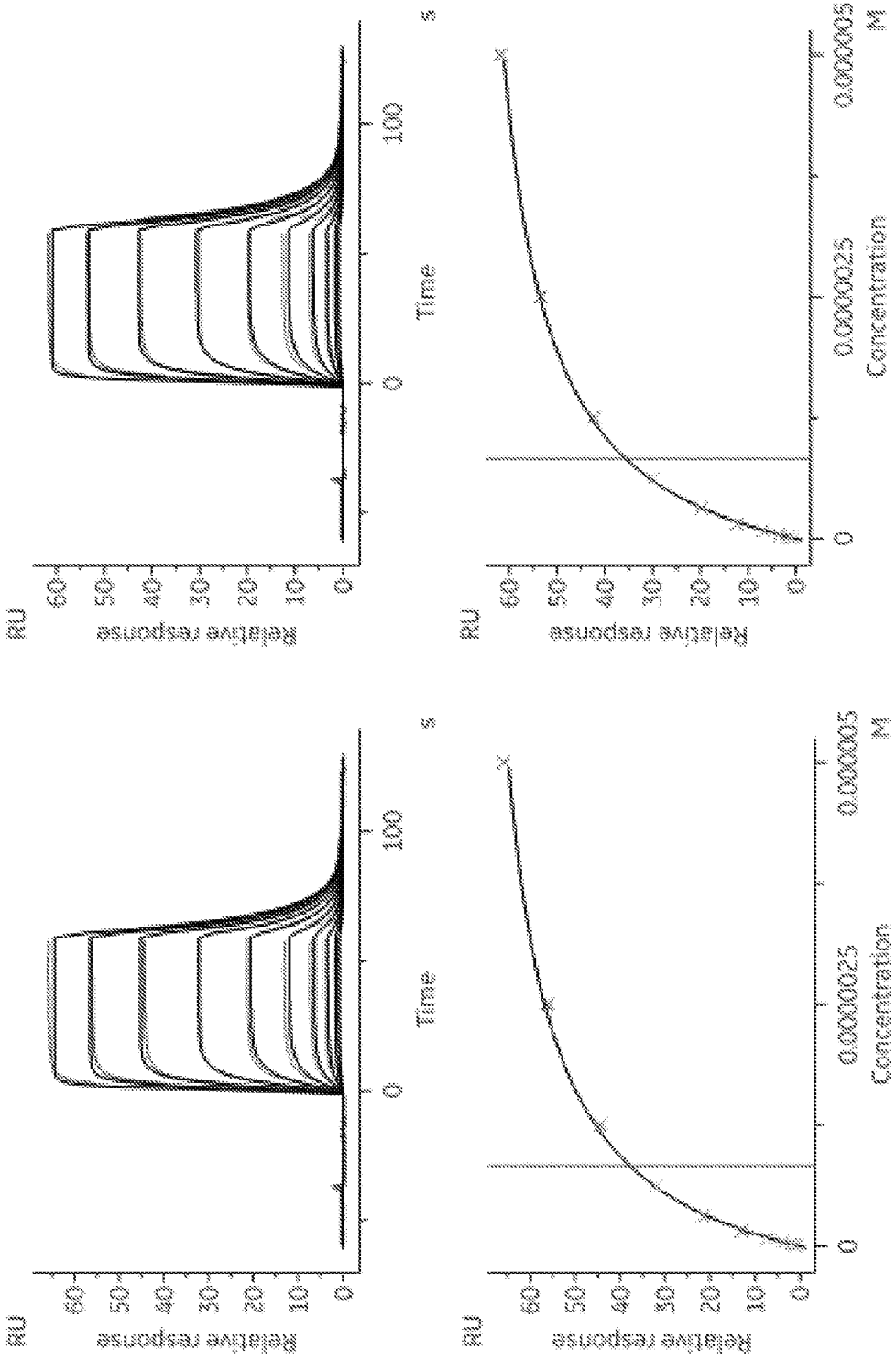

RU
Relative response
60
50
40
30
20
10
0
Time
s
0
100
RU
Relative response
60
50
40
30
20
10
0
0
0.0000025
0.0000005
Concentration
M

FIG. 57

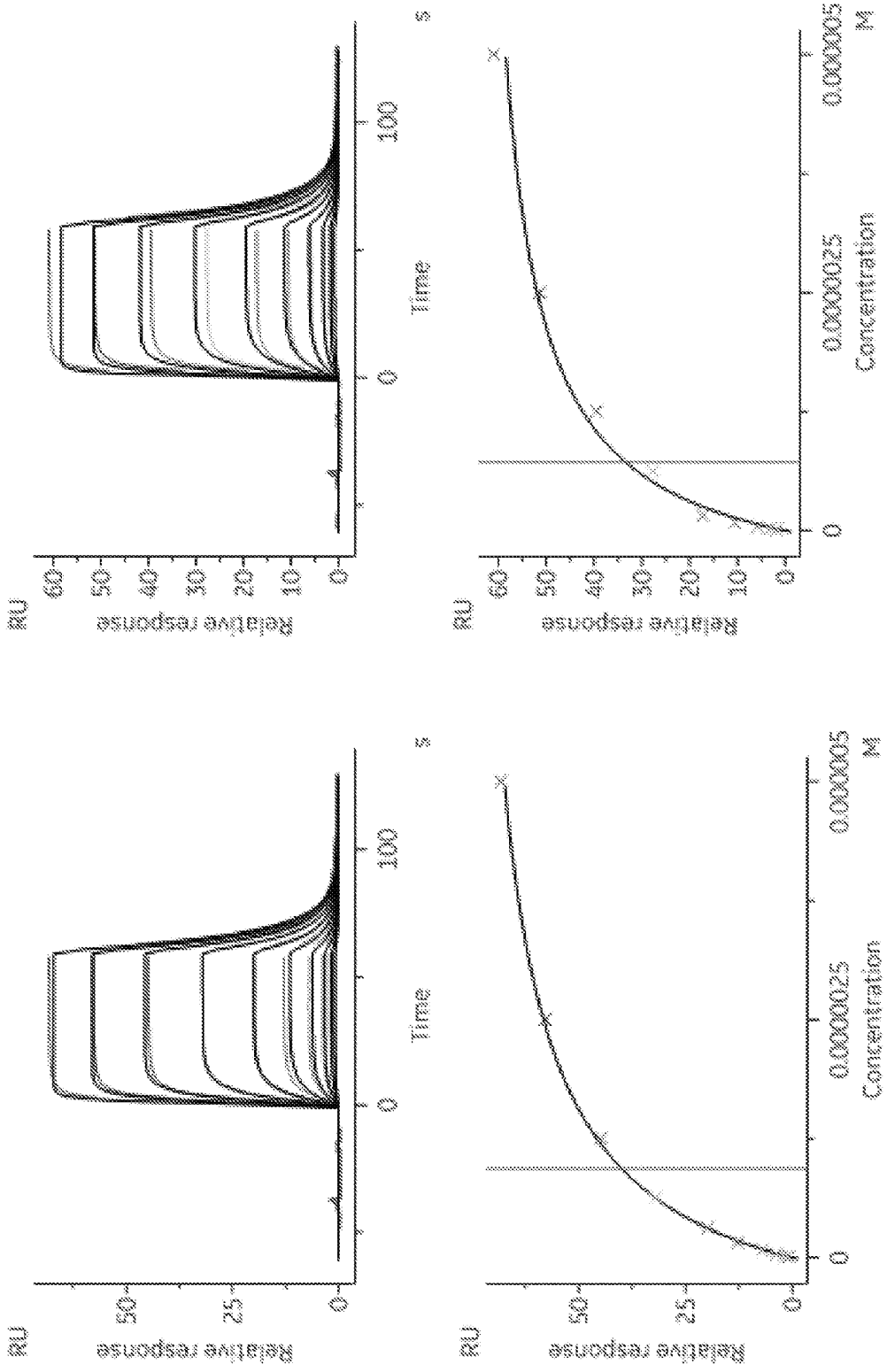


FIG. 57 Continued

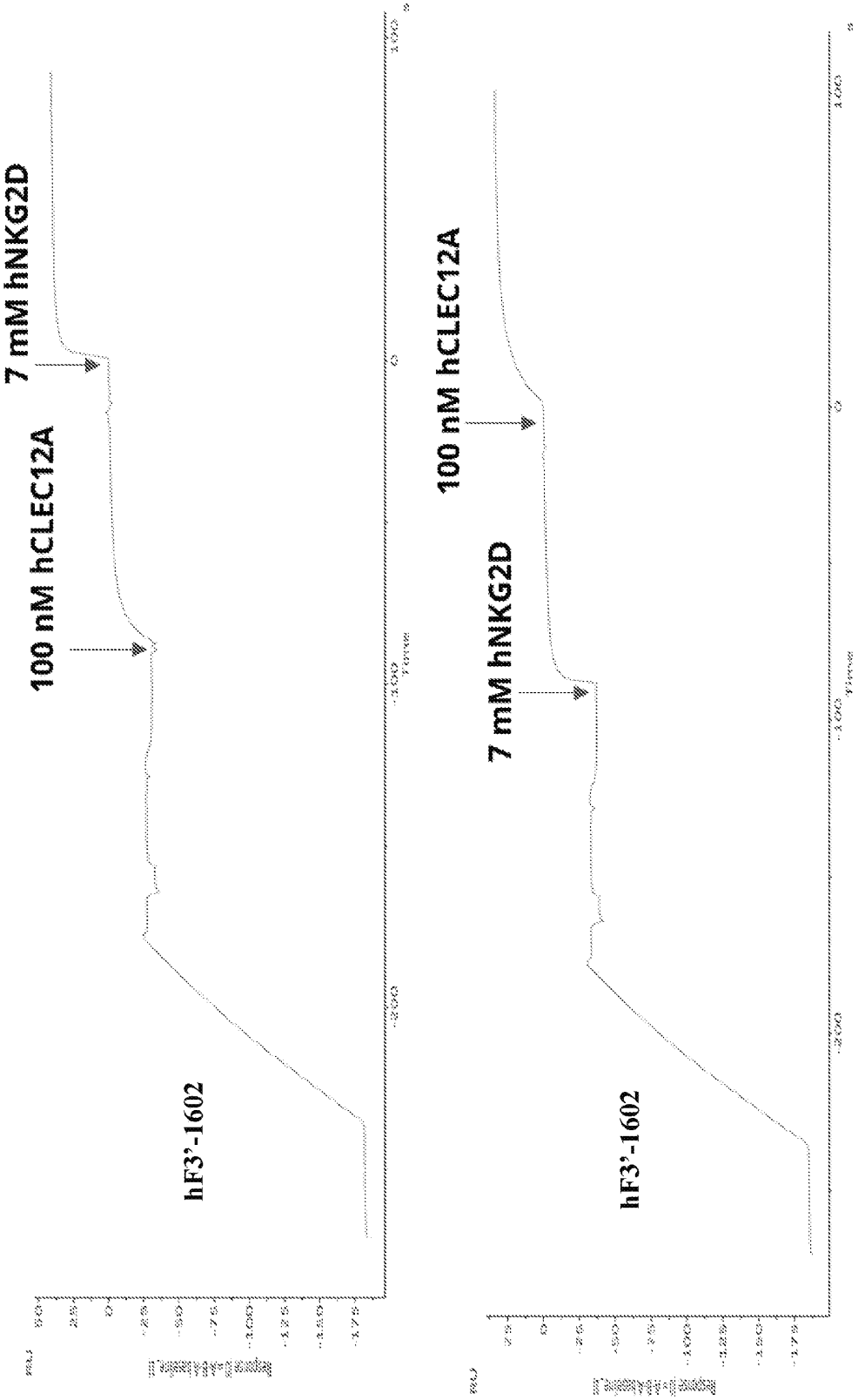

7 mM hNKG2D
100 nM hCLEC12A
hF3'-1602
100 nM hCLEC12A
7 mM hNKG2D
hF3'-1602

FIG. 58

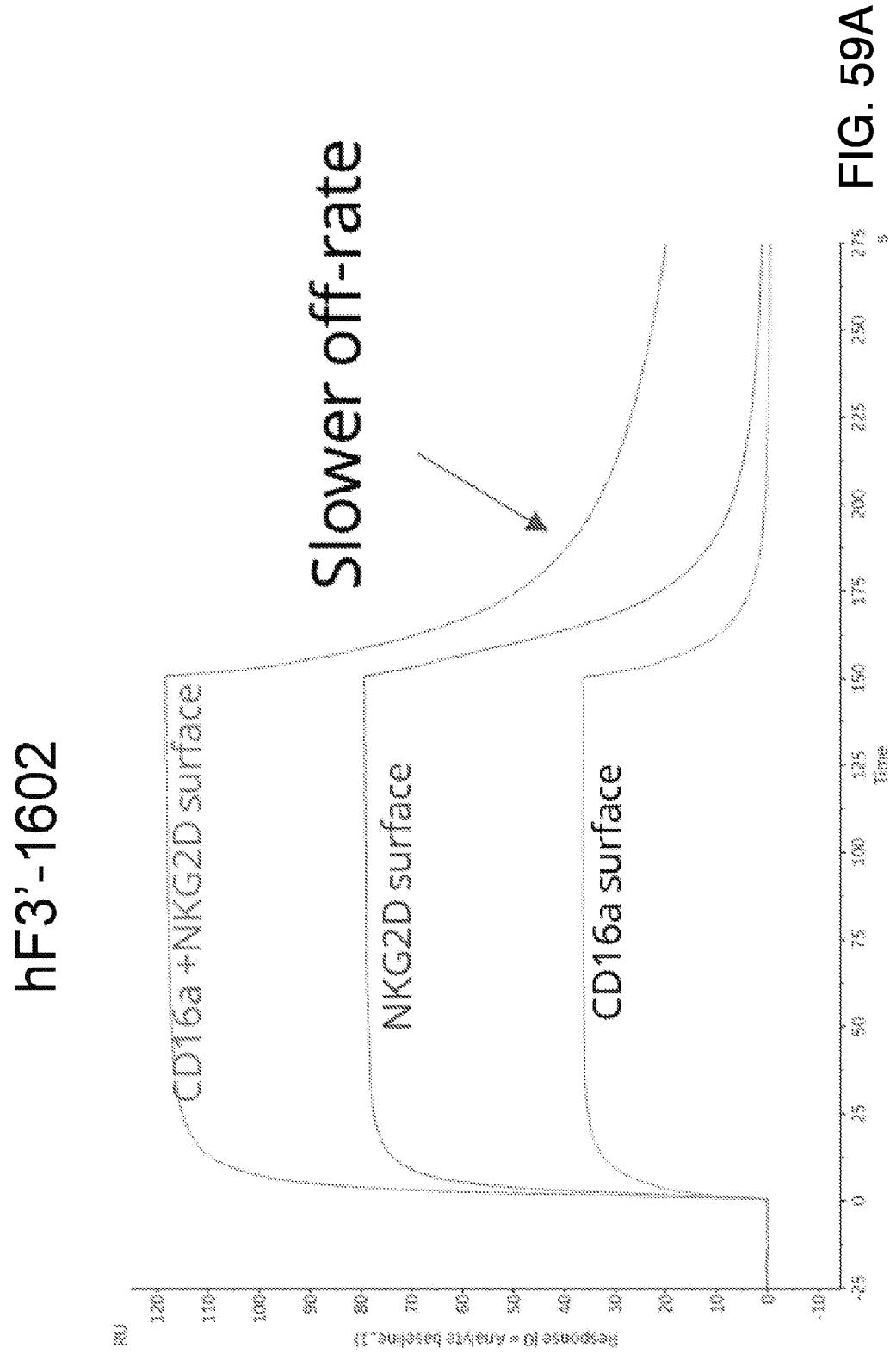

hF3'-1602
Slower off-rate
CD16a +NKG2D surface
NKG2D surface
CD16a surface
Time
RU
Response (Analyte baseline_1)

FIG. 59A

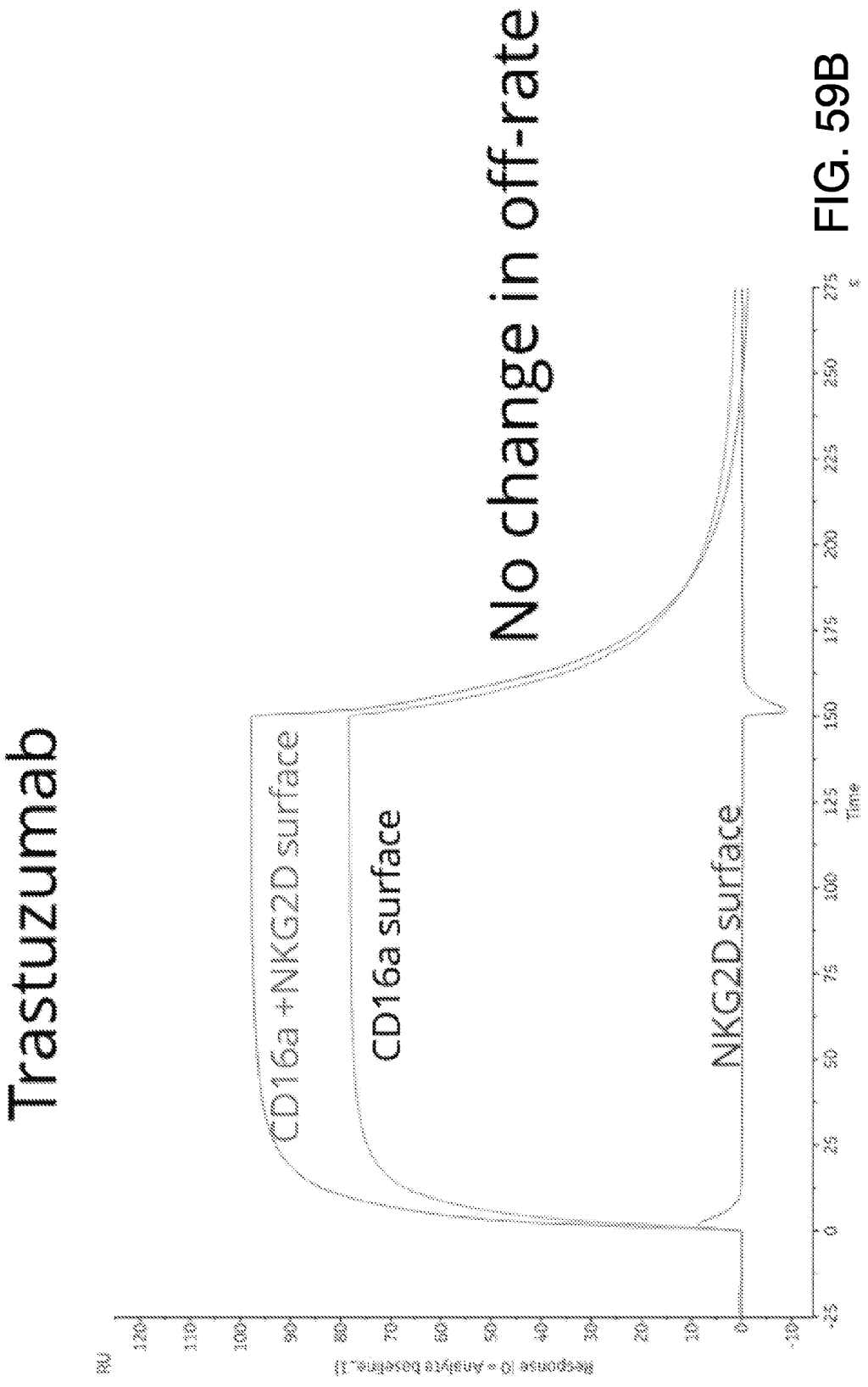

Trastuzumab
No change in off-rate
CD16a +NKG2D surface
CD16a surface
NKG2D surface
RU
Response (Δ = Analyte bonding...)
Time
s

FIG. 59B

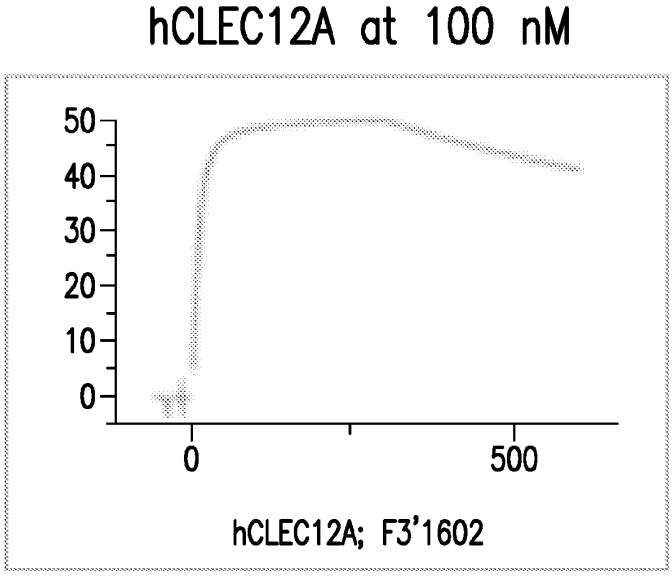


FIG. 60A

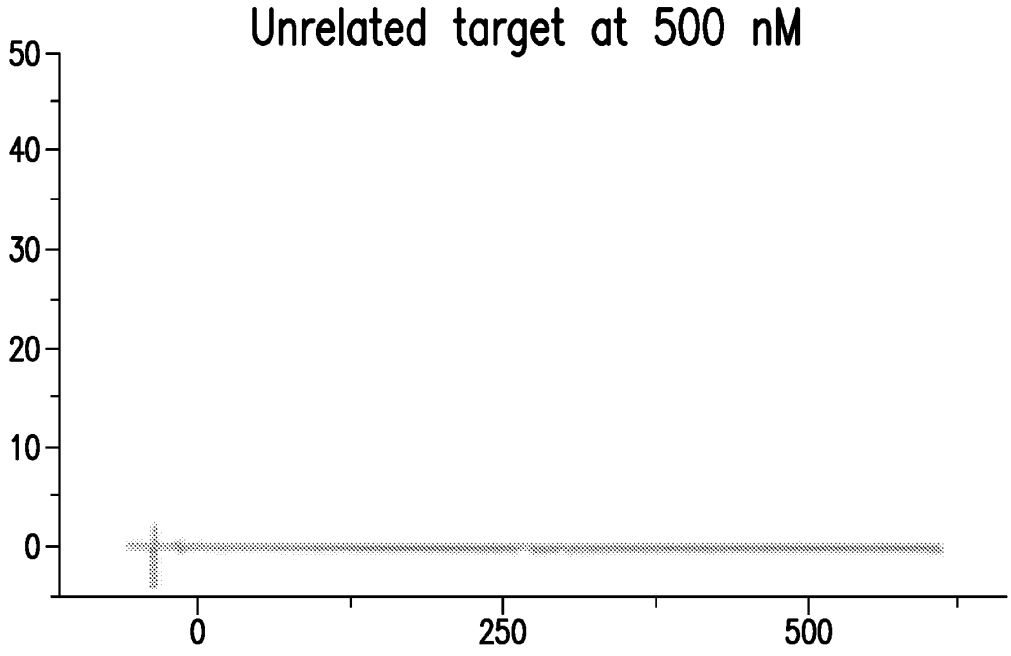


FIG. 60B

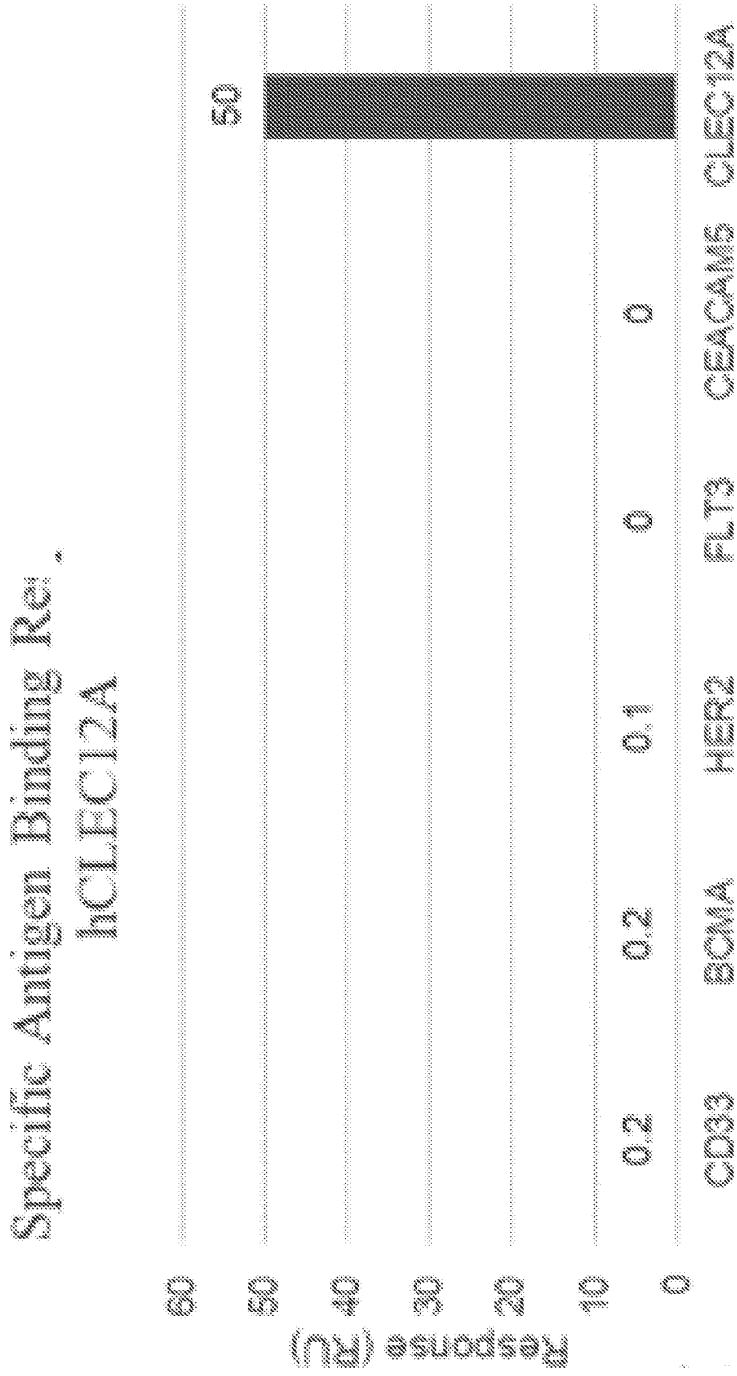

Specific Antigen Binding Ref.
hCLEC12A
Response (RU)
60
50
40
30
20
10
0
50
0
0
0.1
0.2
0.2
CD33
BCMA
HER2
FLT3
CEACAM5
CLEC12A
Antigen

FIG. 60C

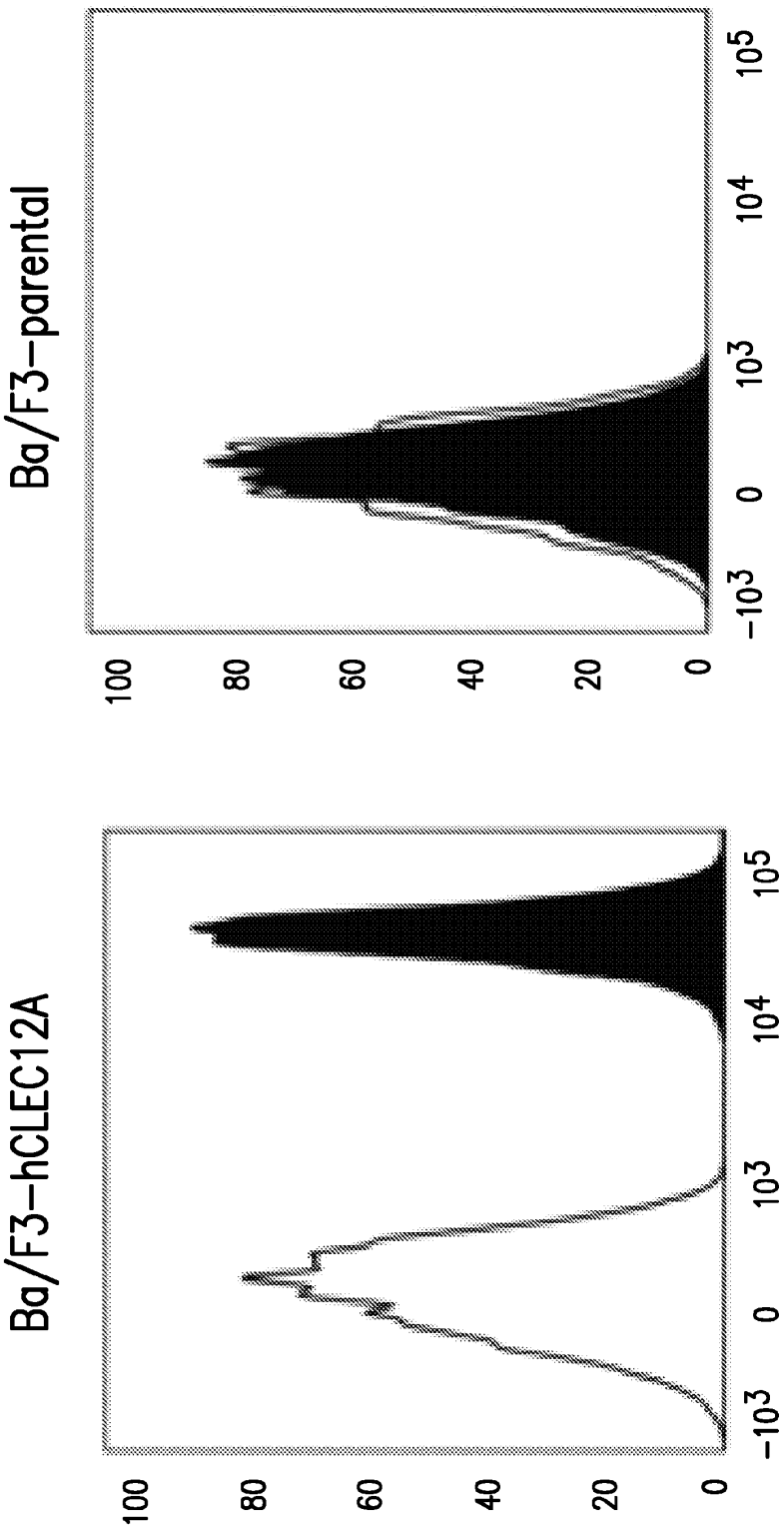


FIG. 60D FIG. 60E

No fill = hIgG1 isotype
Black fill= hF3'-1602

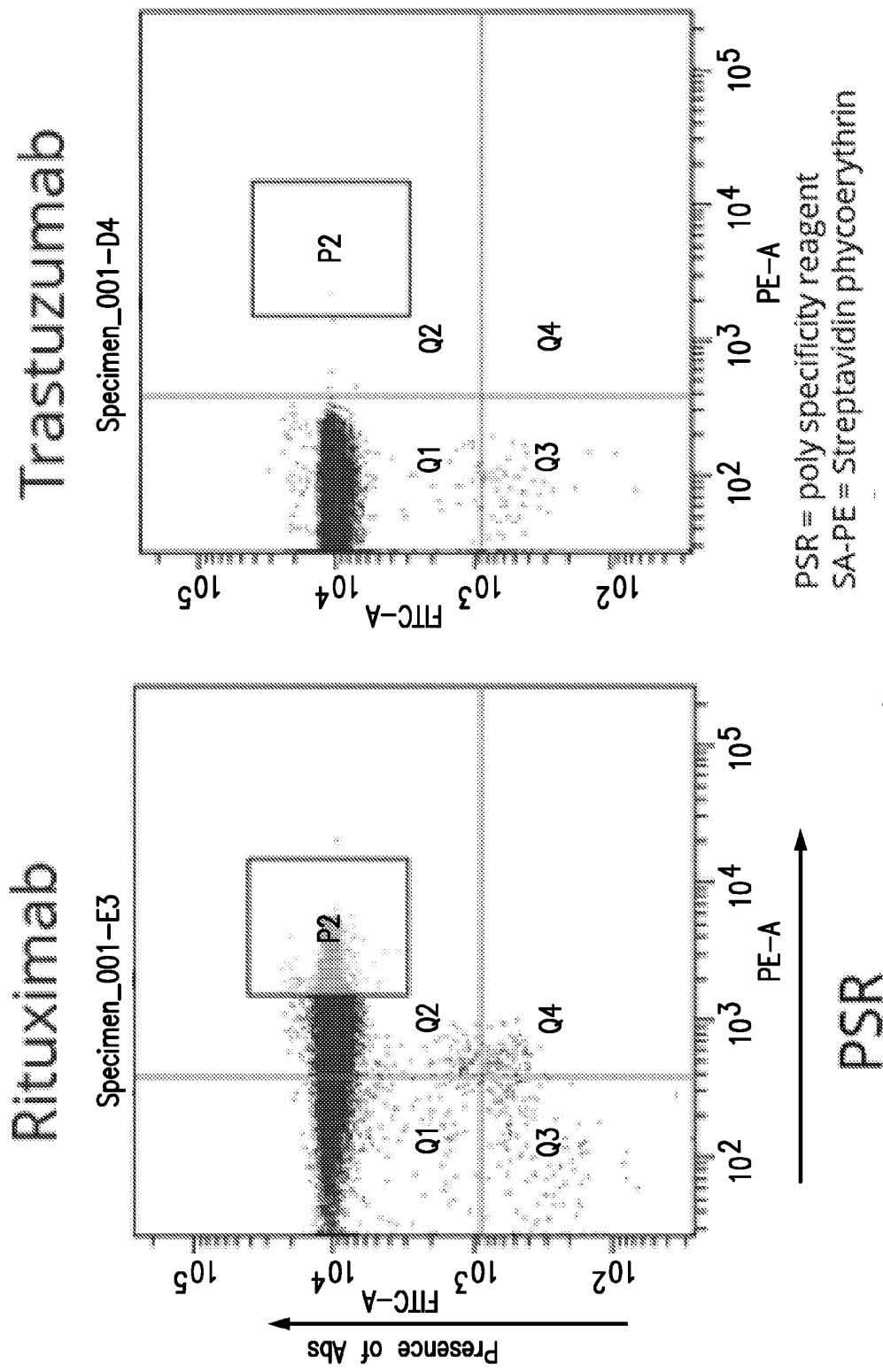

Trastuzumab
Specimen_001-D4
P2
Q1
Q2
Q3
Q4
FITC-A
PE-A
PSR = poly specificity reagent
SA-PE = Streptavidin phycoerythrin
Rituximab
Specimen_001-E3
Presence of Abs
PSR

FIG. 61

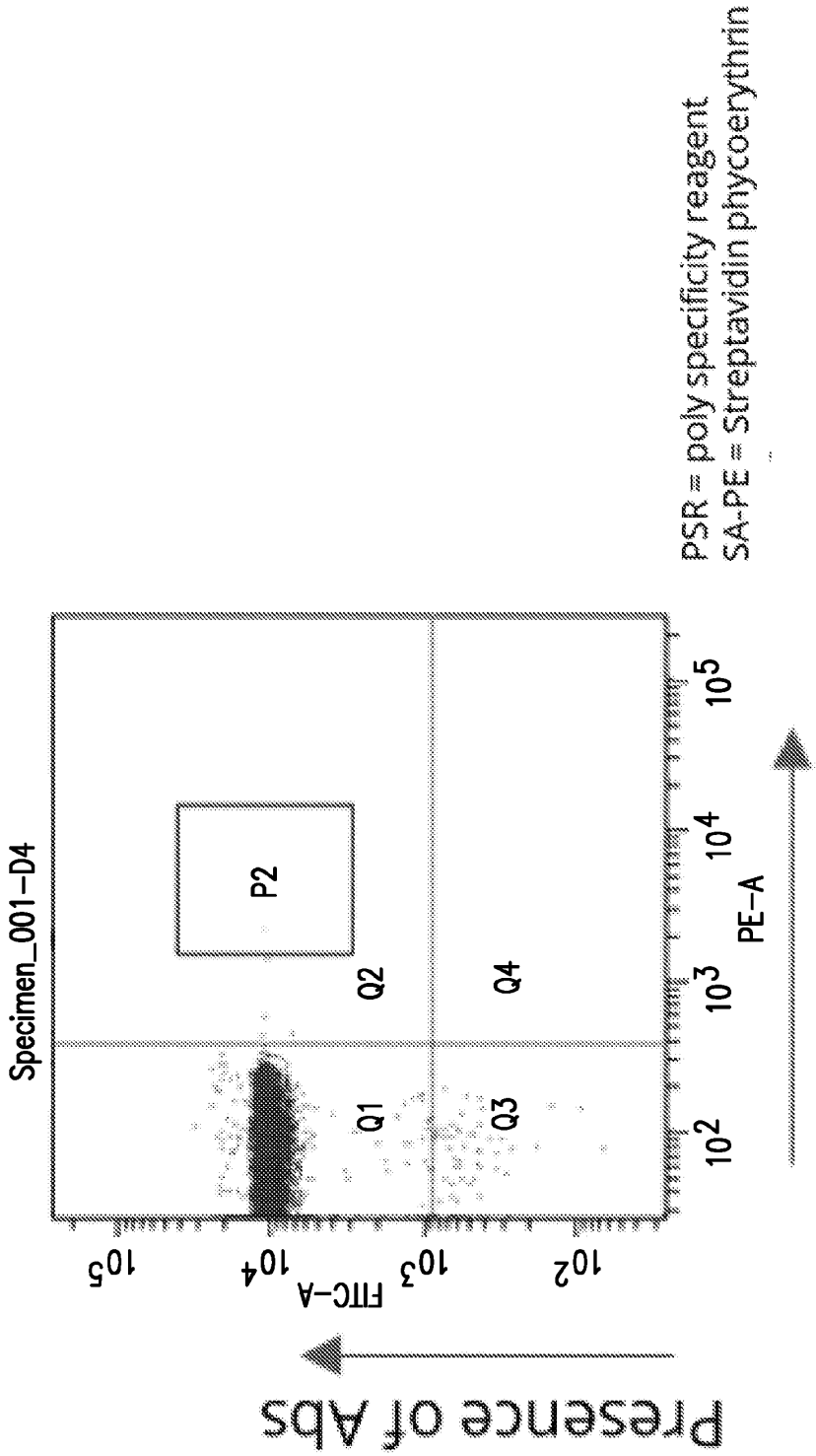


FIG. 61 Continued

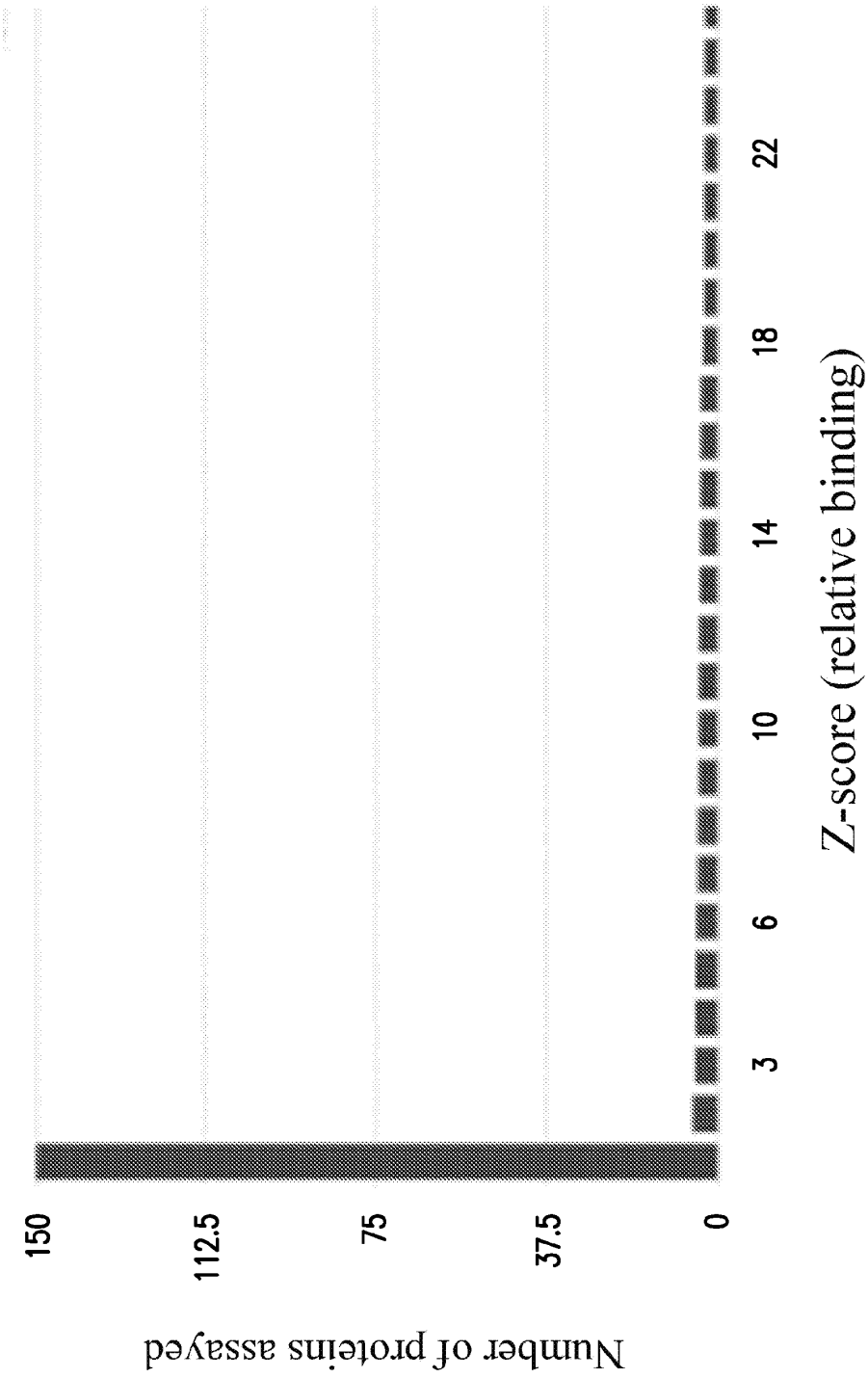
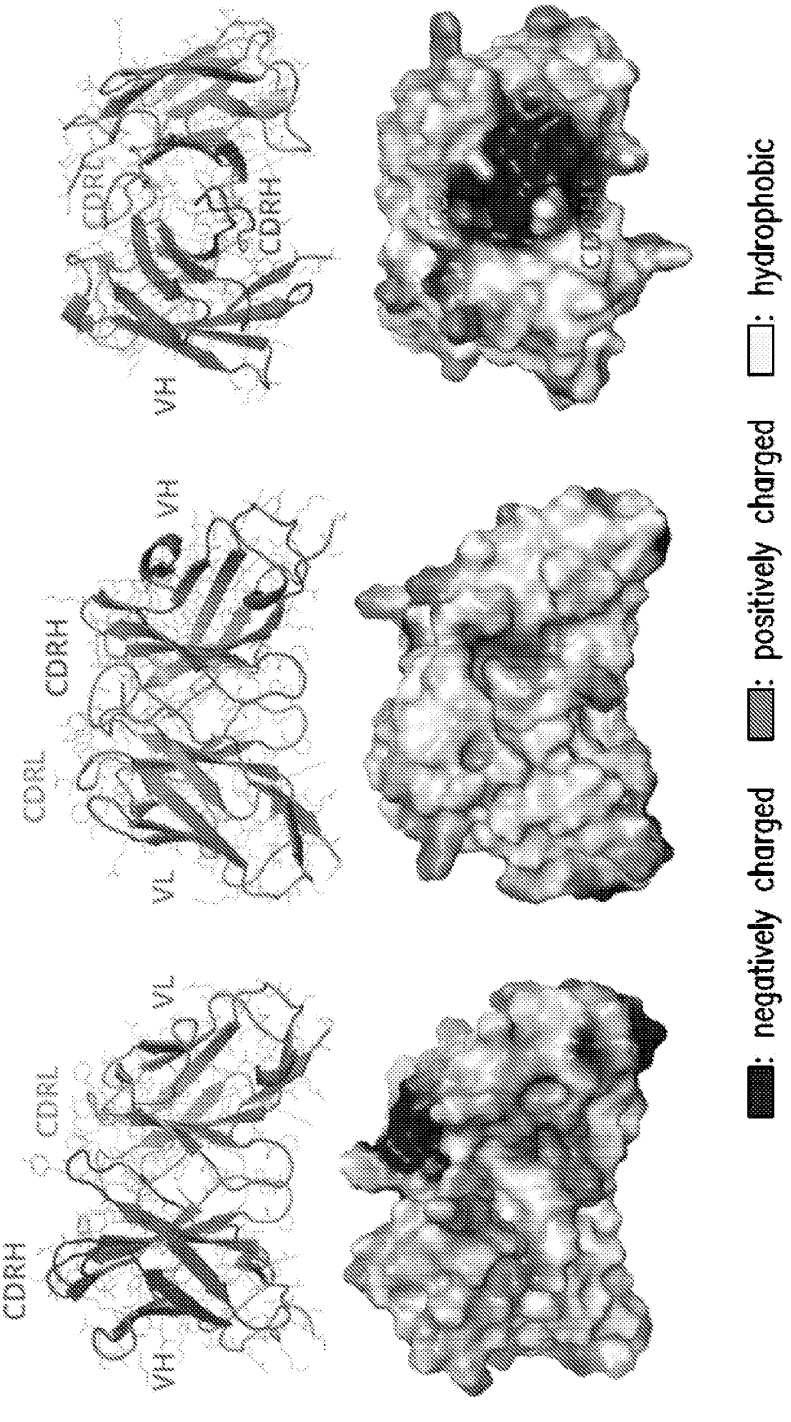

Number of proteins assayed
150
112.5
75
37.5
0
3
6
10
14
18
22
Z-score (relative binding)

FIG. 62

FIG. 63


CDRH
CDRL
VL
VH
CDRL
CDRH
VH
VL
CDRL
CDRH
VH
VL
: negatively charged
: positively charged
: hydrophobic

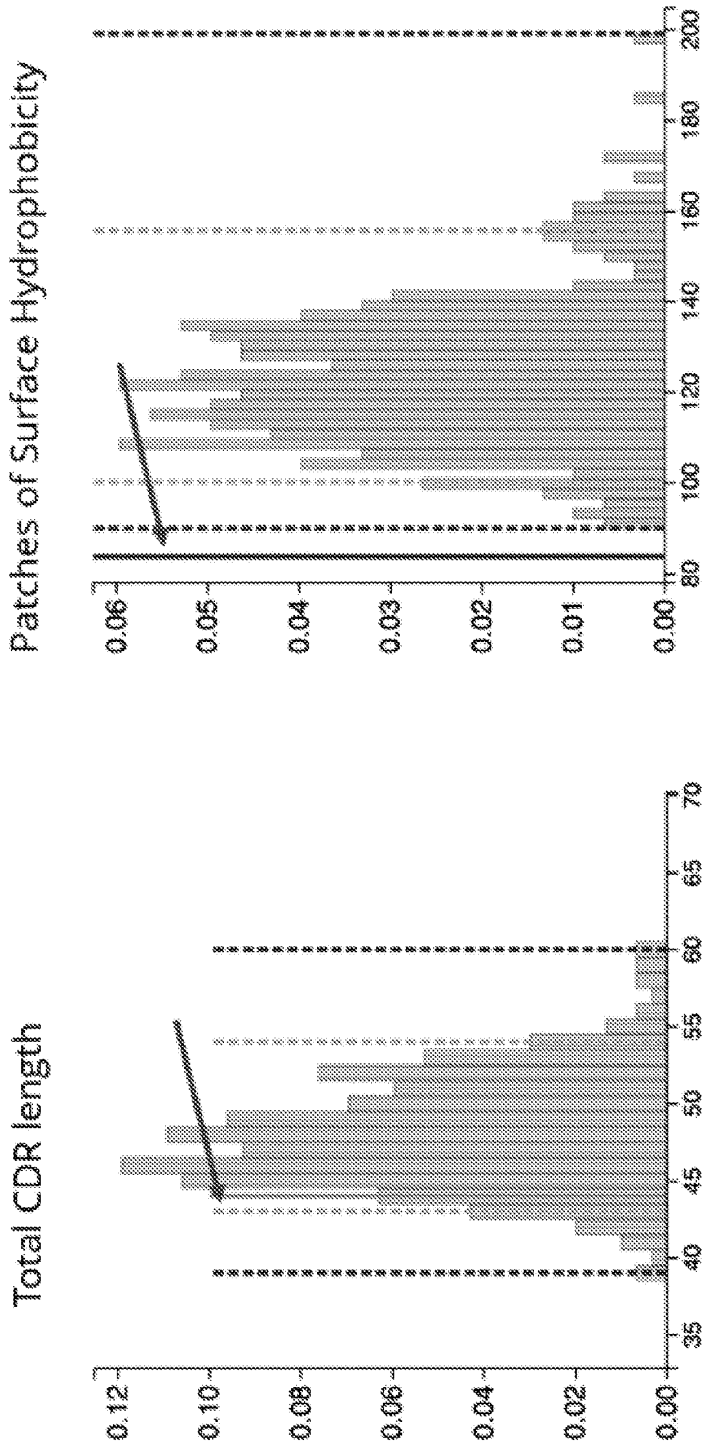

Patches of Surface Hydrophobicity
0.06
0.05
0.04
0.03
0.02
0.01
0.00
80
100
120
140
160
180
200
Total CDR length
0.12
0.10
0.08
0.06
0.04
0.02
0.00
35
40
45
50
55
60
65
70

FIG. 64

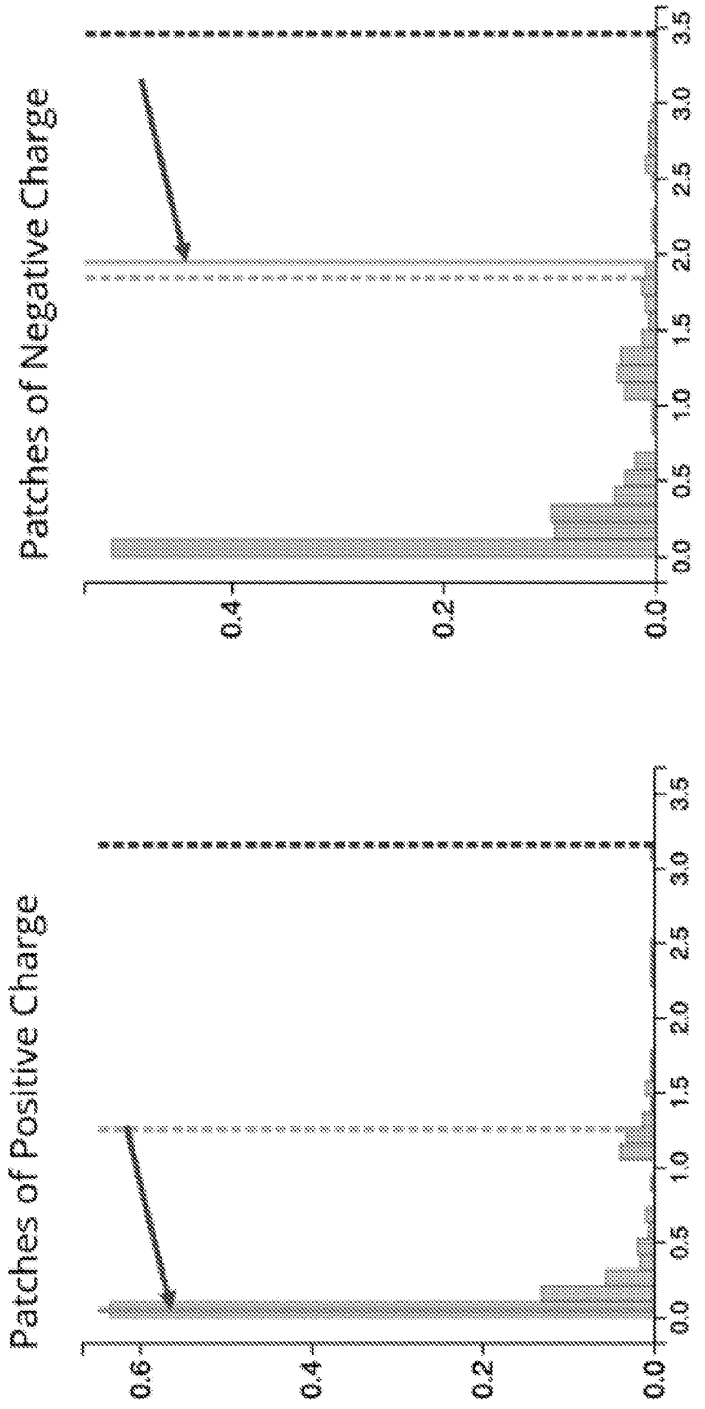


FIG. 64 Continued

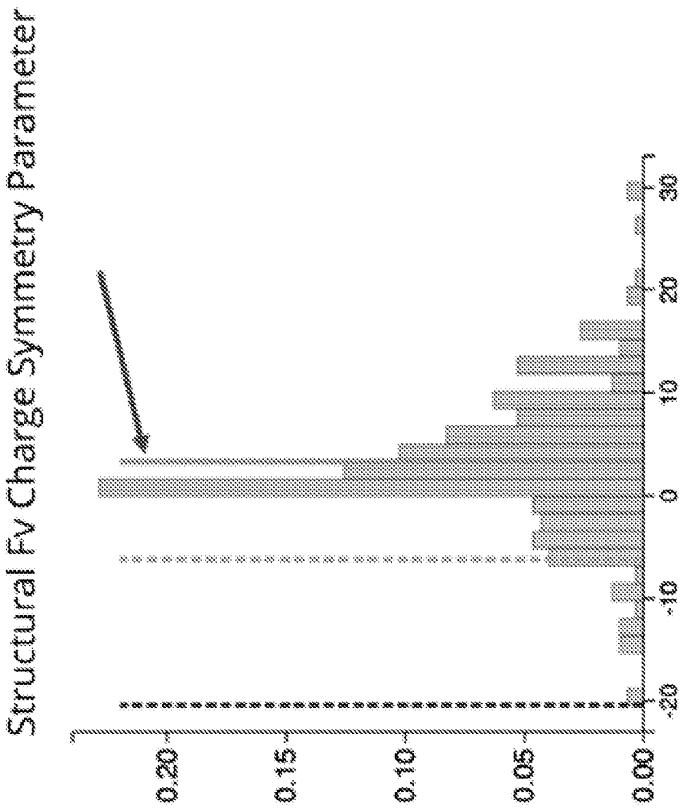
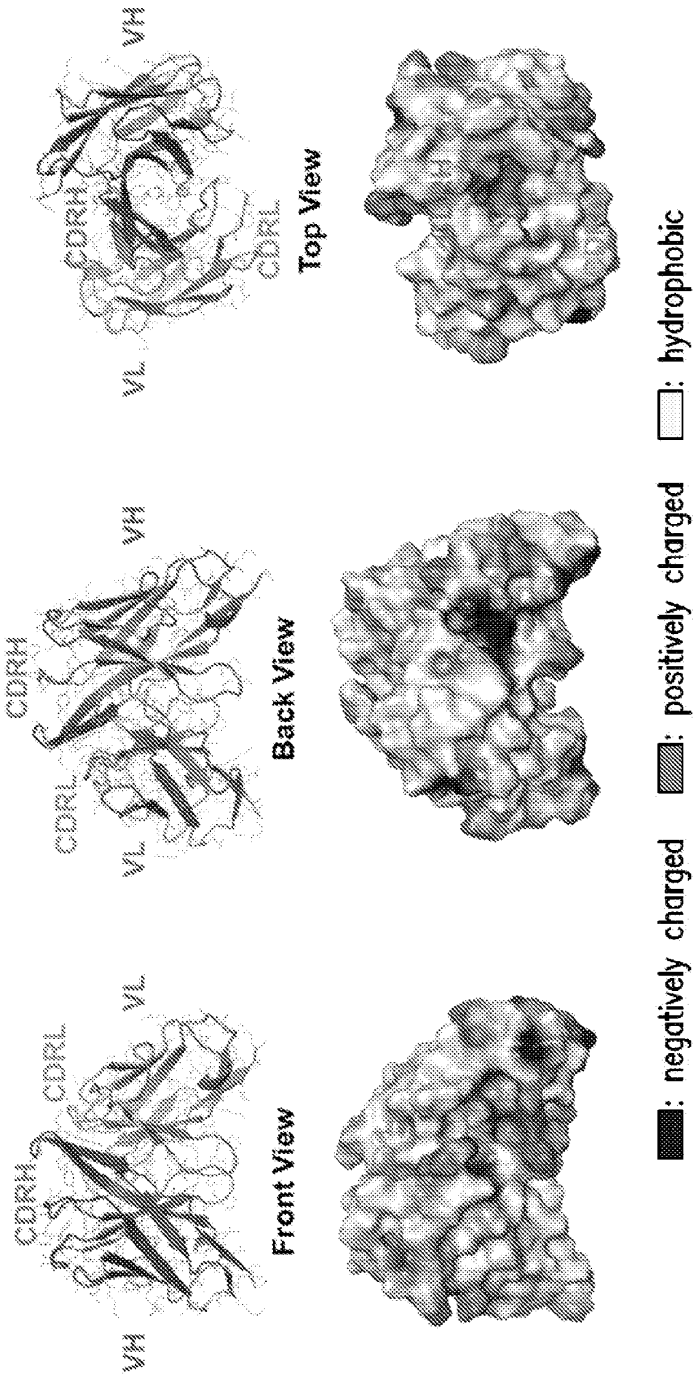


FIG. 64 Continued

FIG. 65


VH
CDRH
CDRL
VL
Front View
VL
CDRL
CDRH
VH
Back View
VH
CDRH
CDRL
VL
Top View
■: negatively charged
▨: positively charged
□: hydrophobic

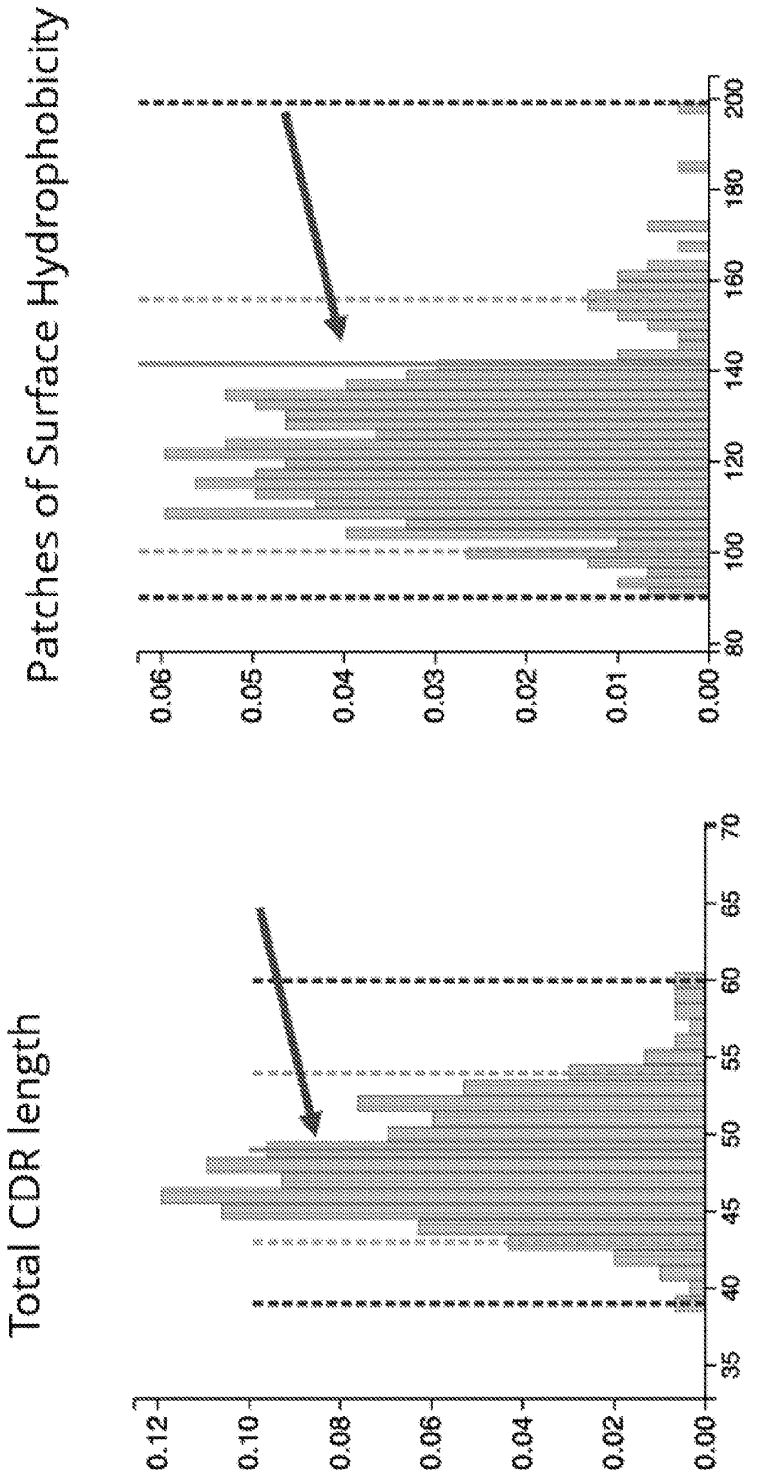

Patches of Surface Hydrophobicity
0.06
0.05
0.04
0.03
0.02
0.01
0.00
80
100
120
140
160
180
200
Total CDR length
0.12
0.10
0.08
0.06
0.04
0.02
0.00
35
40
45
50
55
60
65
70

FIG. 66

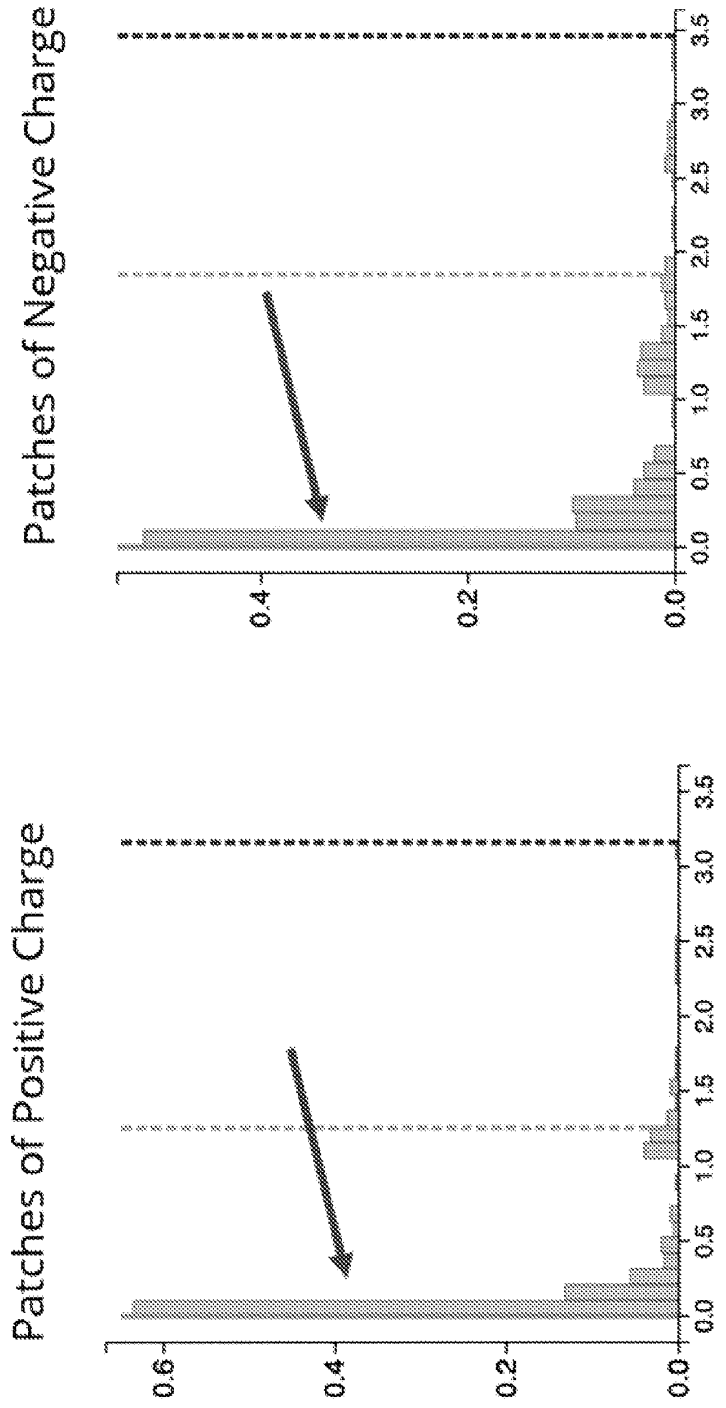


FIG. 66 Continued

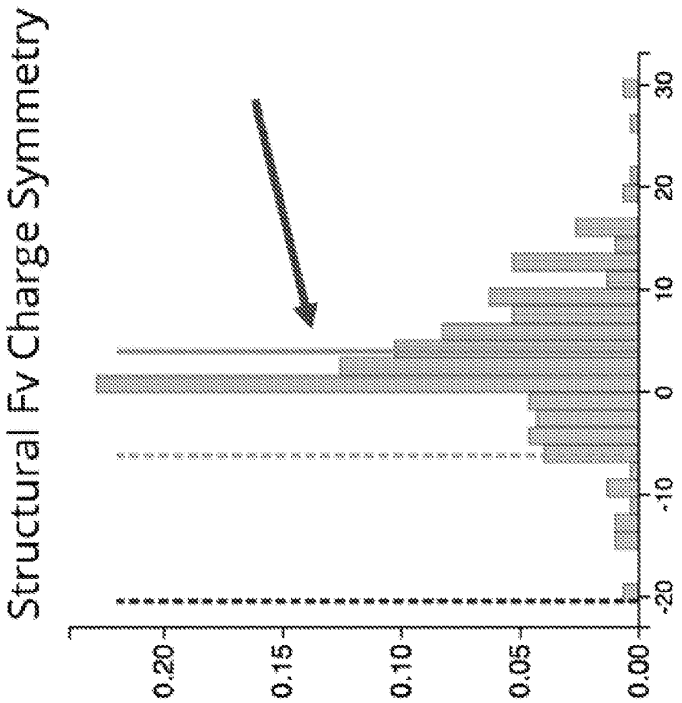


FIG. 66 Continued

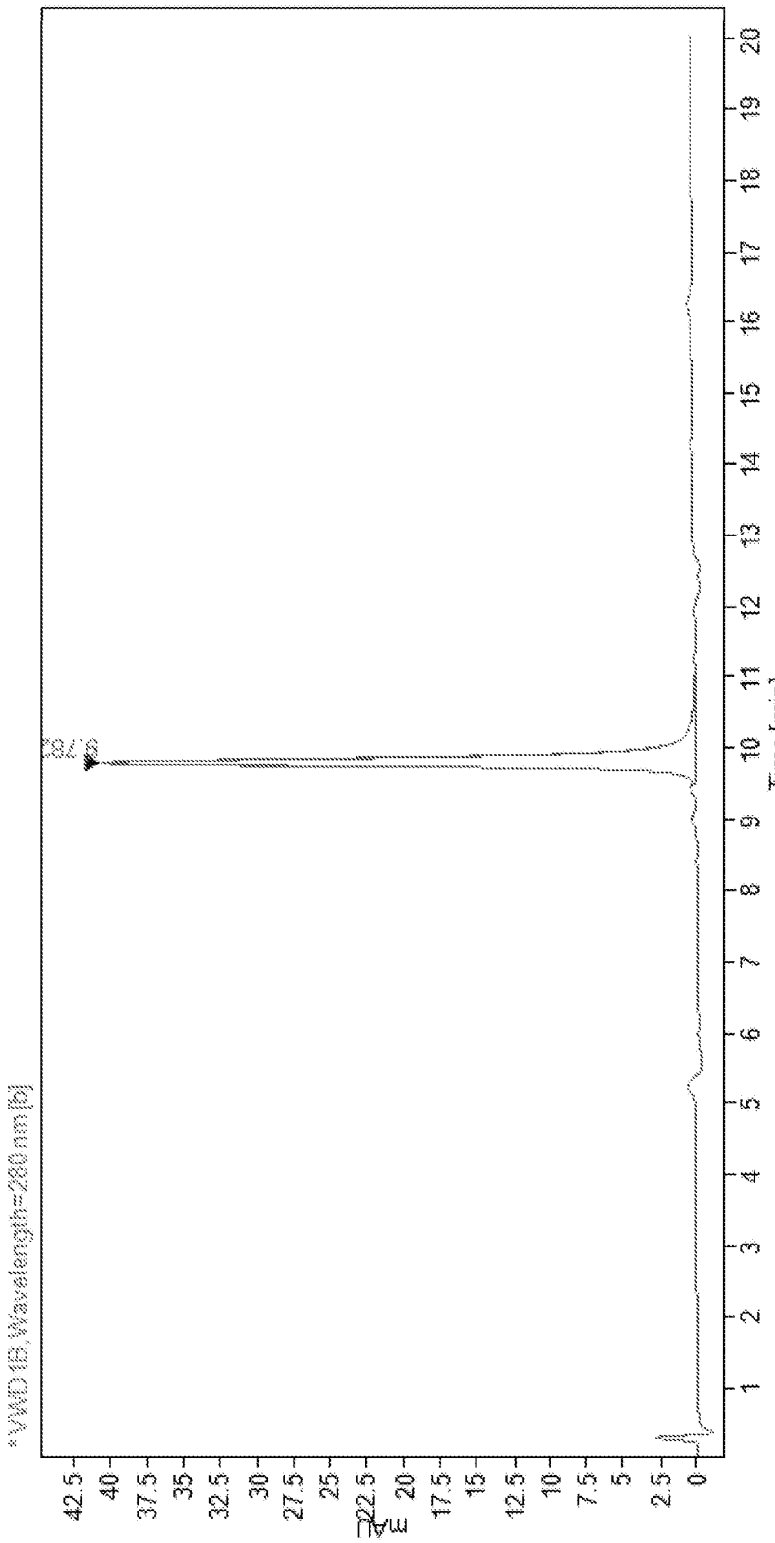


FIG. 67

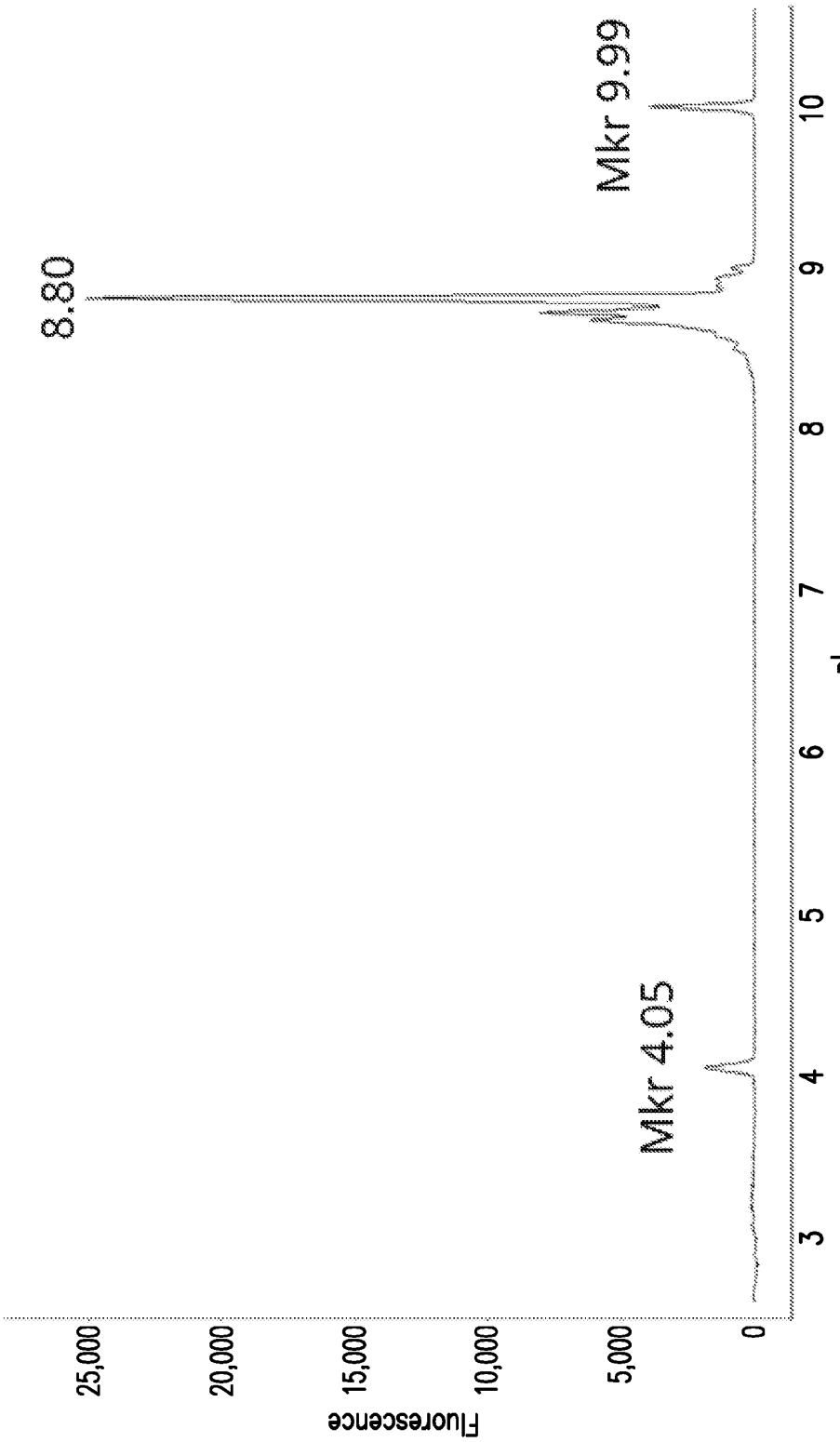

Fluorescence
25,000
20,000
15,000
10,000
5,000
0
3
4
5
6
7
8
9
10
pI
8.80
Mkr 9.99
Mkr 4.05

FIG. 68

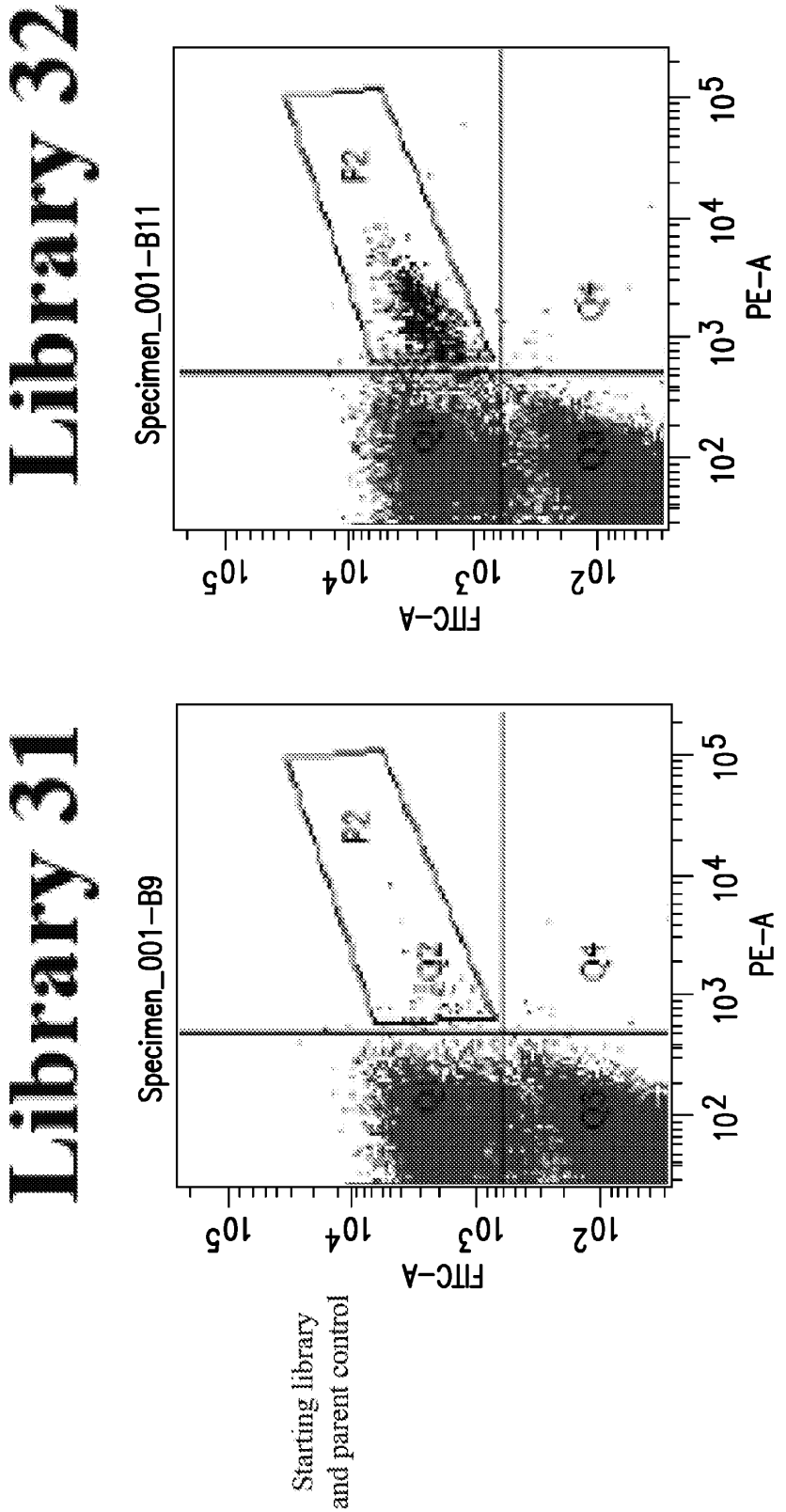

Library 32
Specimen_001-B11
P2
Q4
FITC-A
PE-A
10^2
10^3
10^4
10^5
Library 31
Specimen_001-B9
P2
Q2
Q4
FITC-A
PE-A
10^2
10^3
10^4
10^5
Starting library
and parent control

FIG. 69

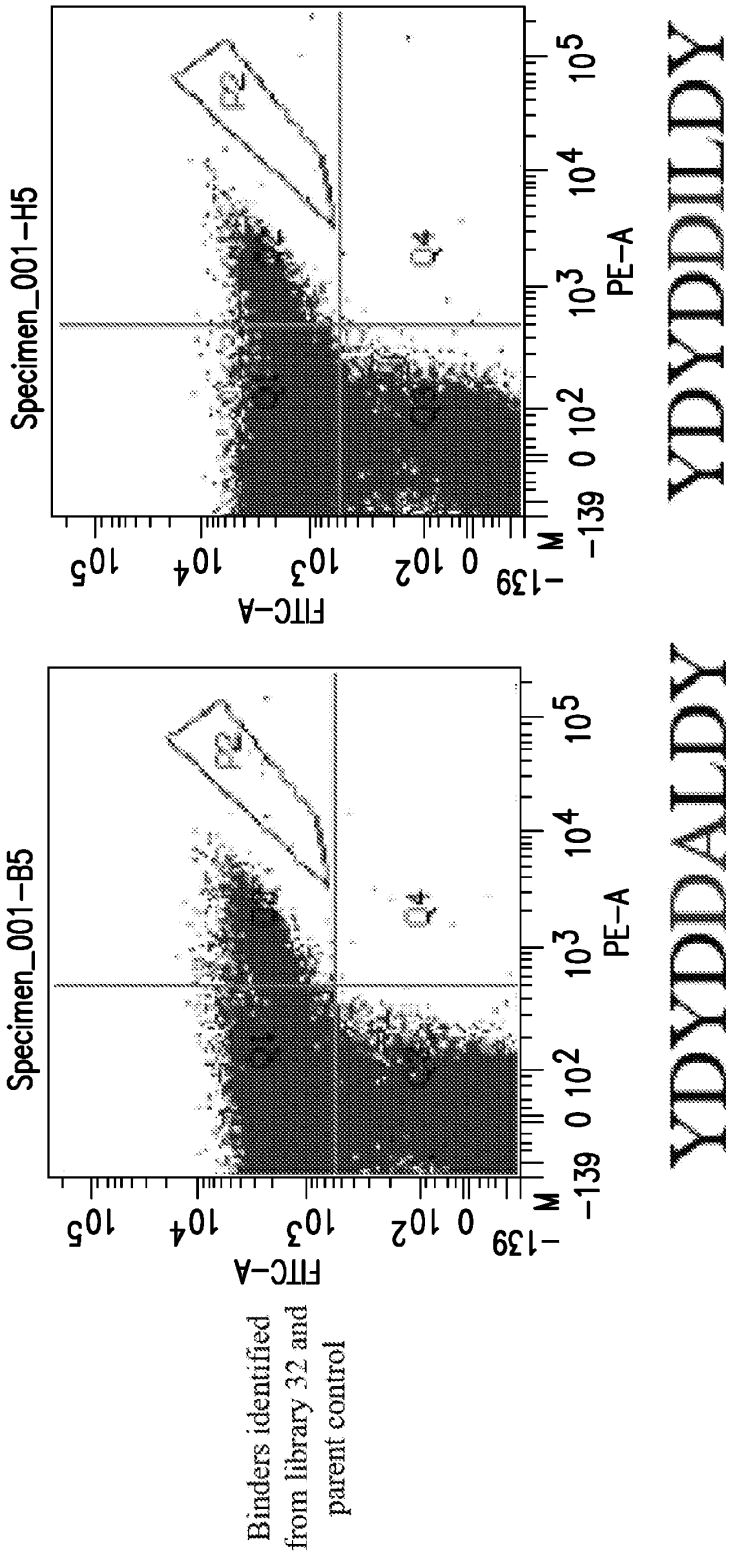


FIG. 69 Continued

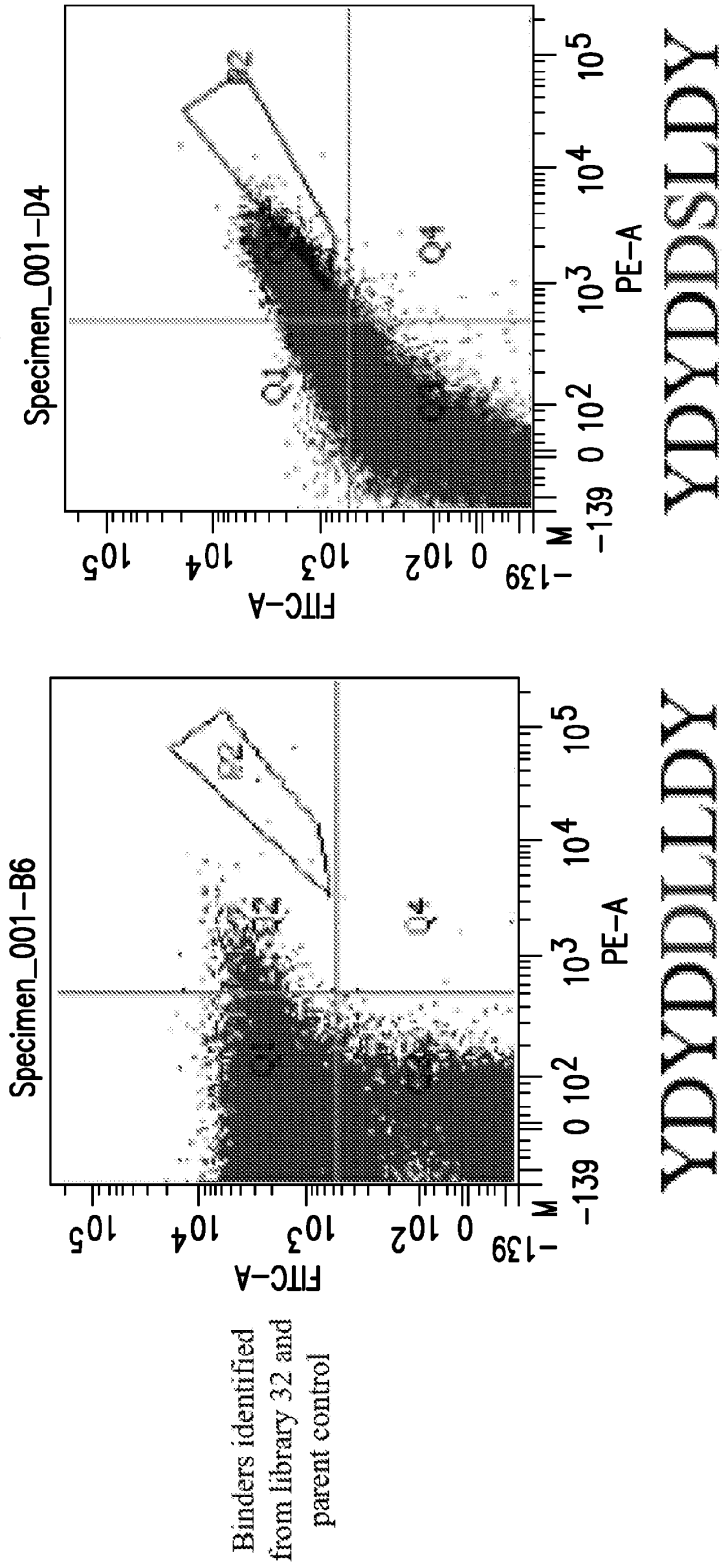


FIG. 69 Continued

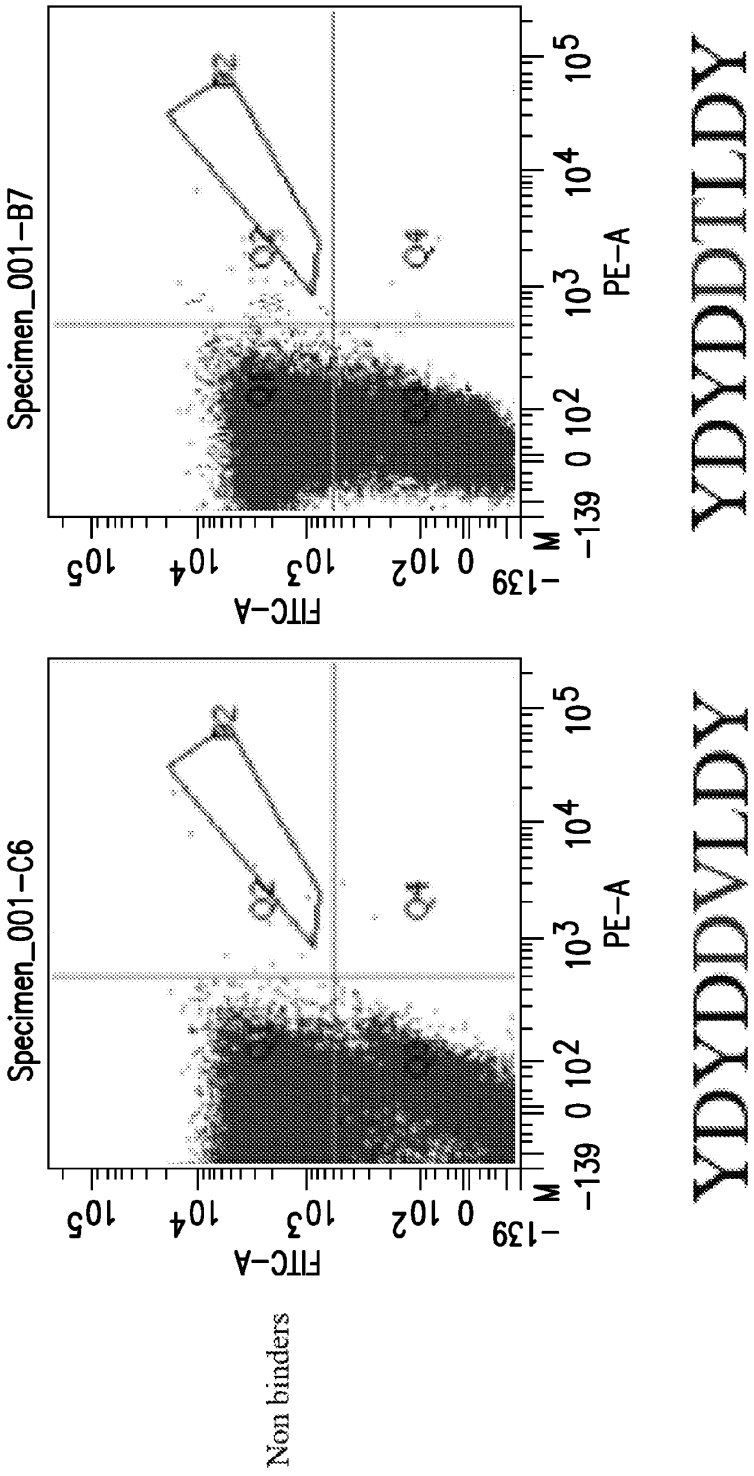
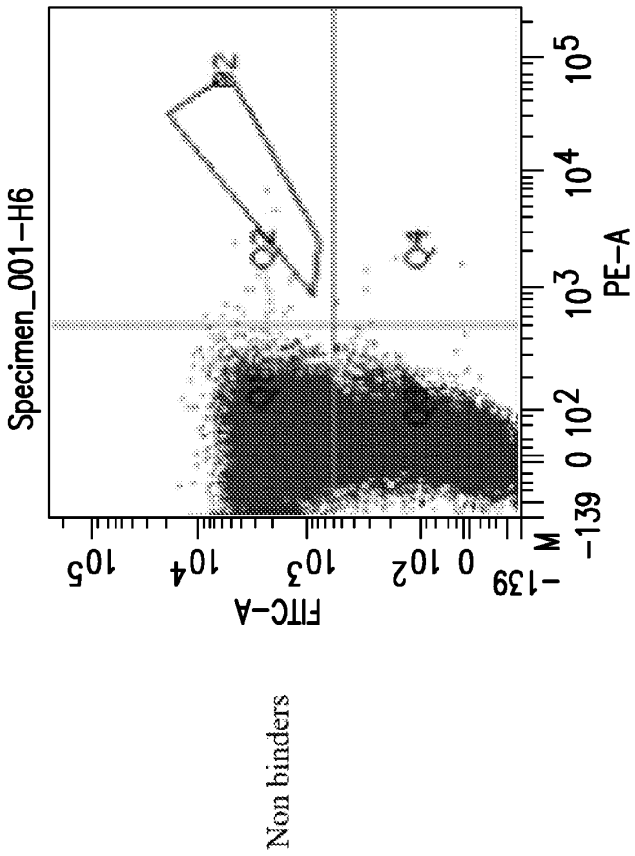


FIG. 69 Continued



Non binders

YDYDESLDY

FIG. 69 Continued

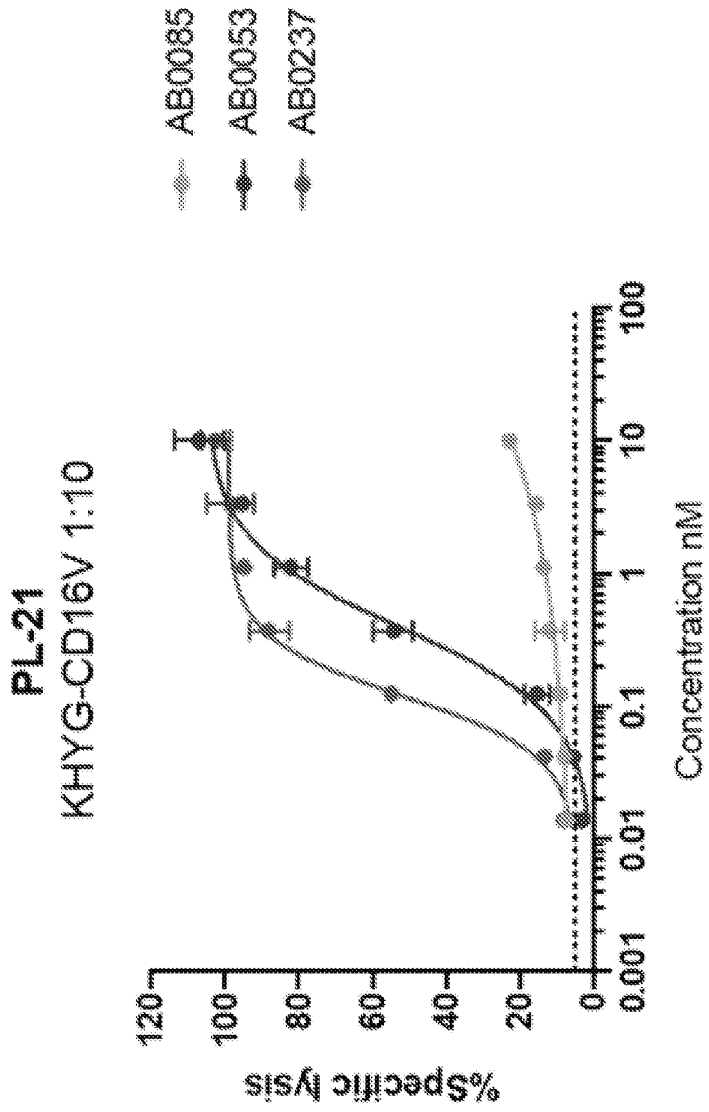
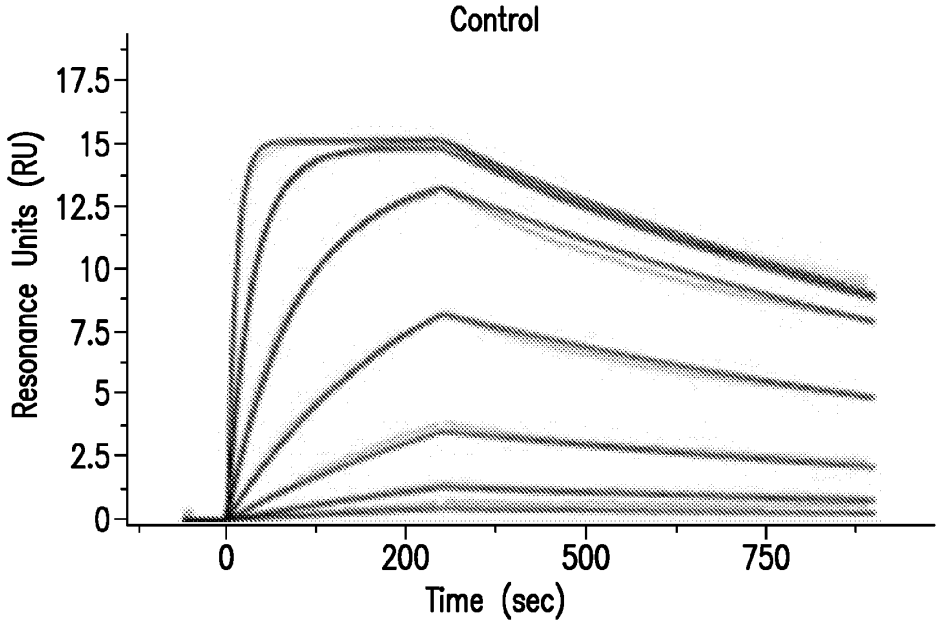

PL-21
KHYG-CD16V 1:10
AB0085
AB0053
AB0237
%Specific lysis
120
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Concentration nM

FIG. 70


Control
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
0
200
500
750
Time (sec)

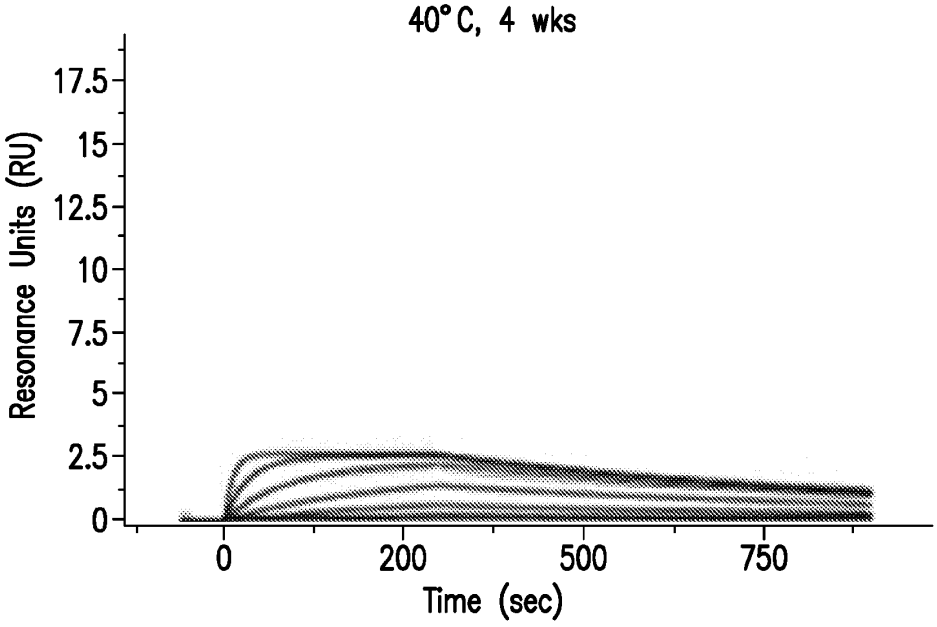
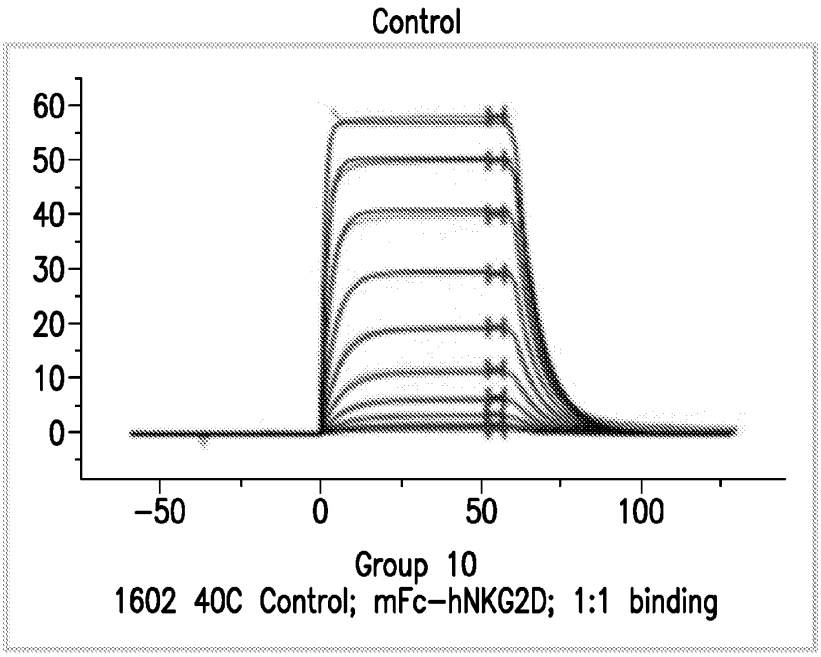

40° C, 4 wks
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
0
200
500
750
Time (sec)

FIG. 71


Control
60
50
40
30
20
10
0
−50
0
50
100
Group 10
1602 40C Control; mFc−hNKG2D; 1:1 binding

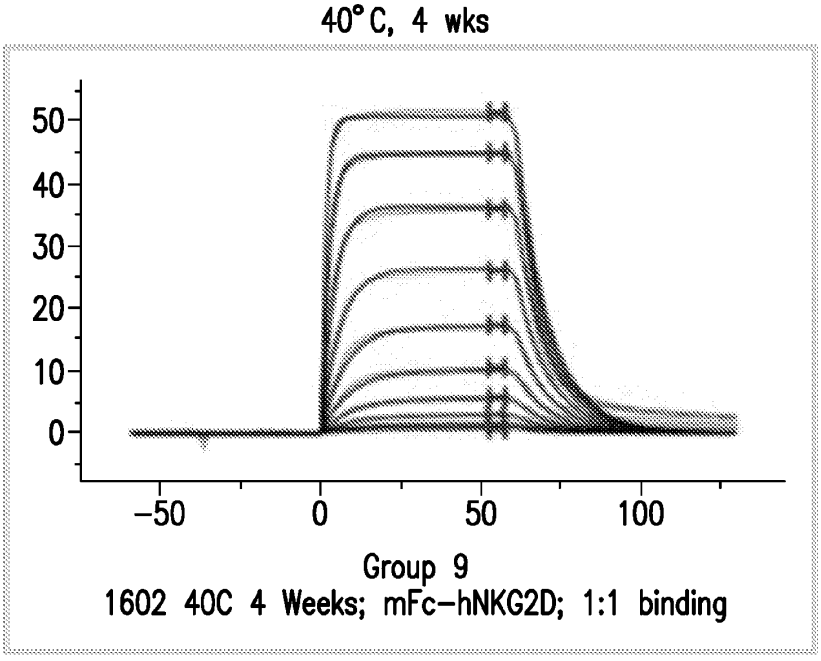
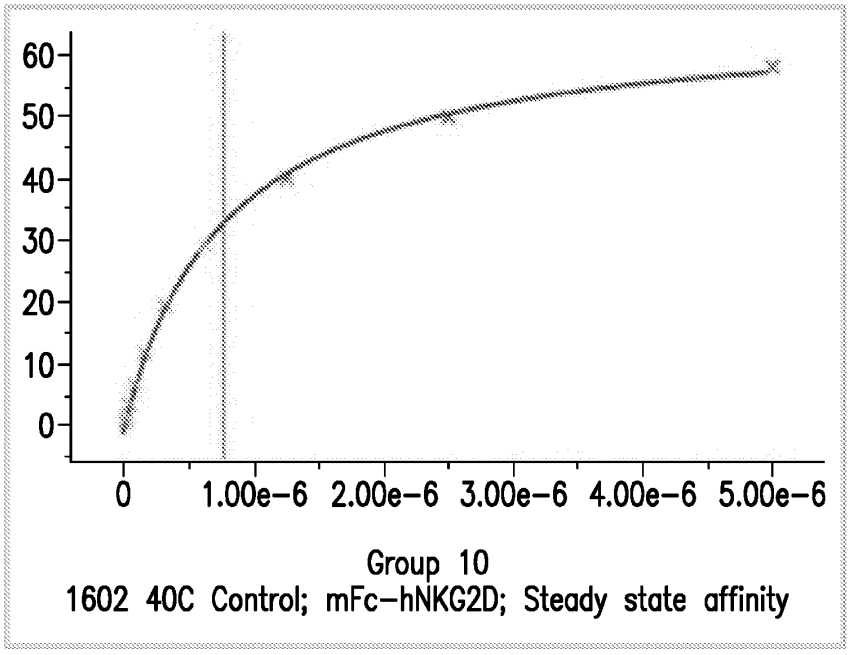

40° C, 4 wks
50
40
30
20
10
0
−50
0
50
100
Group 9
1602 40C 4 Weeks; mFc−hNKG2D; 1:1 binding

FIG. 72



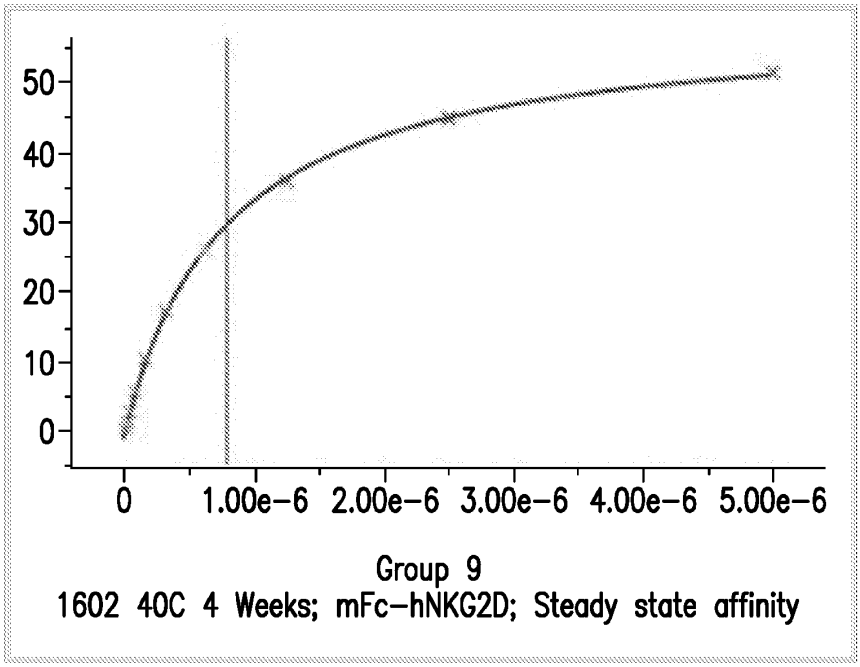


FIG. 72 Continued

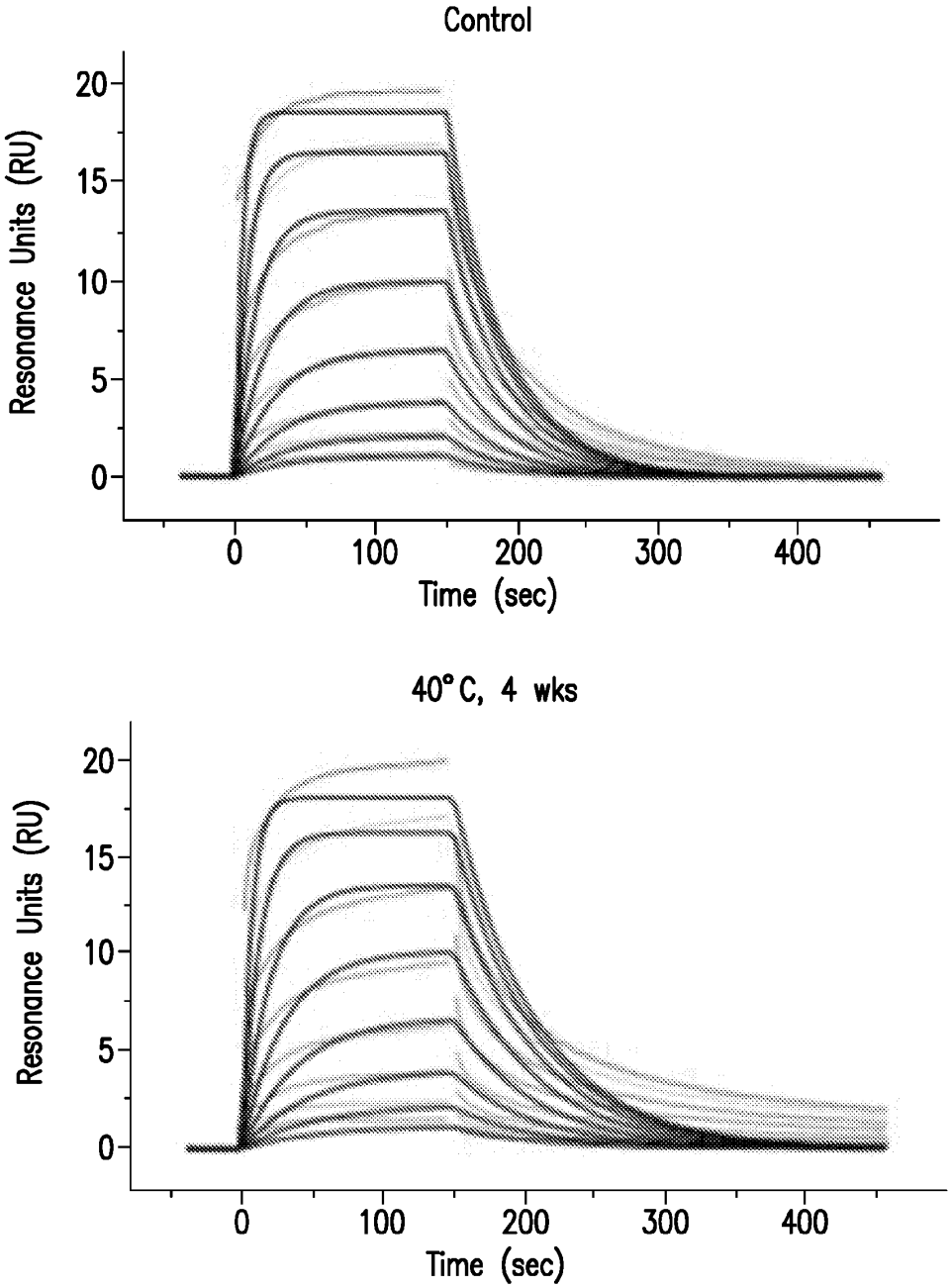
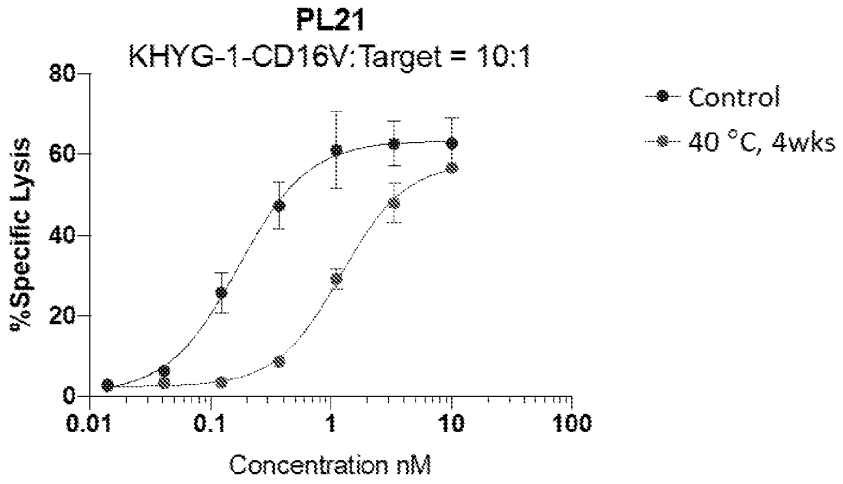

Control
Resonance Units (RU)
20
15
10
5
0
0
100
200
300
400
Time (sec)
40° C, 4 wks
Resonance Units (RU)
20
15
10
5
0
0
100
200
300
400
Time (sec)

FIG. 73

FIG. 74


PL21
KHYG-1-CD16V:Target = 10:1
%Specific Lysis
80
60
40
20
0
0.01
0.1
1
10
100
Concentration nM
Control
40 °C, 4wks

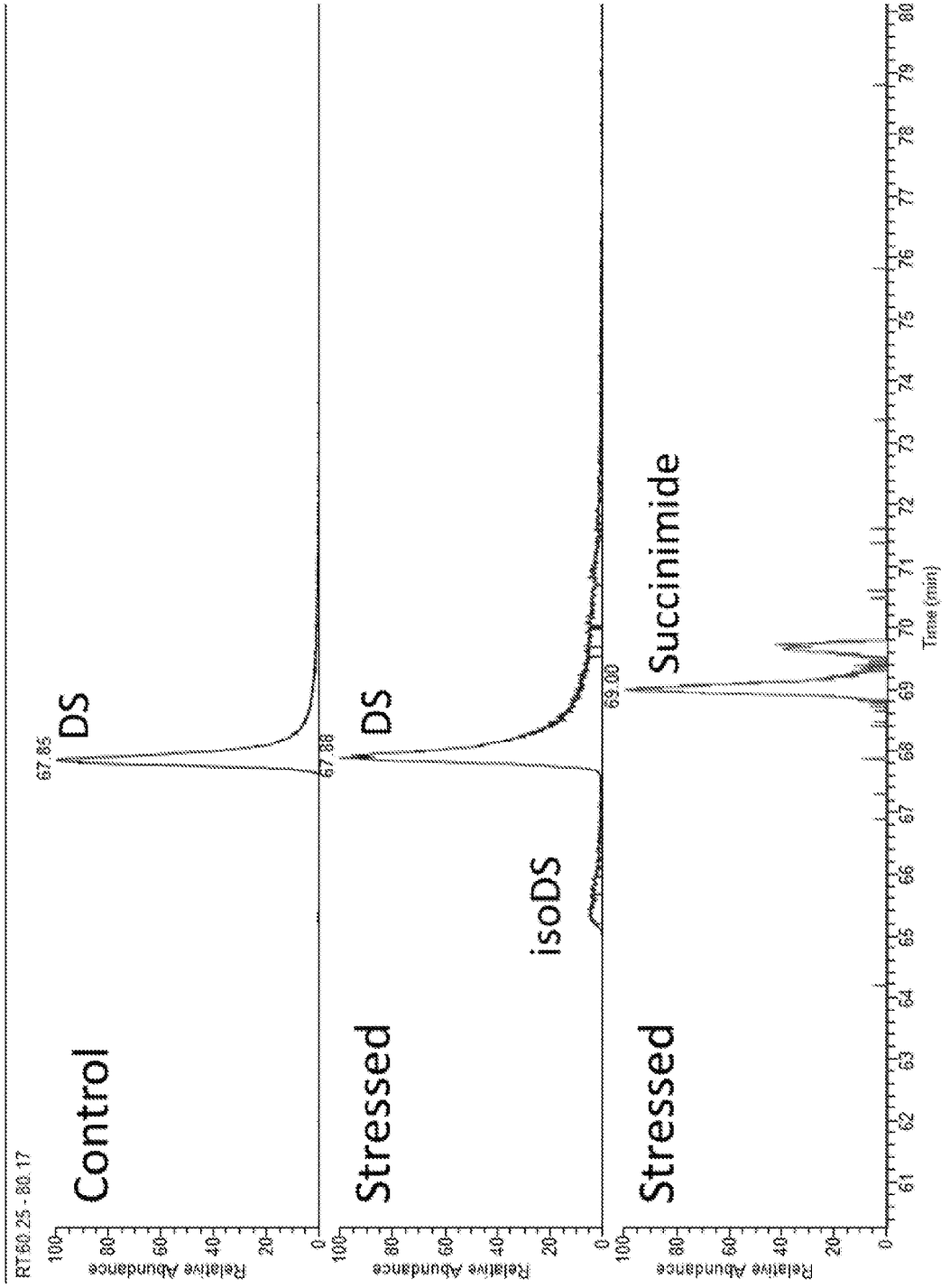

RT:60.25 - 80.17
Control
DS
67.85
88.79
Stressed
DS
isoDS
69.00
Succinimide
Stressed
Relative Abundance
Time (min)

FIG. 75

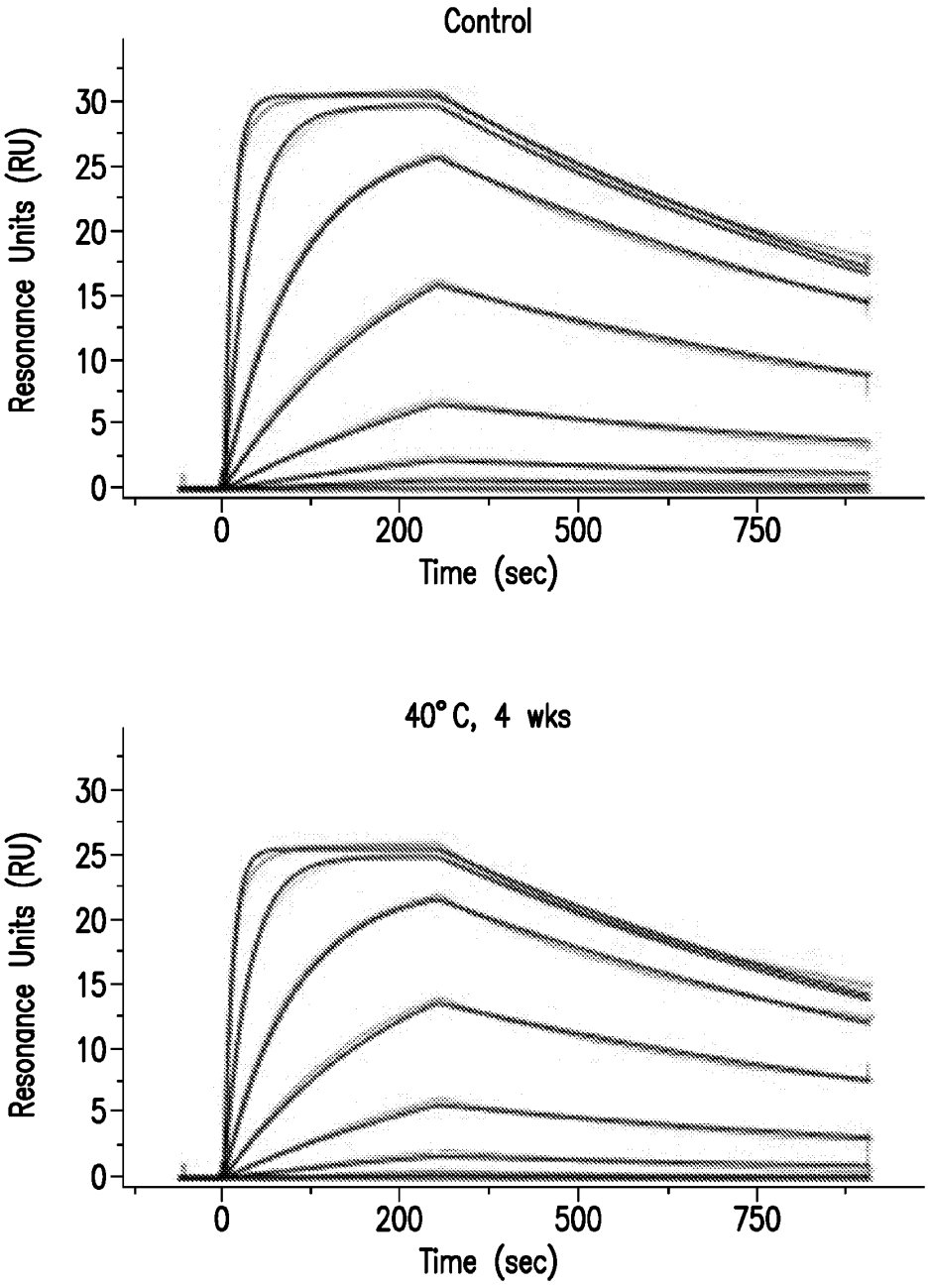
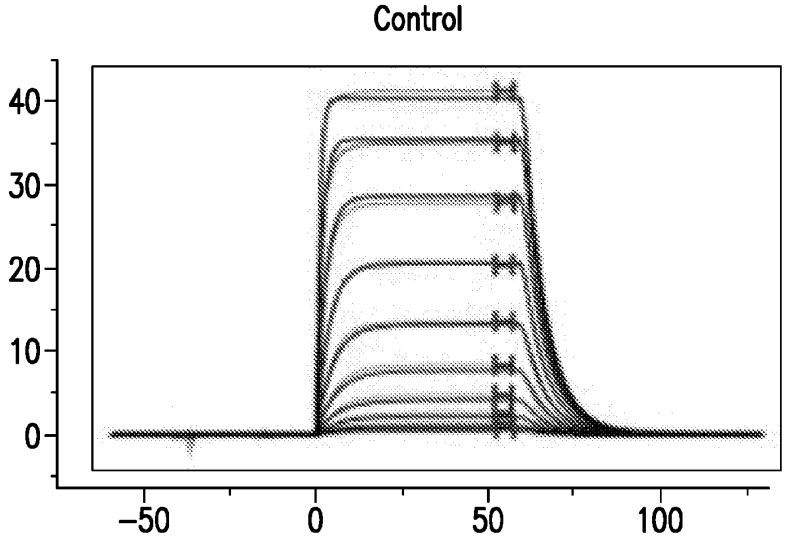
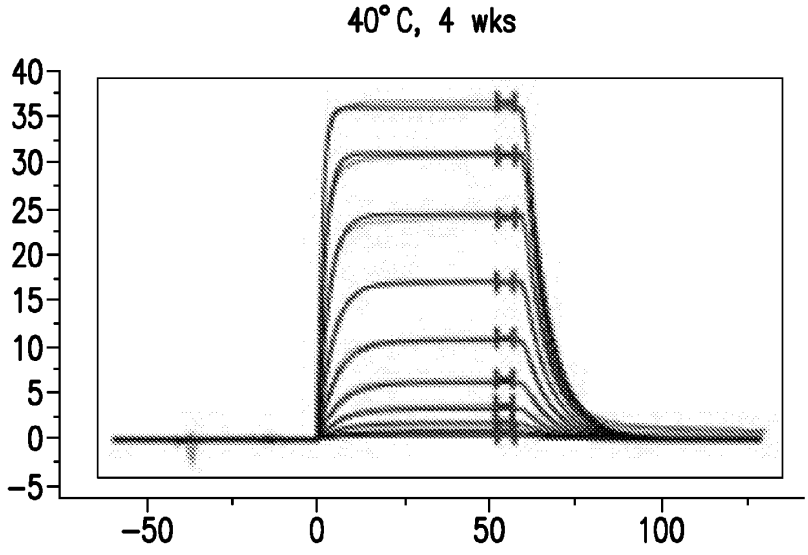

Control
Resonance Units (RU)
30
25
20
15
10
5
0
0
200
500
750
Time (sec)
40° C, 4 wks
Resonance Units (RU)
30
25
20
15
10
5
0
0
200
500
750
Time (sec)

FIG. 76

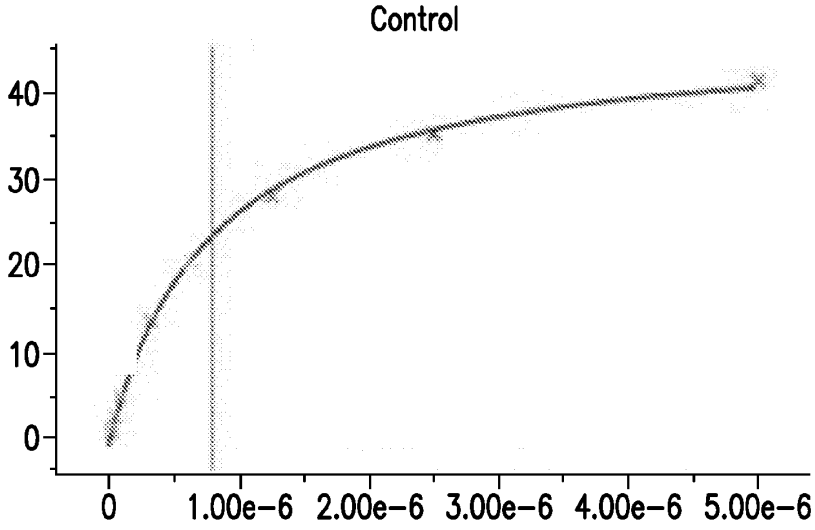


Group 4
40C Buffer Control; mFc−hNKG2D; 1:1 binding

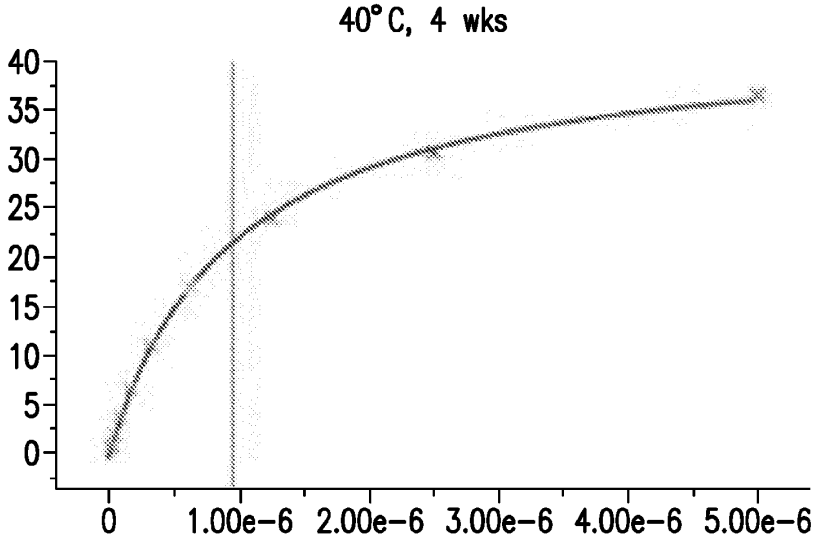


Group 14
NaCl 40C 4 Wks; mFc−hNKG2D; 1:1 binding

FIG. 77



Group 4
40C Buffer Control; mFc-hNKG2D; Steady state affinity



Group 14
NaCl 40C 4 Wks; mFc-hNKG2D; Steady state affinity

FIG. 77 Continued

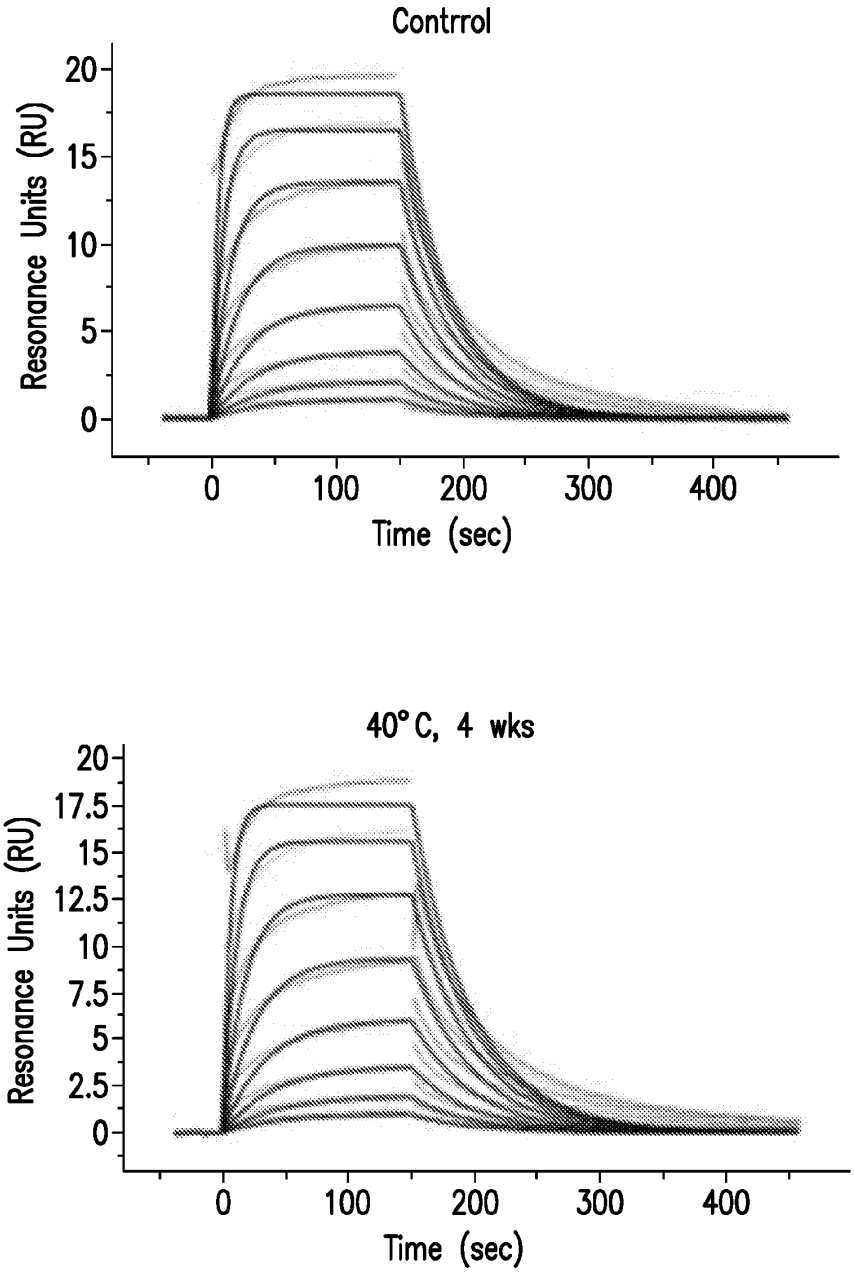
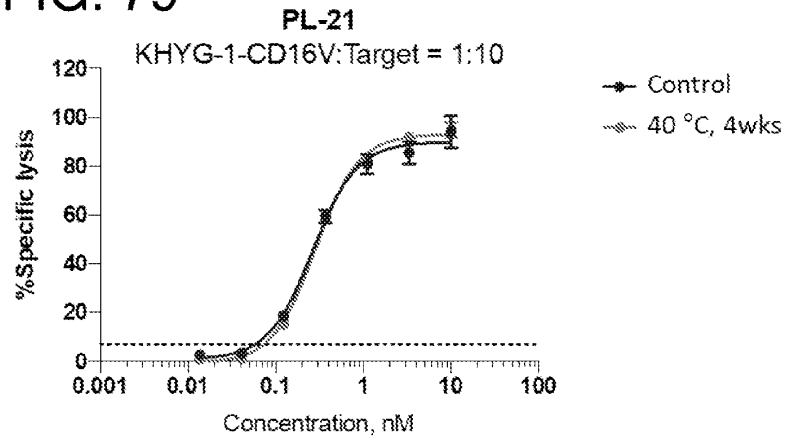

Contrrol
Resonance Units (RU)
20
15
10
5
0
0
100
200
300
400
Time (sec)
40° C, 4 wks
Resonance Units (RU)
20
17.5
15
12.5
10
7.5
5
2.5
0
0
100
200
300
400
Time (sec)

FIG. 78

FIG. 79


PL-21
KHYG-1-CD16V:Target = 1:10
%Specific lysis
120
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Concentration, nM
Control
40 °C, 4wks

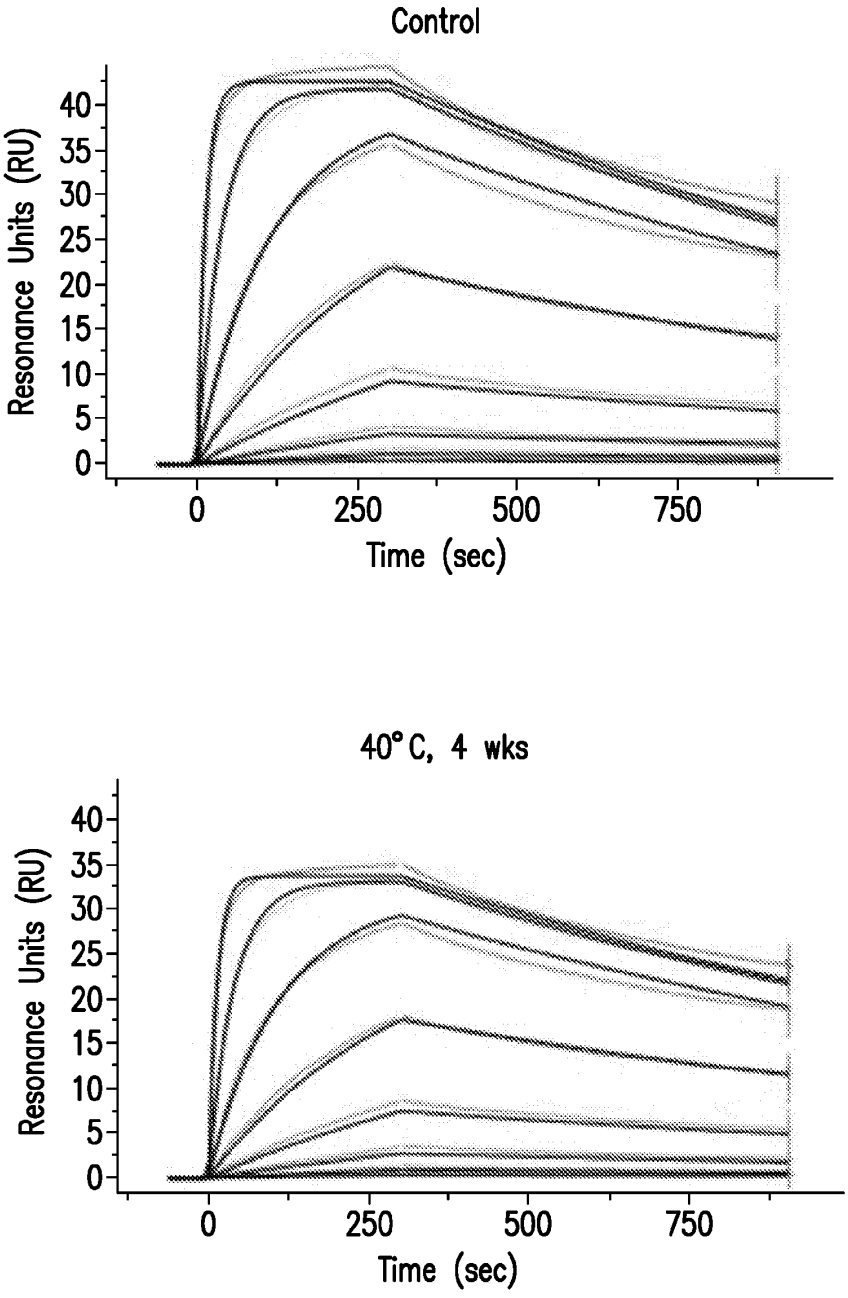

Control
Resonance Units (RU)
0
5
10
15
20
25
30
35
40
0
250
500
750
Time (sec)
40° C, 4 wks
Resonance Units (RU)
0
5
10
15
20
25
30
35
40
0
250
500
750
Time (sec)

FIG. 80

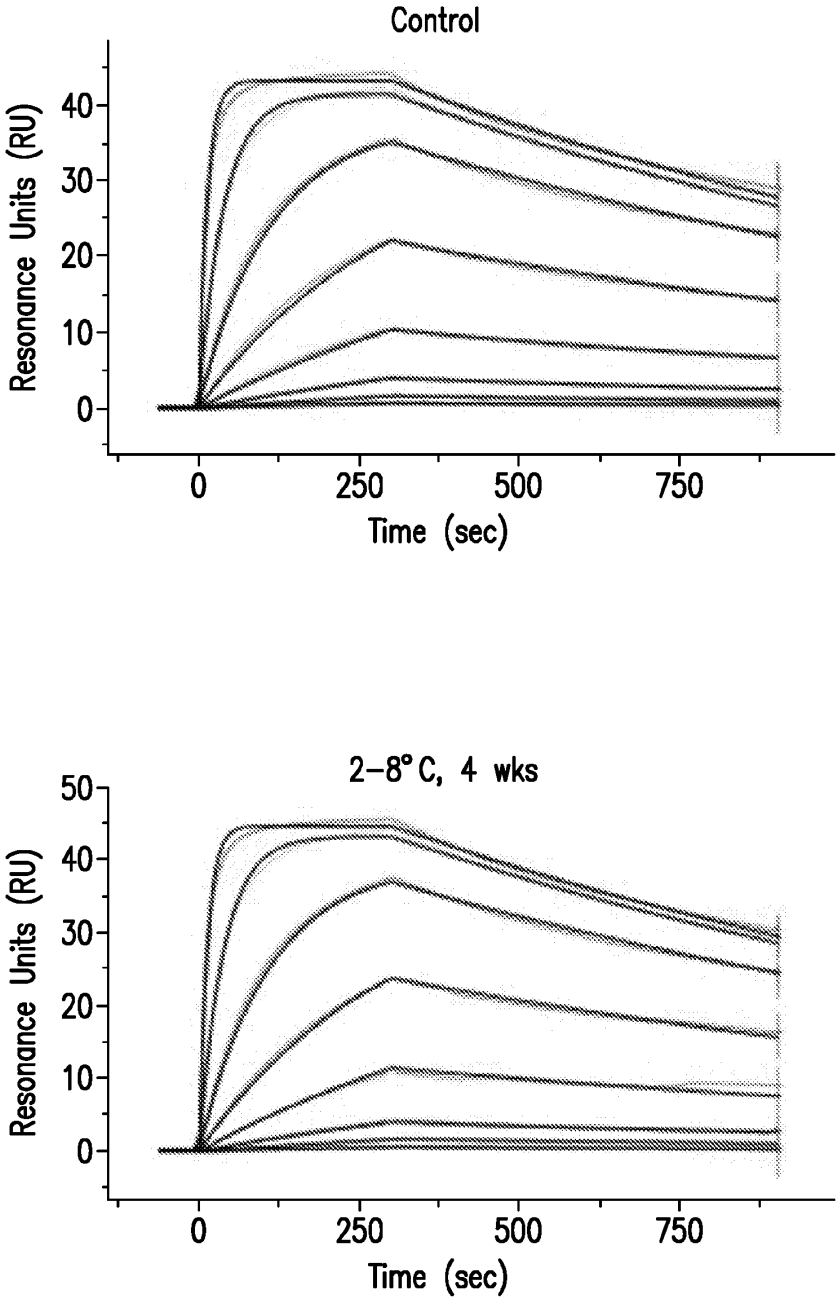

Control
Resonance Units (RU)
0
10
20
30
40
0
250
500
750
Time (sec)
2–8° C, 4 wks
Resonance Units (RU)
0
10
20
30
40
50
0
250
500
750
Time (sec)

FIG. 81

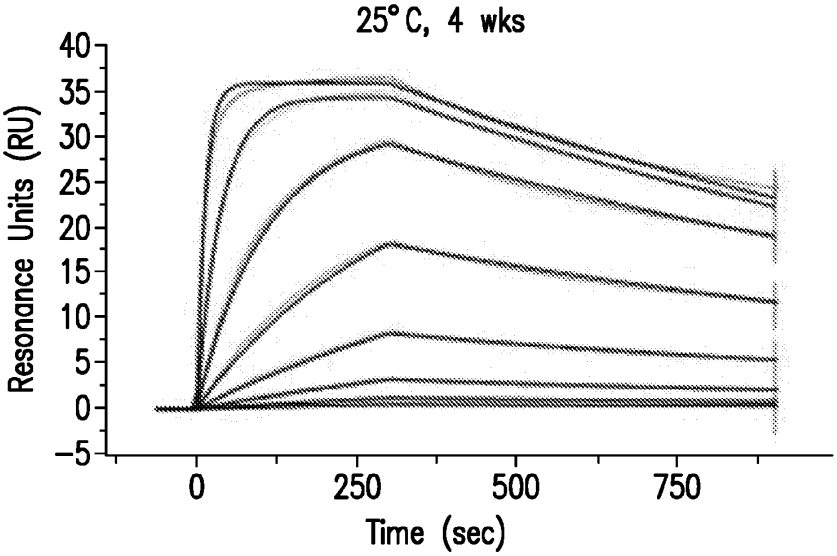
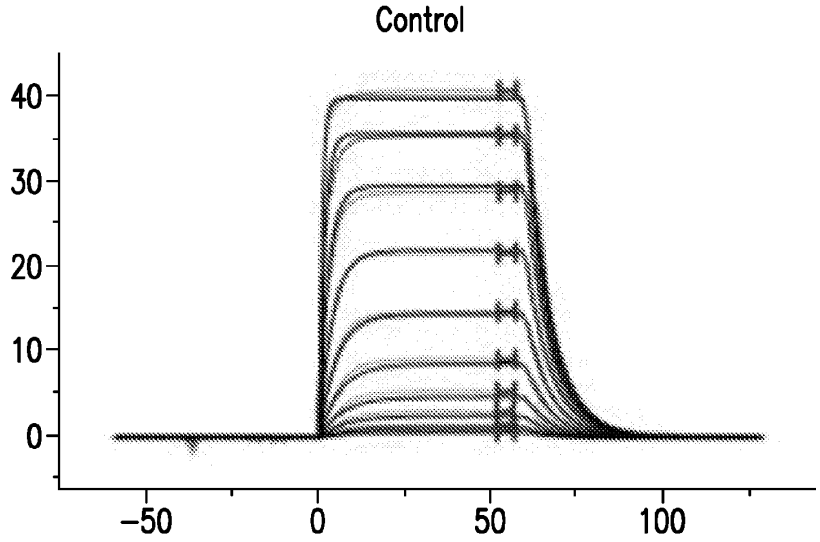
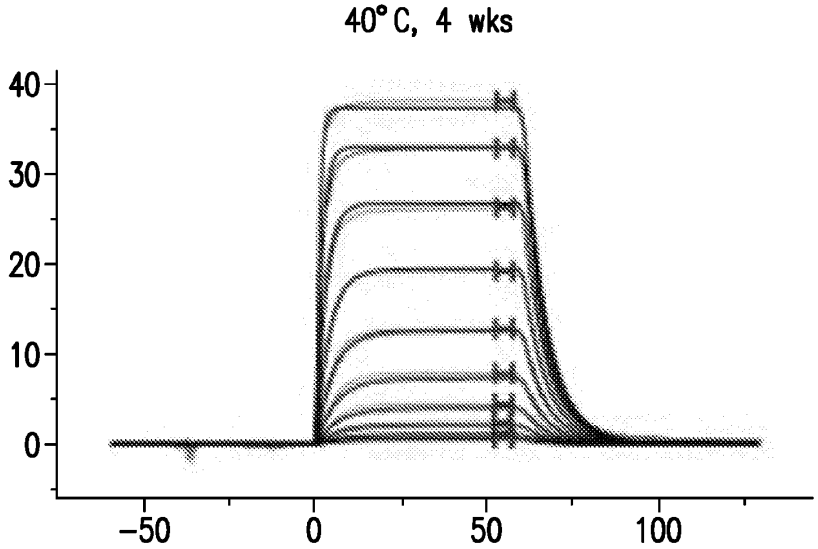


FIG. 81 Continued

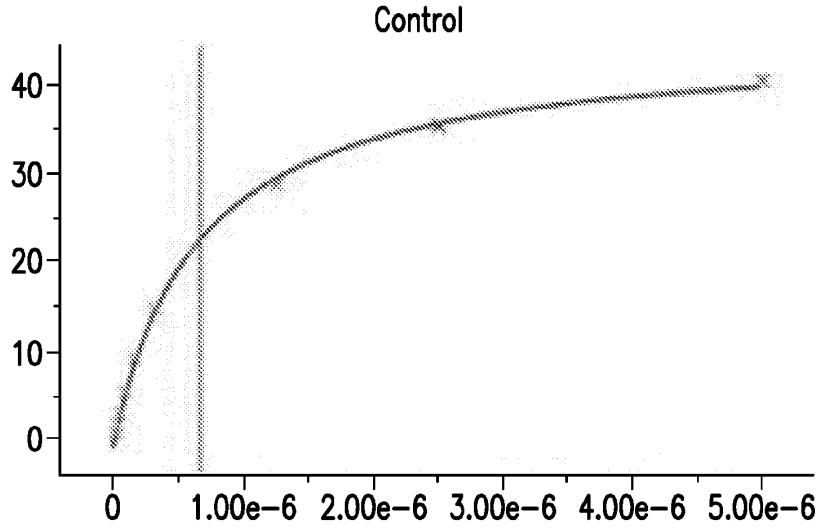


Group 5
40C Control; mFc−hNKG2D; 1:1 binding

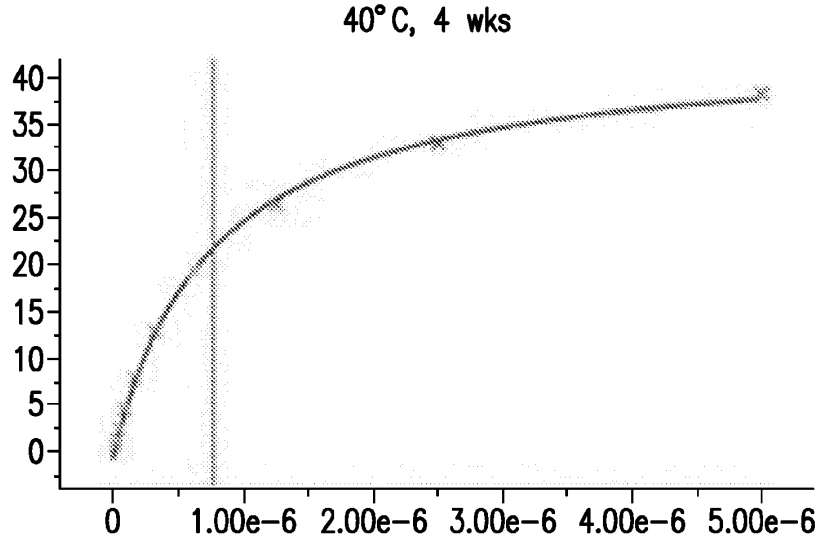


Group 1
NaCl 40C 4 Wks; mFc−hNKG2D; 1:1 binding

FIG. 82



Group 5
40C Control; mFc-hNKG2D; Steady state affinity



Group 1
NaCl 40C 4 Wks; mFc-hNKG2D; Steady state affinity

FIG. 82 Continued

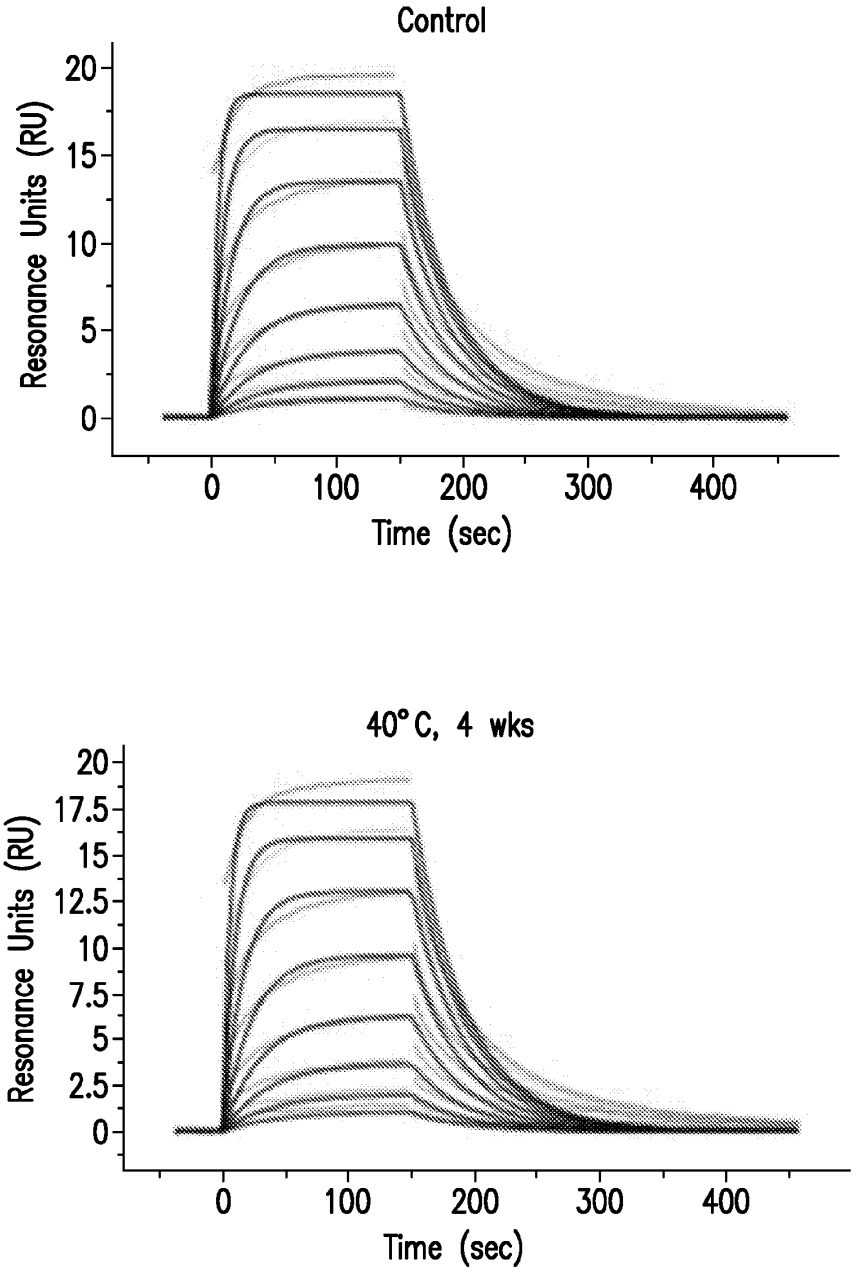
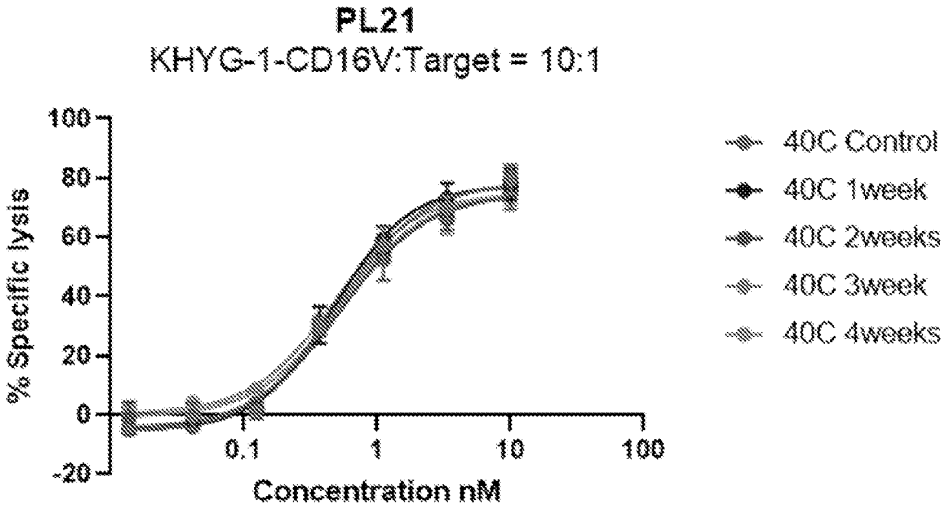

Control
Resonance Units (RU)
20
15
10
5
0
0
100
200
300
400
Time (sec)
40° C, 4 wks
Resonance Units (RU)
20
17.5
15
12.5
10
7.5
5
2.5
0
0
100
200
300
400
Time (sec)

FIG. 83

FIG. 84


PL21
KHYG-1-CD16V:Target = 10:1
% Specific lysis
100
80
60
40
20
0
-20
0.1
1
10
100
Concentration nM
40C Control
40C 1week
40C 2weeks
40C 3week
40C 4weeks

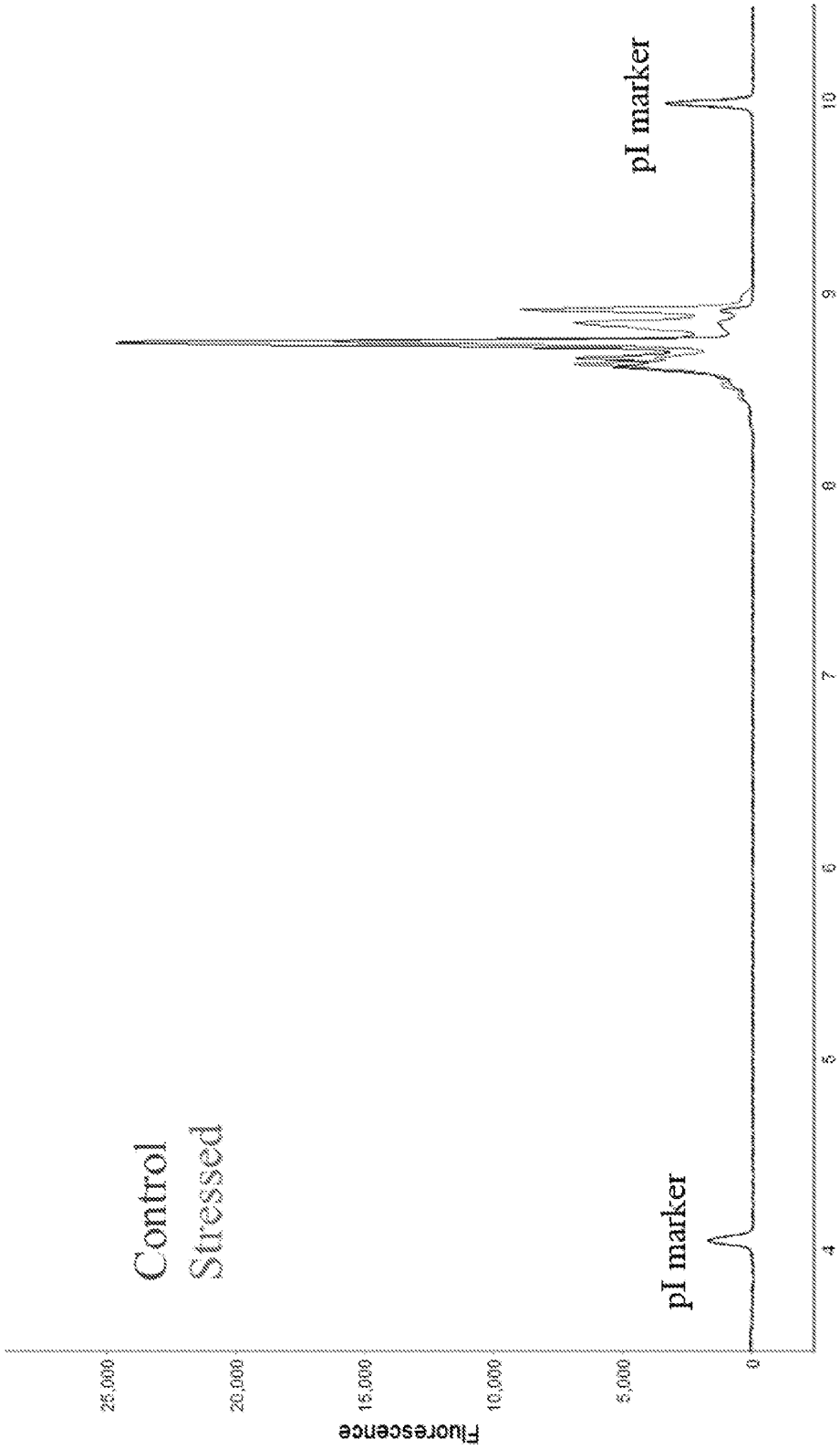

Control
Stressed
pI marker
pI marker
Fluorescence
25,000
20,000
15,000
10,000
5,000
0
4
5
6
7
8
9
10
pI

FIG. 85

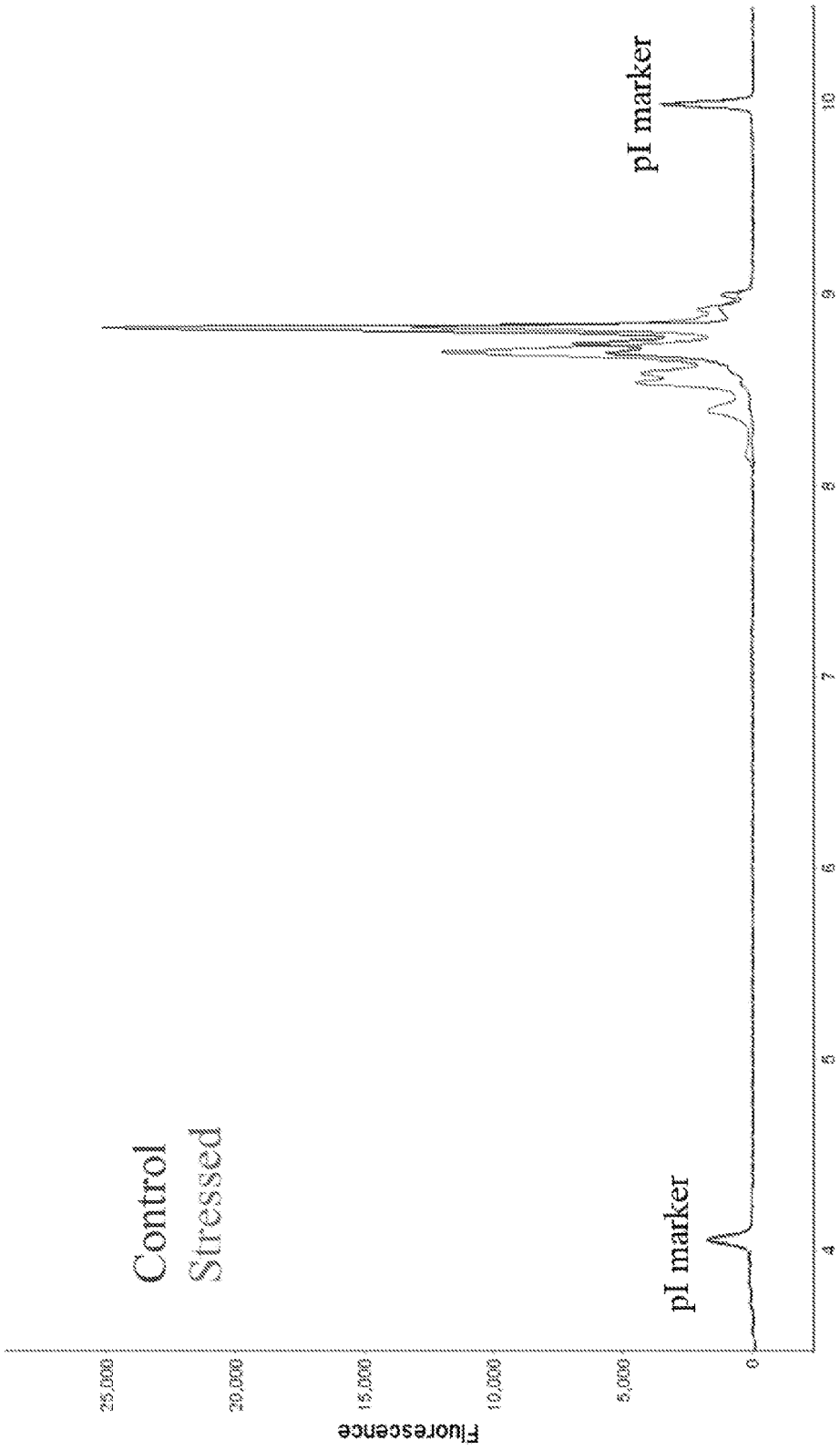
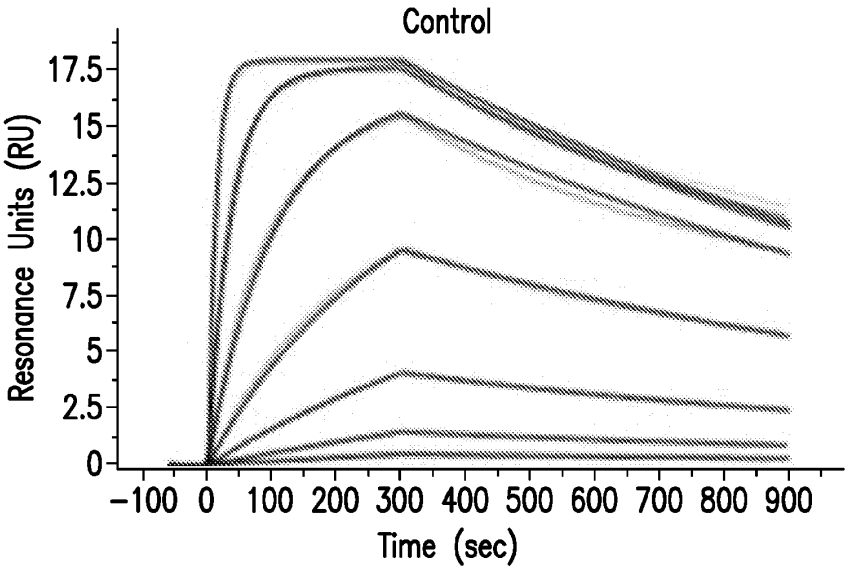

Fluorescence
25,000
20,000
15,000
10,000
5,000
0
4
5
6
7
8
9
10
pI
Control
Stressed
pI marker
pI marker

FIG. 86


Control
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
−100 0 100 200 300 400 500 600 700 800 900
Time (sec)

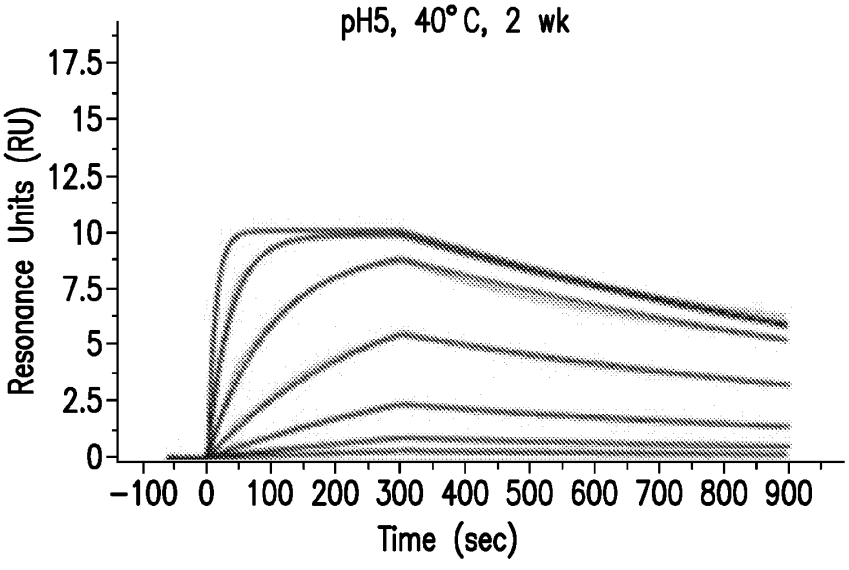

pH5, 40° C, 2 wk
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
−100 0 100 200 300 400 500 600 700 800 900
Time (sec)

FIG. 87

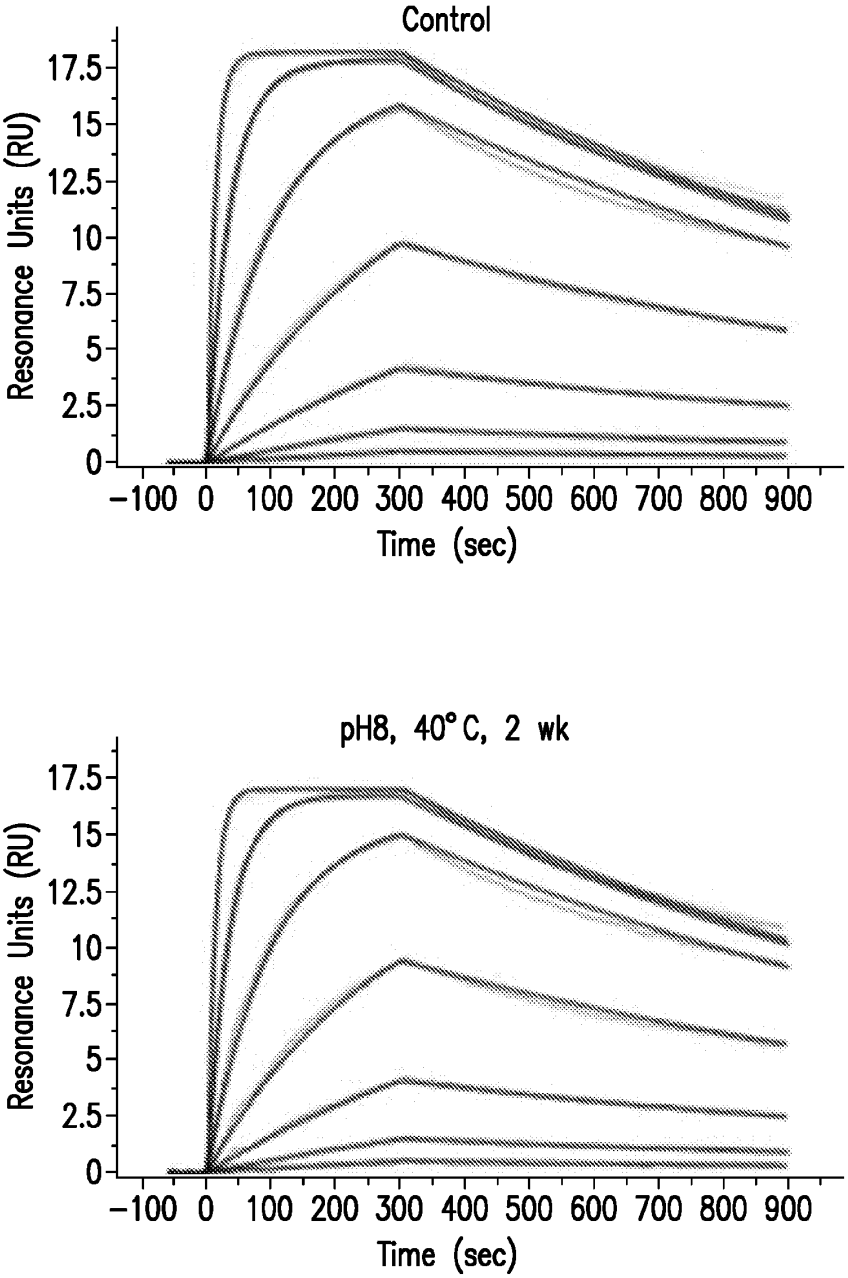
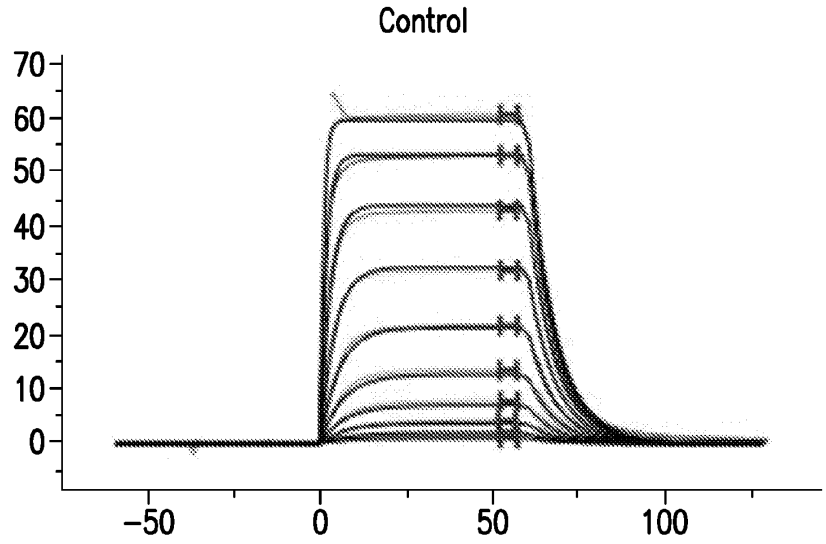
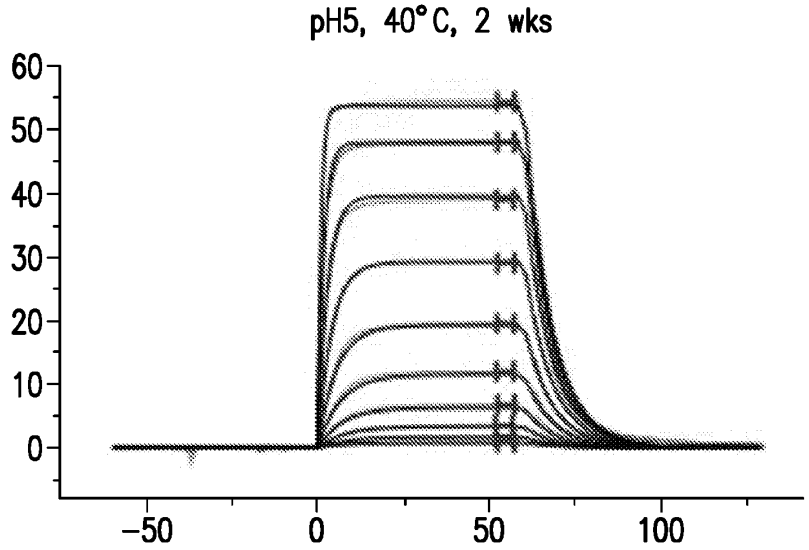

Control
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
−100 0 100 200 300 400 500 600 700 800 900
Time (sec)
pH8, 40° C, 2 wk
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
−100 0 100 200 300 400 500 600 700 800 900
Time (sec)

FIG. 88

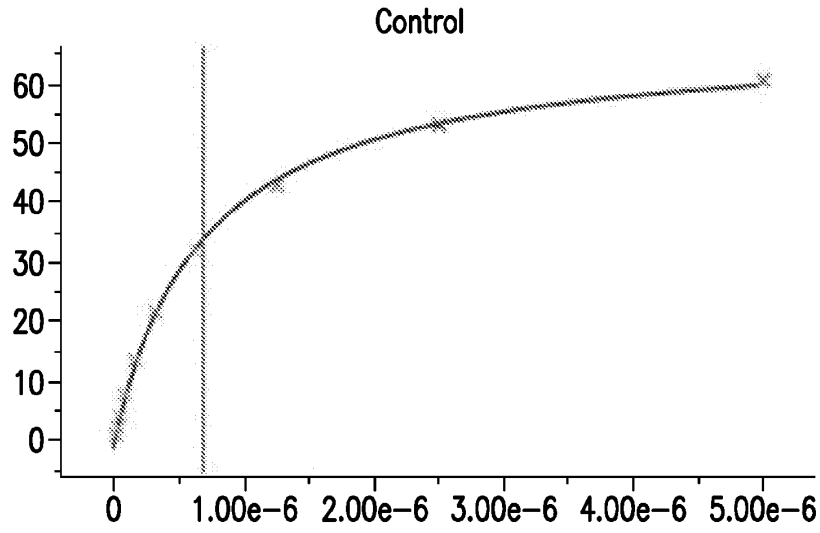


Group 14
1602 pH 5 Control; mFc−hNKG2D; 1:1 binding

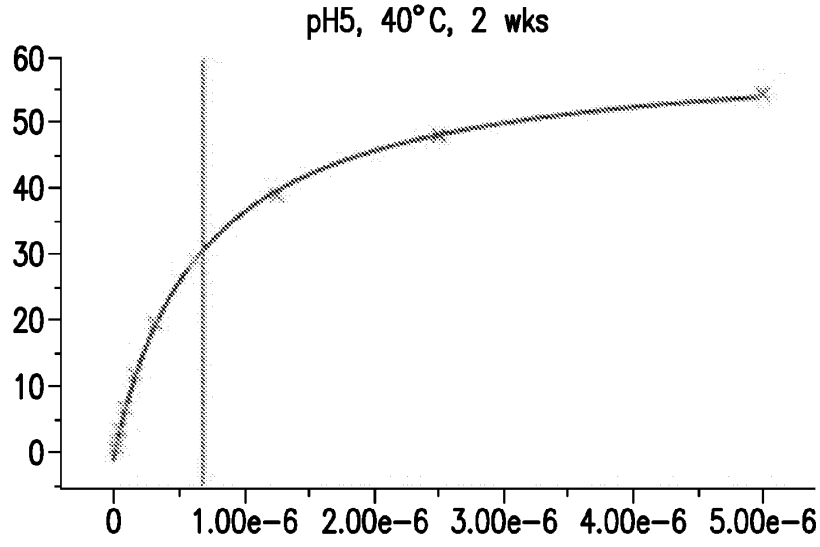


Group 13
1602 pH 5 2 Weeks; mFc−hNKG2D; 1:1 binding

FIG. 89

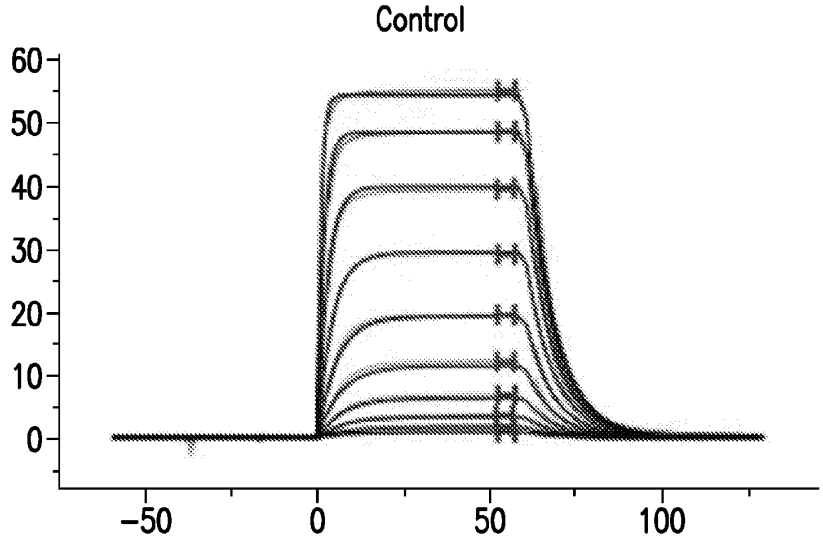


Group 14
1602 pH 5 Control; mFc-hNKG2D; Steady state affinity

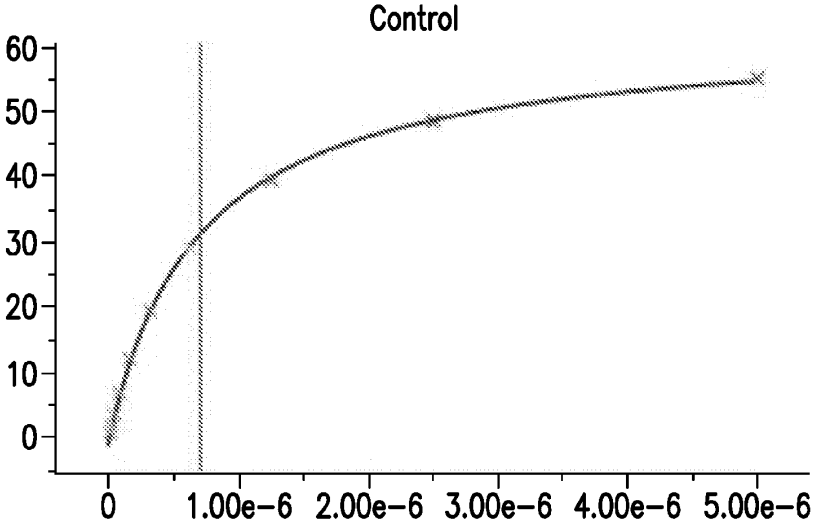


Group 13
1602 pH 5 2 Weeks; mFc-hNKG2D; Steady state affinity

FIG. 89 Continued

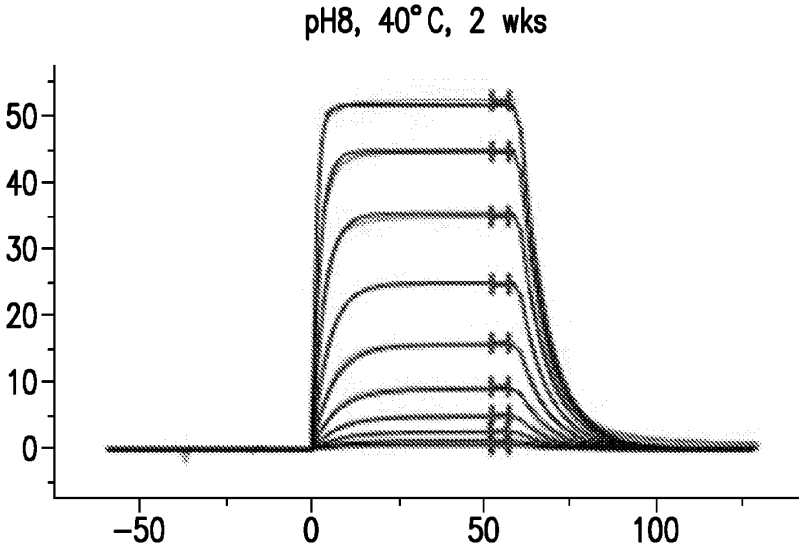


Group 16
1602 pH 8 Control; mFc-hNKG2D; 1:1 binding

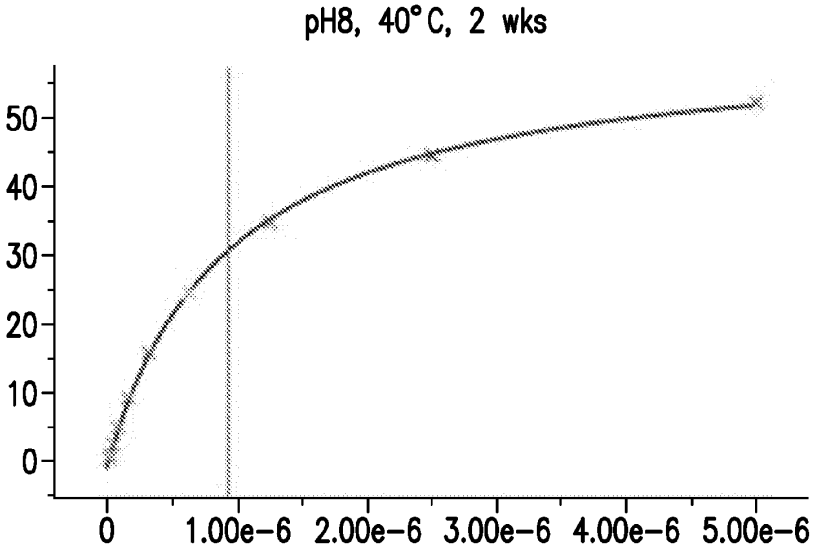


Group 16
1602 pH 8 Control; mFc-hNKG2D; Steady state affinity

FIG. 90



Group 15
1602 pH 8 2 Weeks; mFc−hNKG2D; 1:1 binding



Group 15
1602 pH 8 2 Weeks; mFc−hNKG2D; Steady state affinity

FIG. 90 Continued

FIG. 91

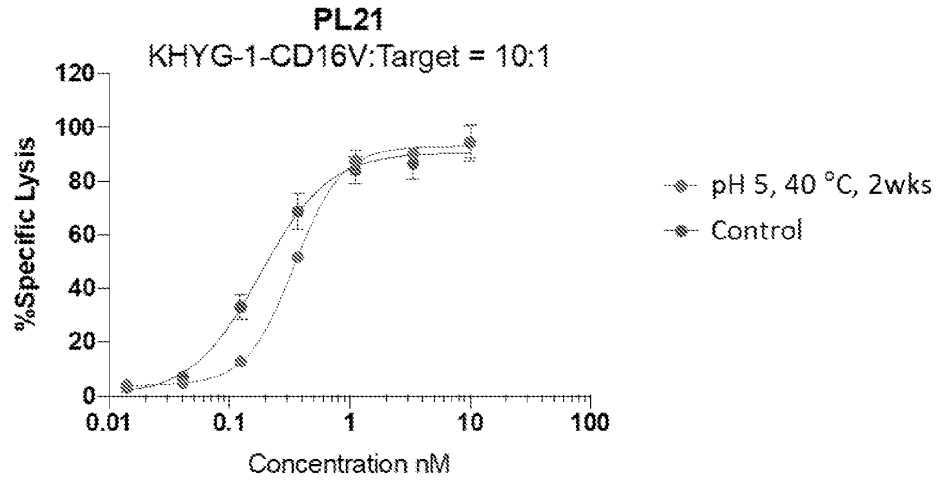
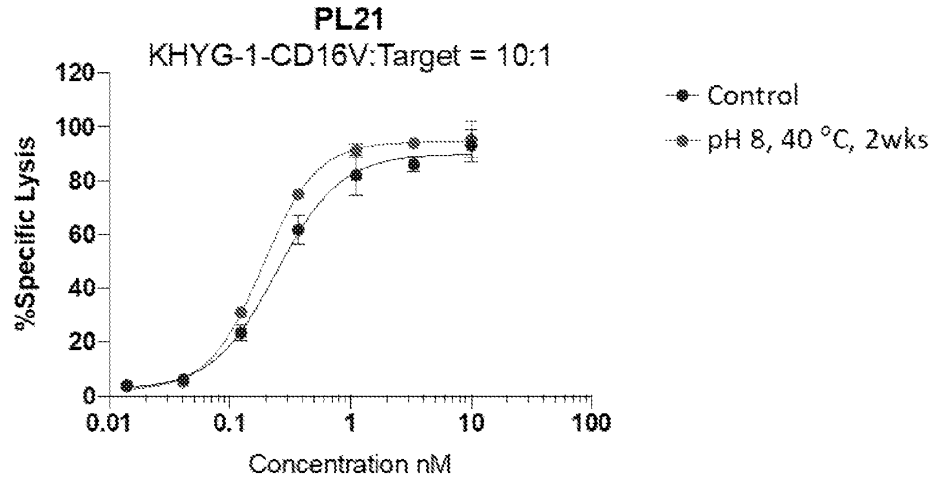

PL21
KHYG-1-CD16V:Target = 10:1
%Specific Lysis
120
100
80
60
40
20
0
0.01
0.1
1
10
100
Concentration nM
pH 5, 40 °C, 2wks
Control

FIG. 92


PL21
KHYG-1-CD16V:Target = 10:1
%Specific Lysis
120
100
80
60
40
20
0
0.01
0.1
1
10
100
Concentration nM
Control
pH 8, 40 °C, 2wks

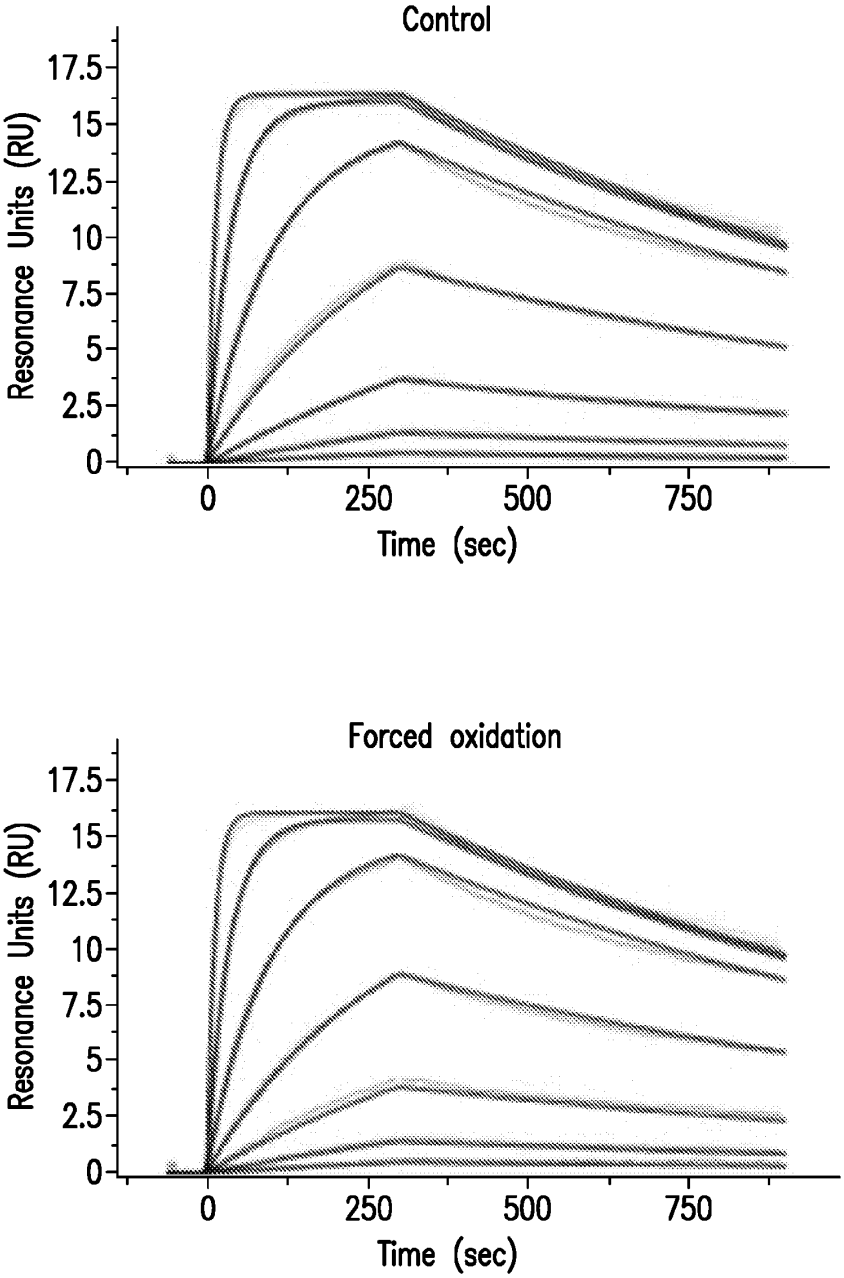
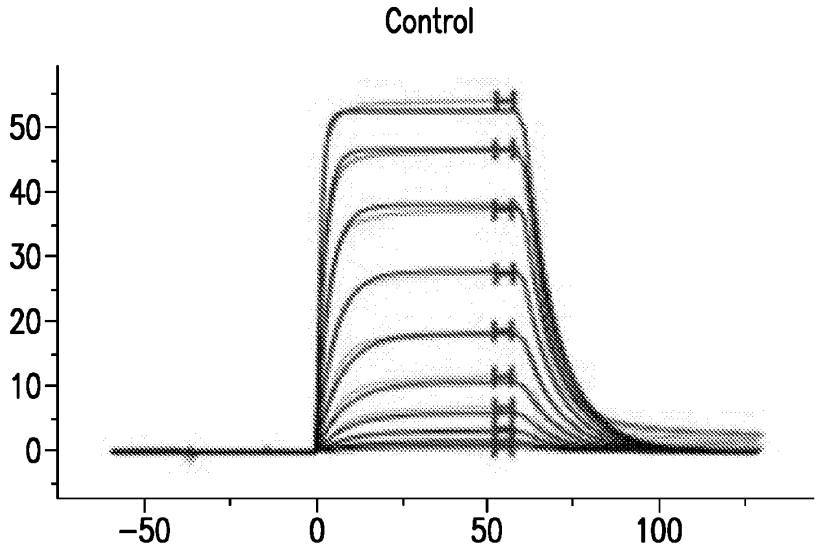
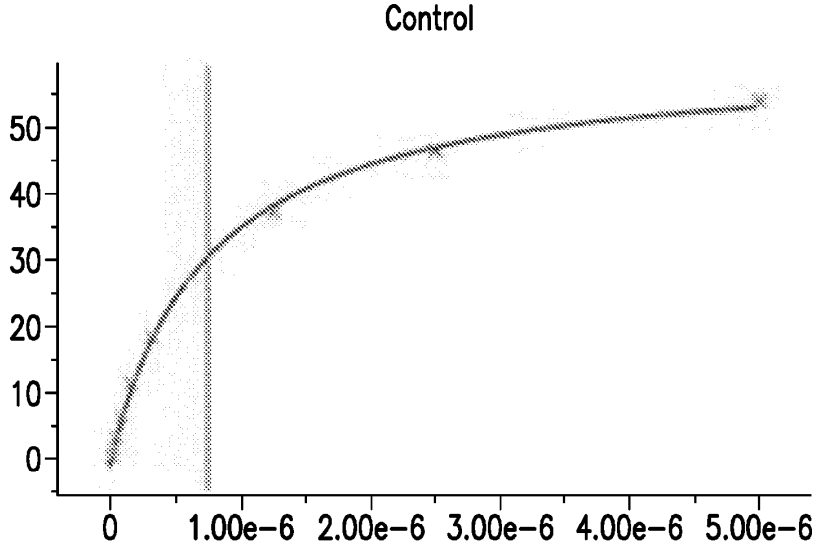

Control
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
0
250
500
750
Time (sec)
Forced oxidation
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
0
250
500
750
Time (sec)

FIG. 93

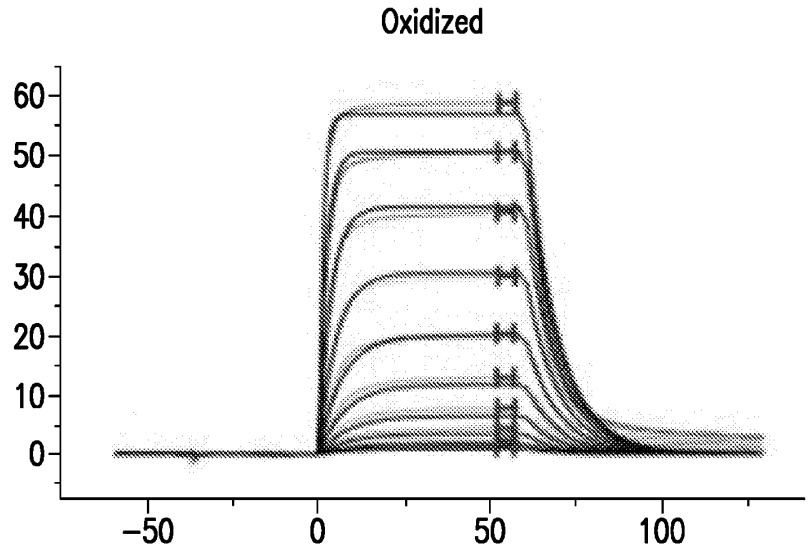


Group 4
Oxidation Control; mFc−hNKG2D; 1:1 binding

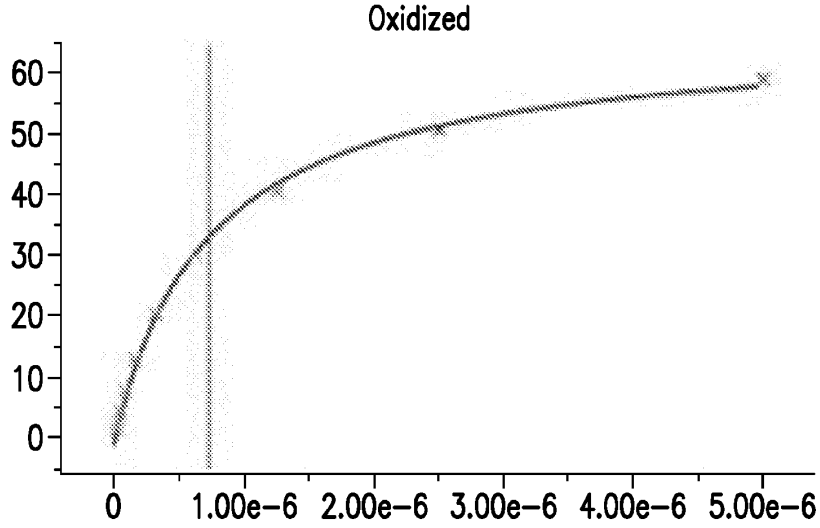


Group 4
Oxidation Control; mFc−hNKG2D; Steady state affinity

FIG. 94



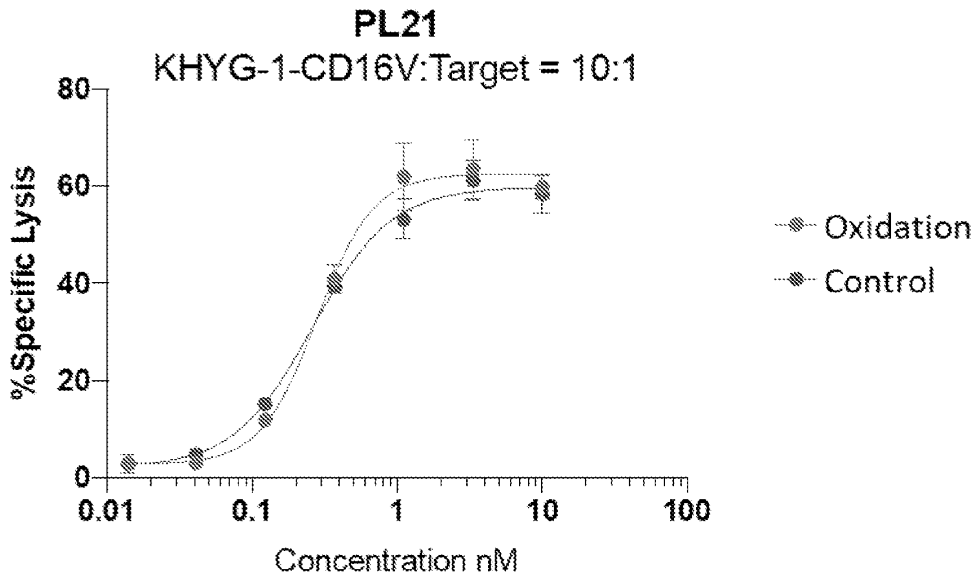
Group 1
Forced Oxidized; mFc−hNKG2D; 1:1 binding

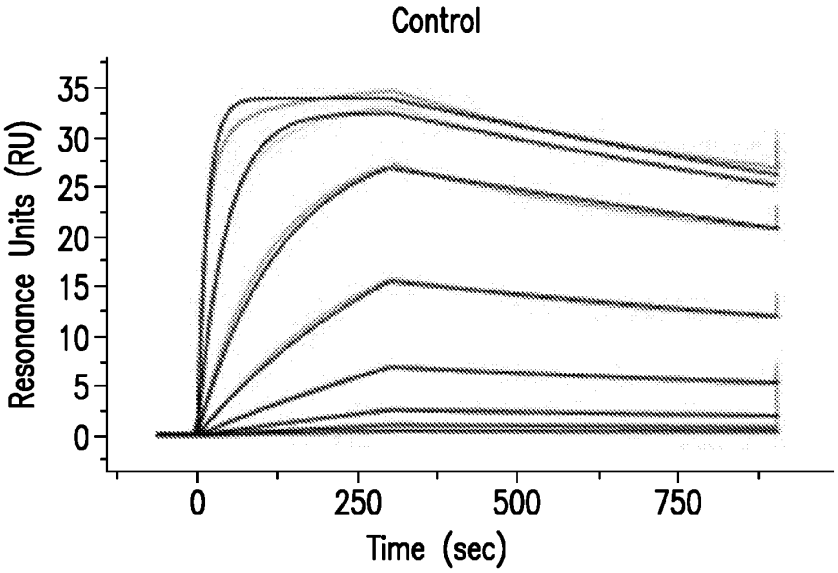


Group 1
Forced Oxidized; mFc−hNKG2D; Steady state affinity

FIG. 94 Continued

FIG. 95


PL21
KHYG-1-CD16V:Target = 10:1
%Specific Lysis
80
60
40
20
0
0.01
0.1
1
10
100
Concentration nM
Oxidation
Control


Control
Resonance Units (RU)
35
30
25
20
15
10
5
0
0
250
500
750
Time (sec)

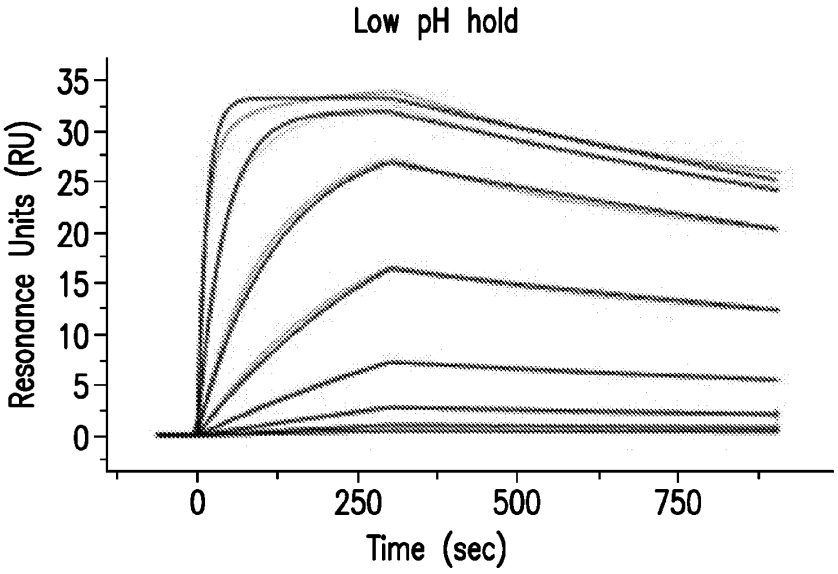
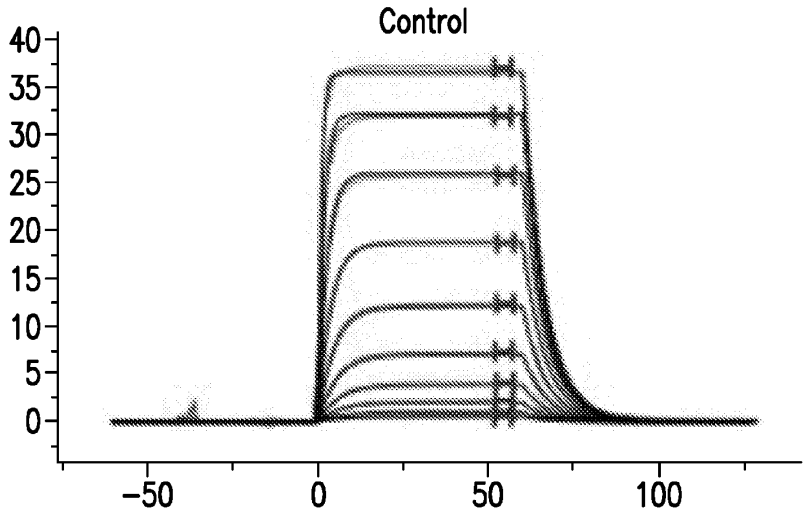
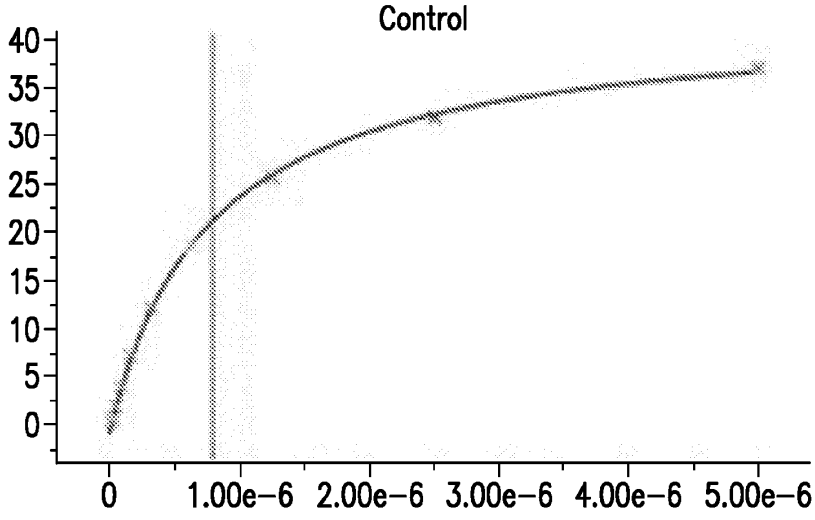

Low pH hold
Resonance Units (RU)
35
30
25
20
15
10
5
0
0
250
500
750
Time (sec)

FIG. 96

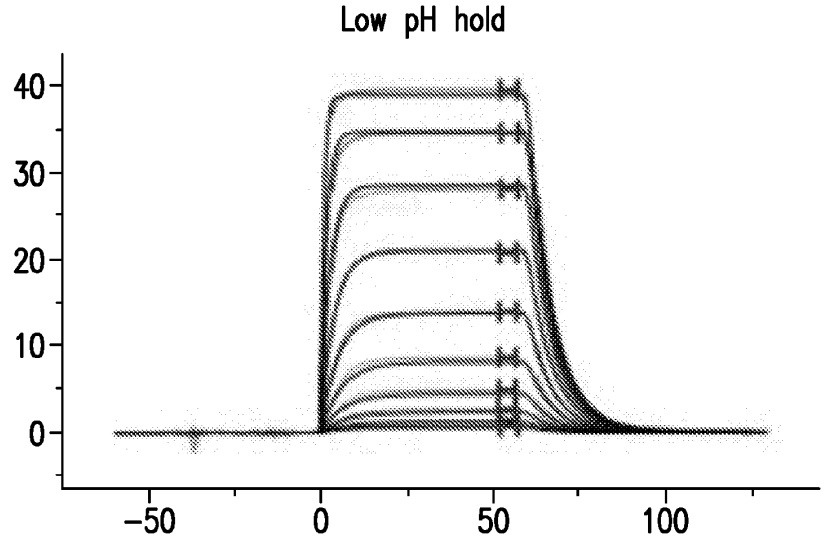


Group 11
Low pH Control; mFc−hNKG2D; 1:1 binding

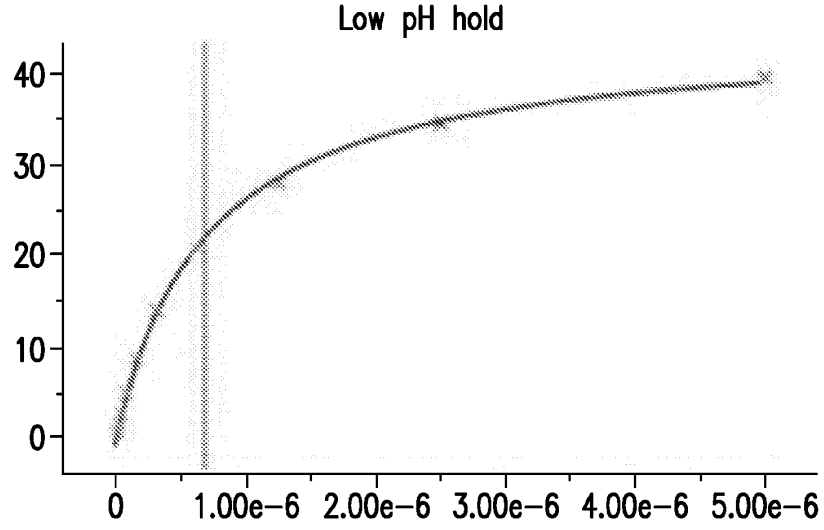


Group 11
Low pH Control; mFc−hNKG2D; Steady state affinity

FIG. 97

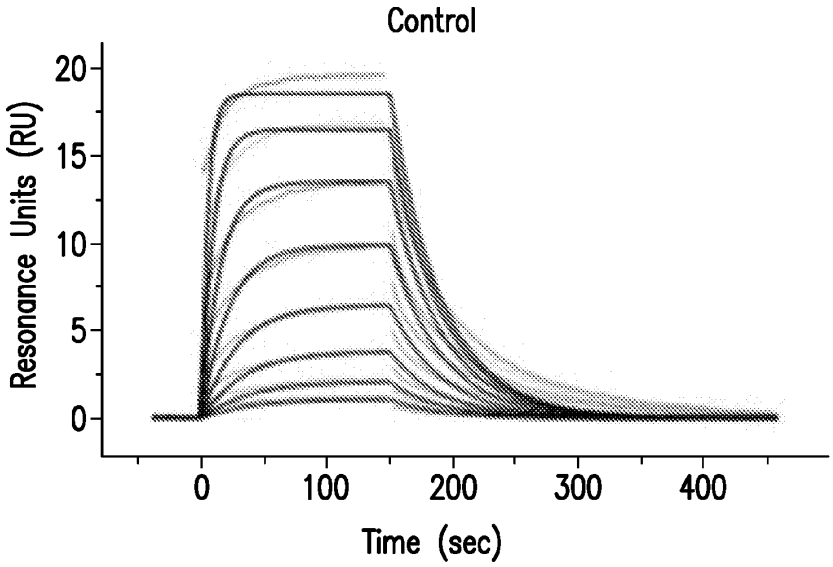


Group 8
Low pH; mFc−hNKG2D; 1:1 binding



Group 8
Low pH; mFc−hNKG2D; Steady state affinity

FIG. 97 Continued


Control
Resonance Units (RU)
20
15
10
5
0
0
100
200
300
400
Time (sec)

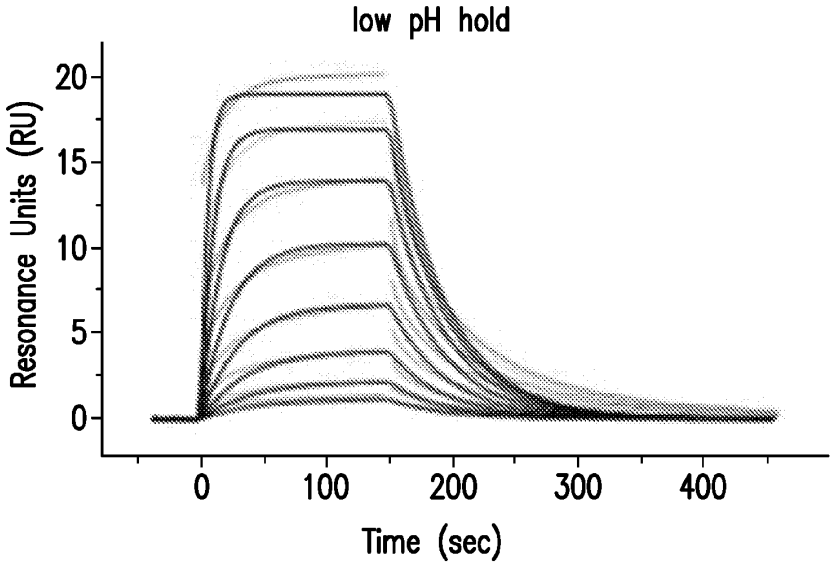

low pH hold
Resonance Units (RU)
20
15
10
5
0
0
100
200
300
400
Time (sec)

FIG. 98

FIG. 99

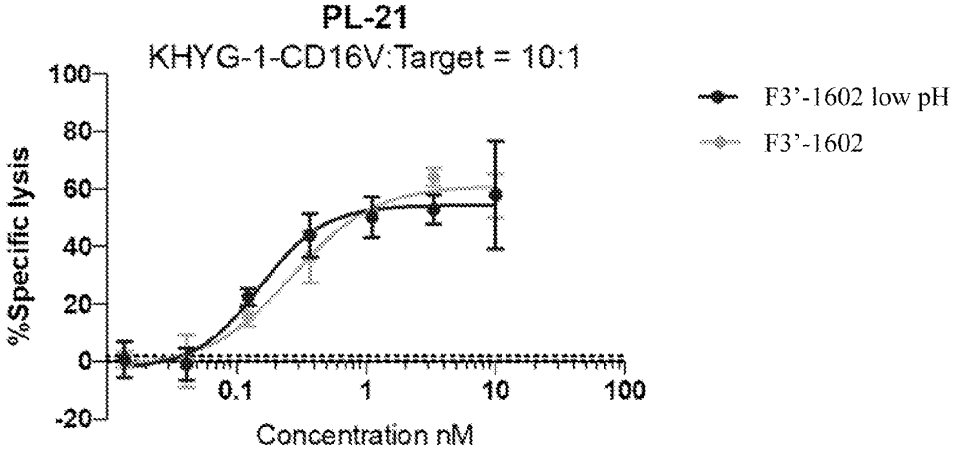


FIG. 100

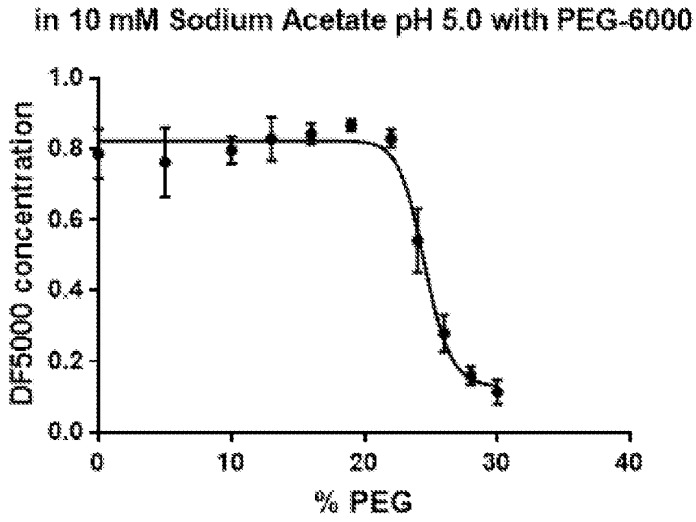


FIG. 101

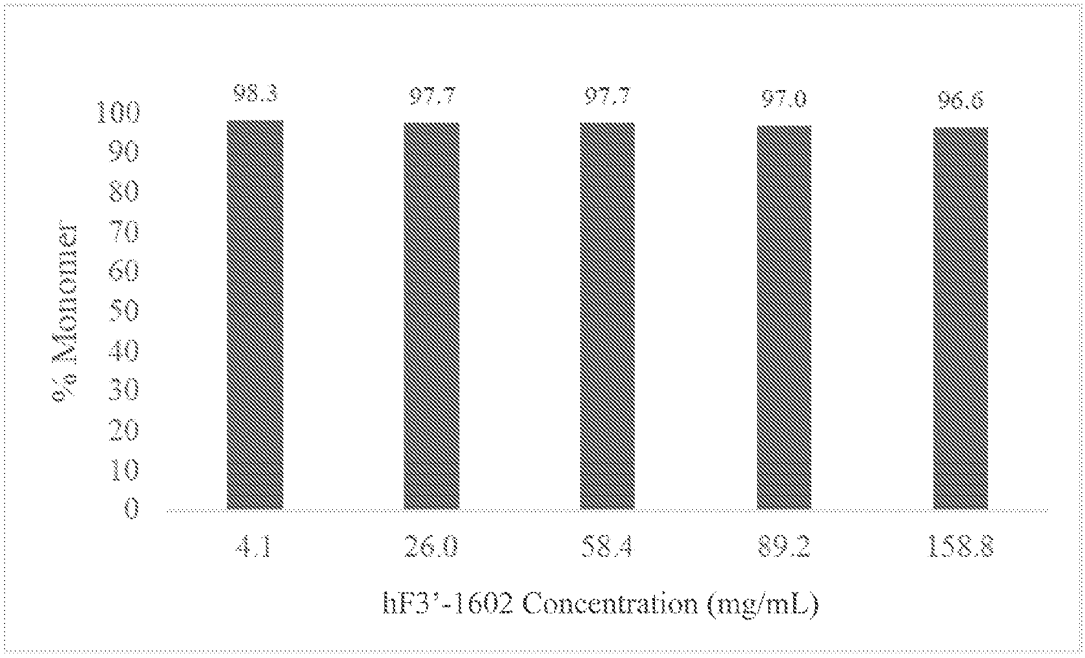

98.3
97.7
97.7
97.0
96.6
100
90
80
70
60
50
40
30
20
10
0
% Monomer
4.1
26.0
58.4
89.2
158.8
hF3'-1602 Concentration (mg/mL)

FIG. 102A

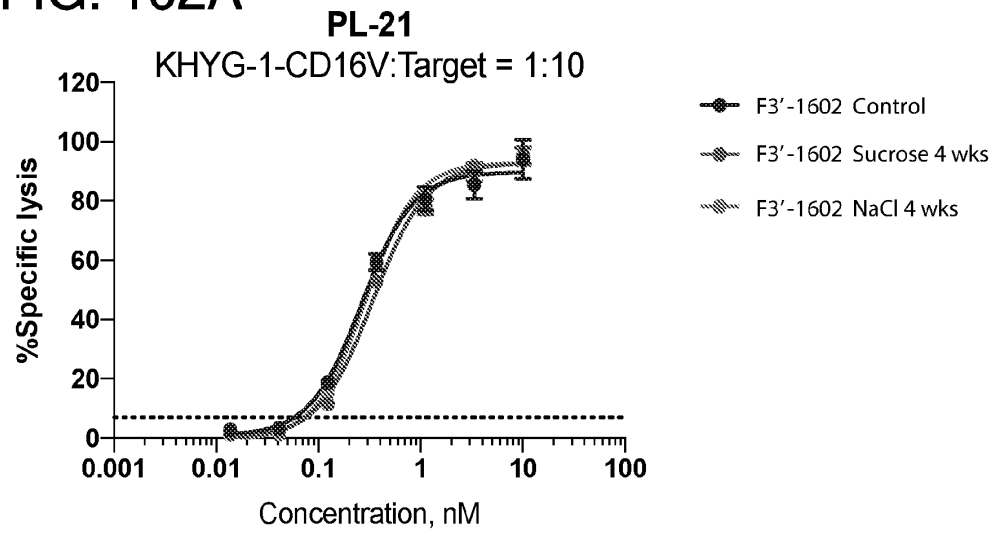


FIG. 102B

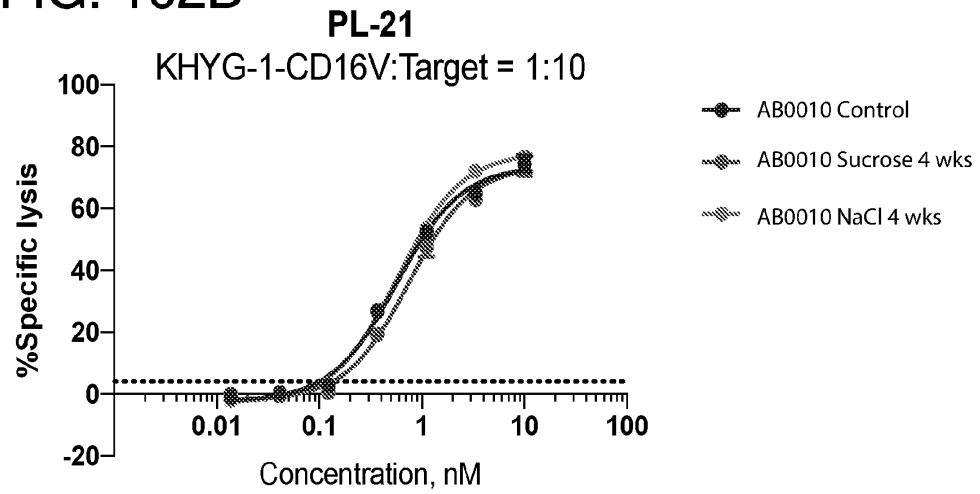


FIG. 103A

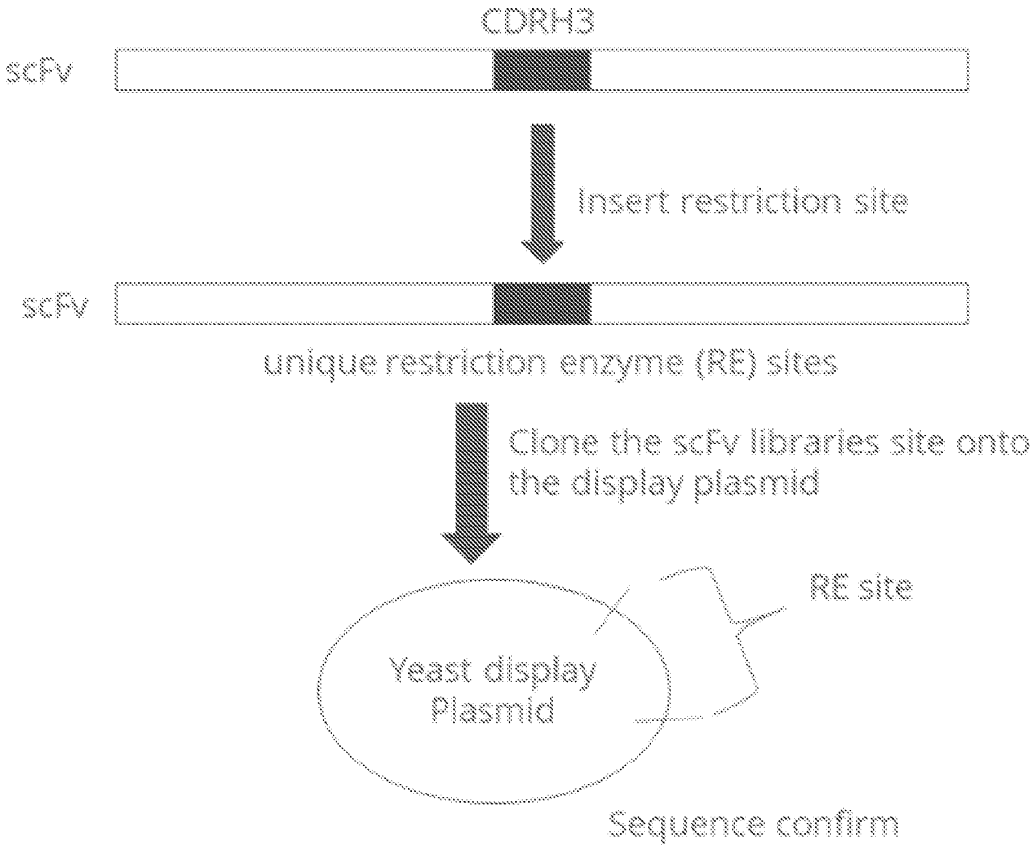
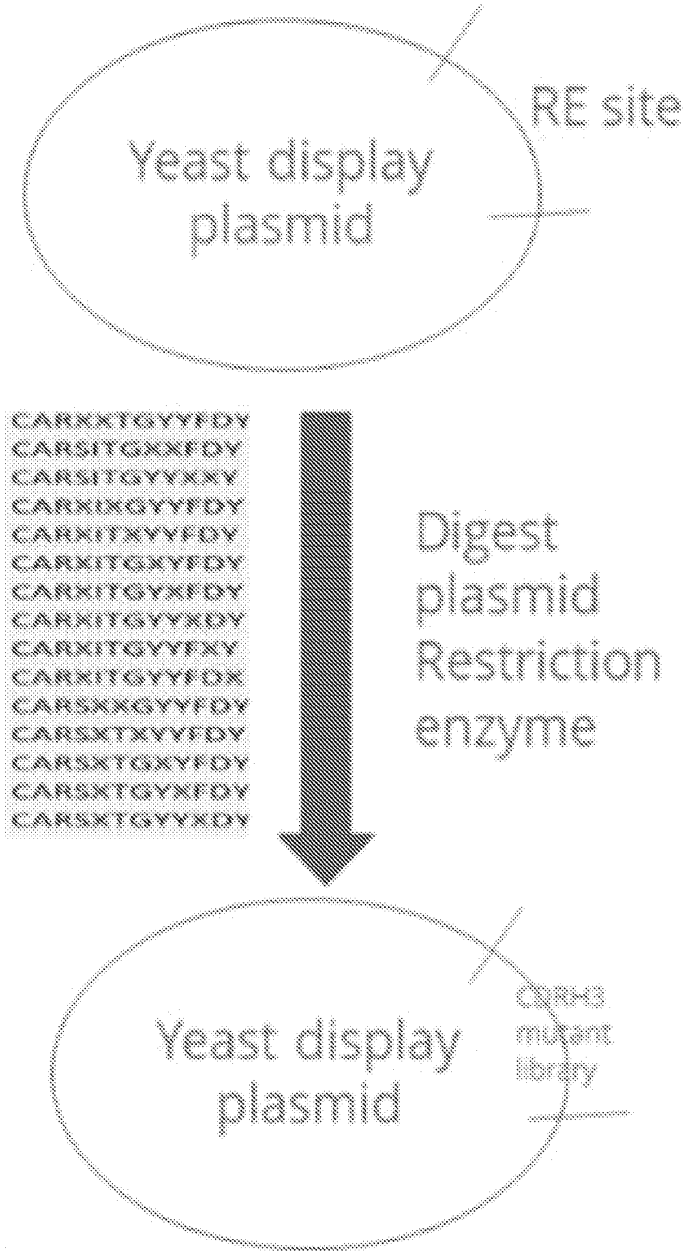

CDRH3
scFv
Insert restriction site
scFv
unique restriction enzyme (RE) sites
Clone the scFv libraries site onto the display plasmid
RE site
Yeast display Plasmid
Sequence confirm

FIG. 103B


RE site
Yeast display plasmid
CARXXTGYYFDY
CARSITGXXFDY
CARSITGYYXXY
CARXIXGYYFDY
CARXITXYYFDY
CARXITGXYFDY
CARXITGYXFDY
CARXITGYYXDY
CARXITGYYFXY
CARXITGYYFDX
CARSXXGYYFDY
CARSXTXYYFDY
CARSXTGXYFDY
CARSXTGYXFDY
CARSXTGYYXDY
Digest plasmid Restriction enzyme
Yeast display plasmid
CDRH3 mutant library

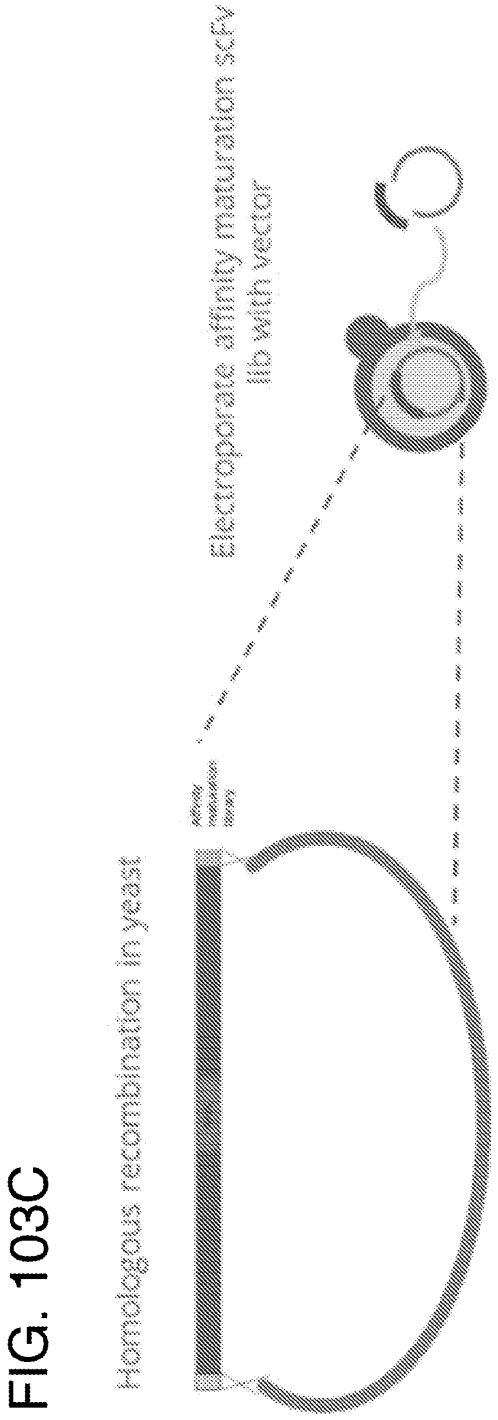
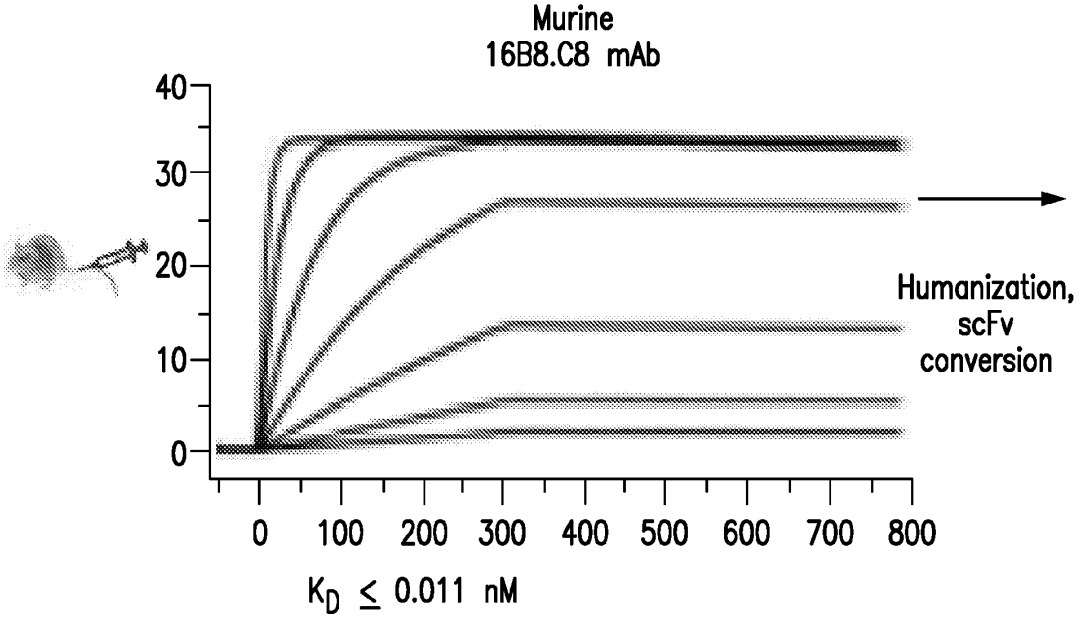

Electroporate affinity maturation scFv lib with vector
Homologous recombination in yeast

FIG. 103C


Murine
16B8.C8 mAb
40
30
20
10
0
0
100
200
300
400
500
600
700
800
$K_D \leq 0.011$ nM
Humanization,
scFv
conversion

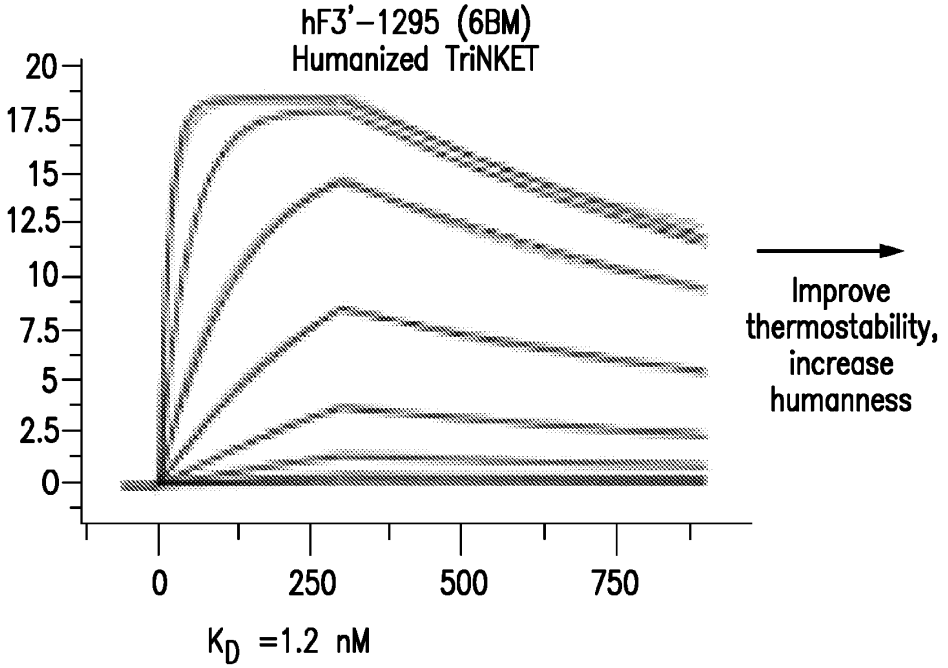
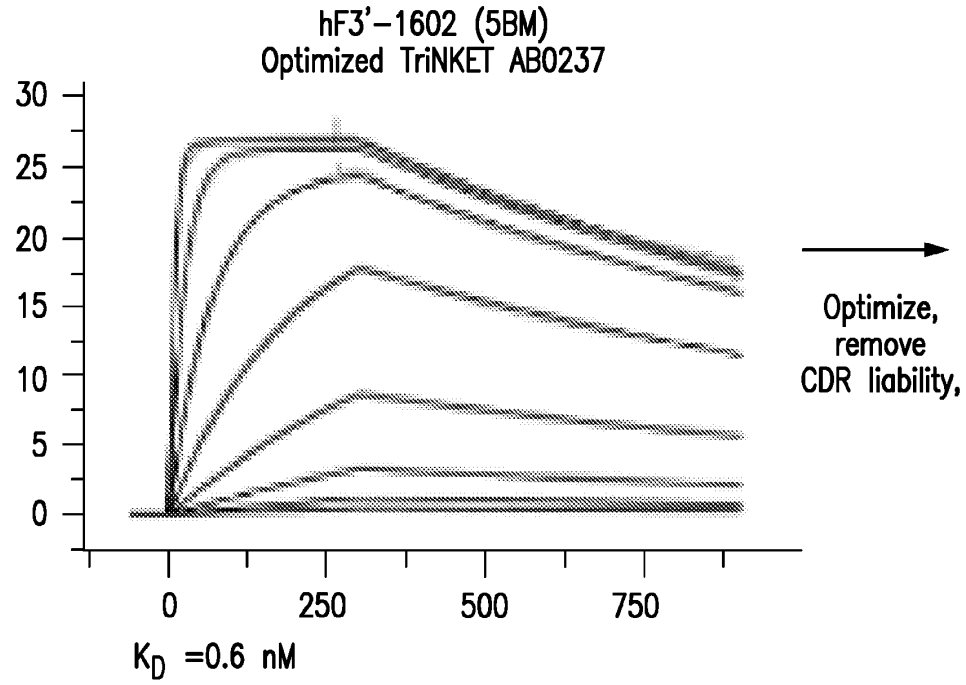

hF3'-1295 (6BM)
Humanized TriNKET
20
17.5
15
12.5
10
7.5
5
2.5
0
0
250
500
750
K_D =1.2 nM
Improve
thermostability,
increase
humanness

FIG. 104



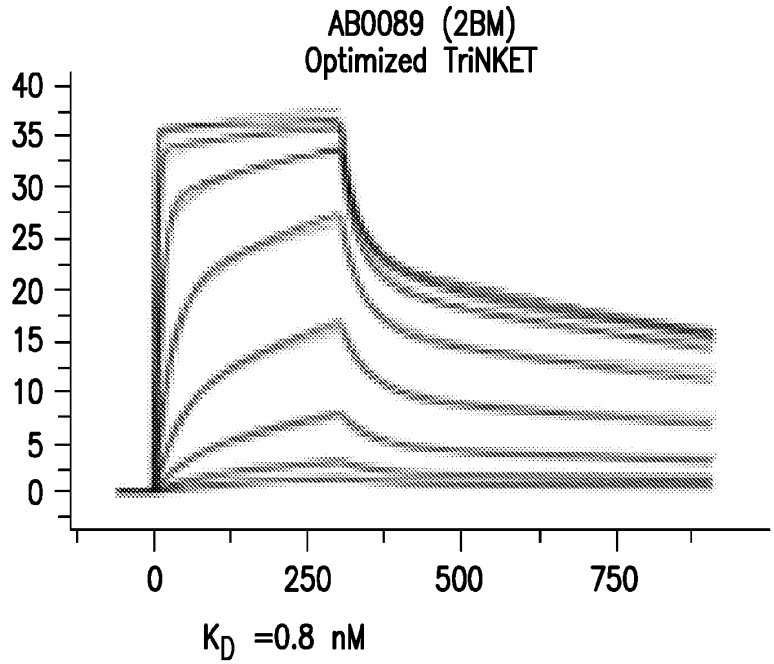
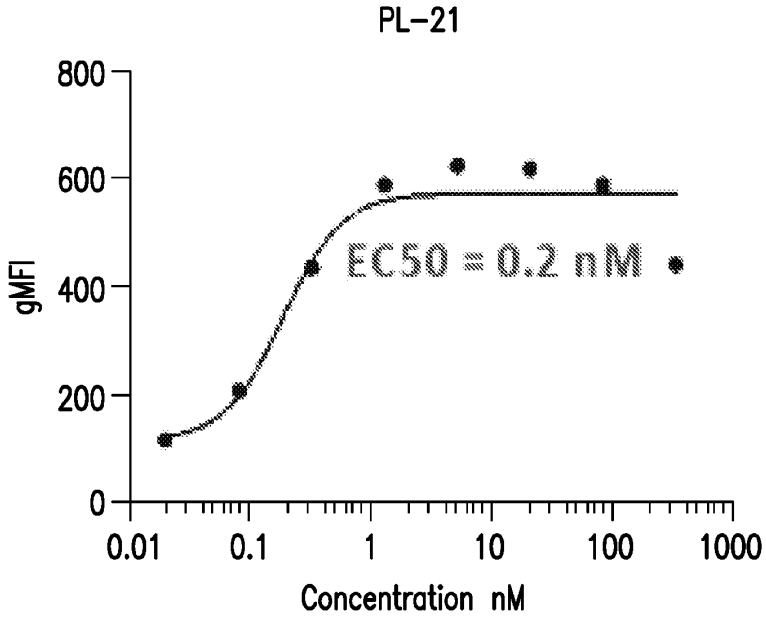


FIG. 104 Continued



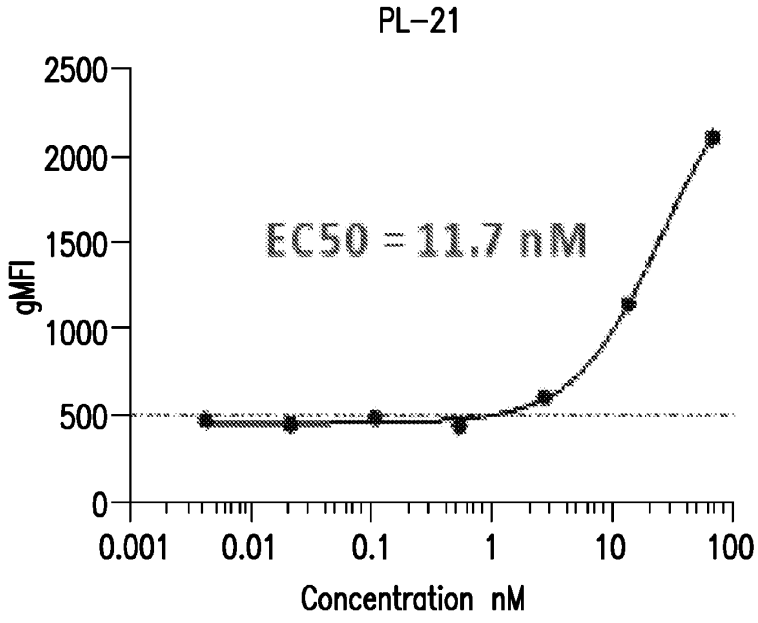
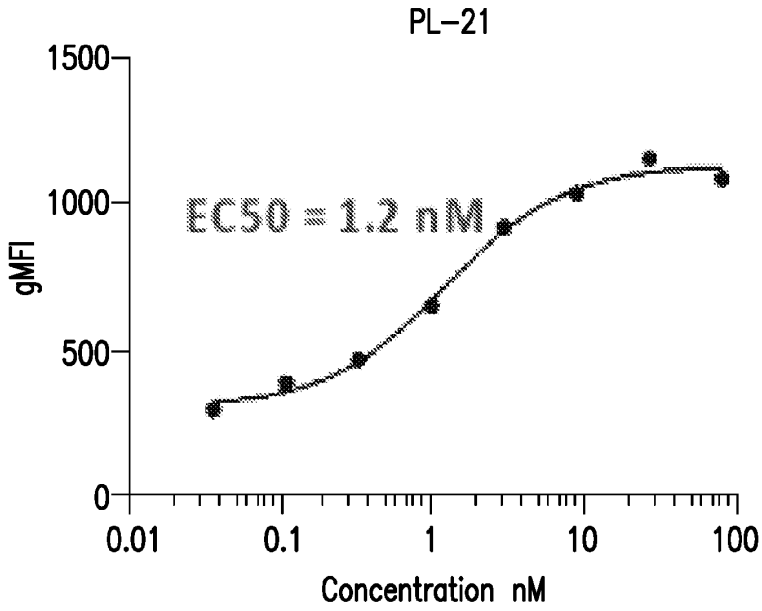


FIG. 104 Continued



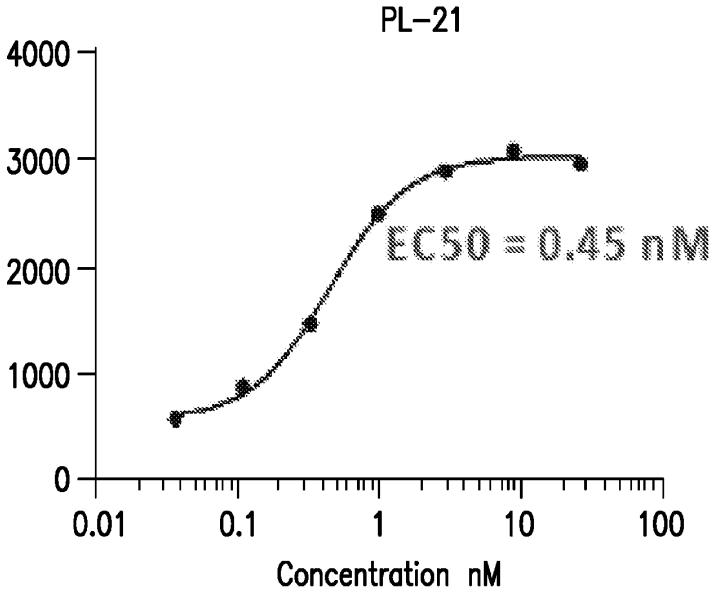


FIG. 104 Continued

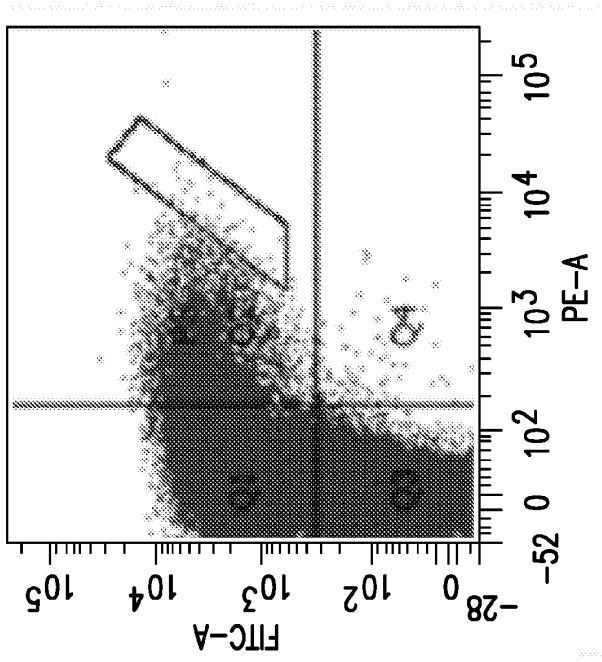


FIG. 105B

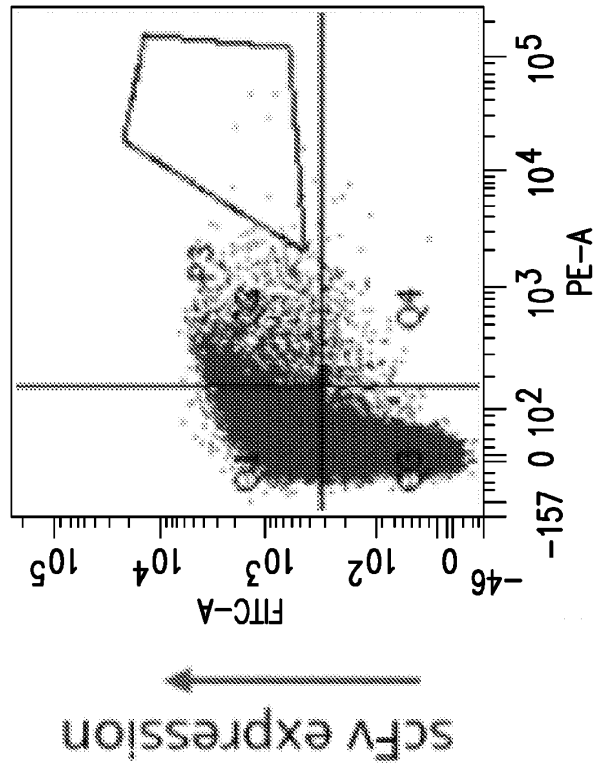


FIG. 105A

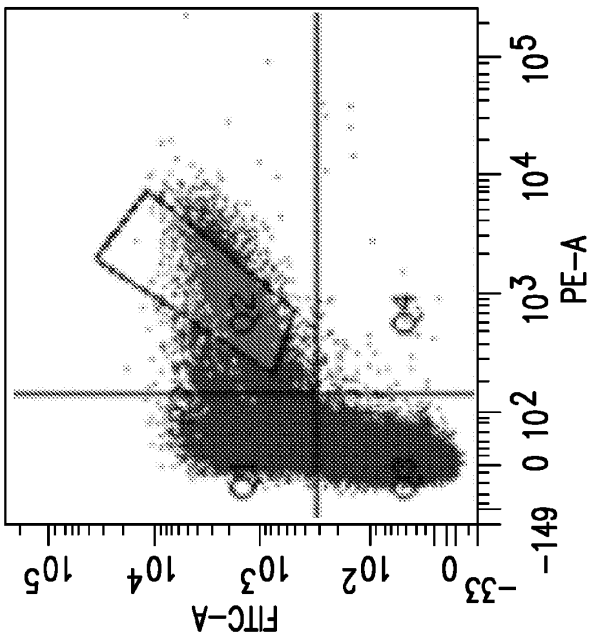
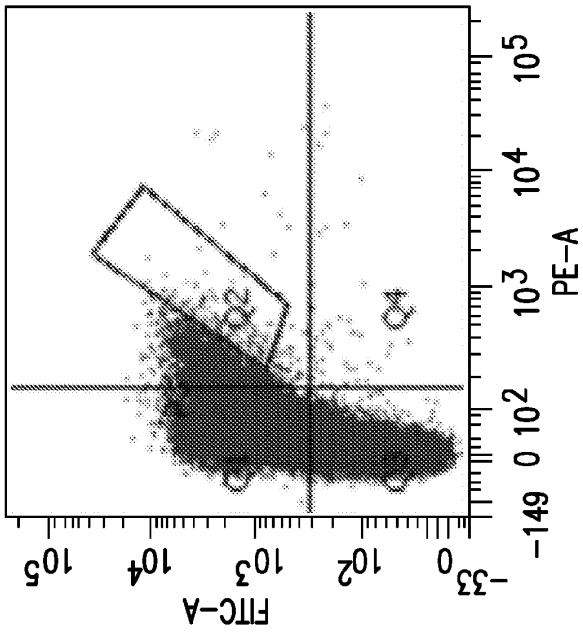


FIG. 105D



scFv expression

Binding to Clec12a-his

FIG. 105C

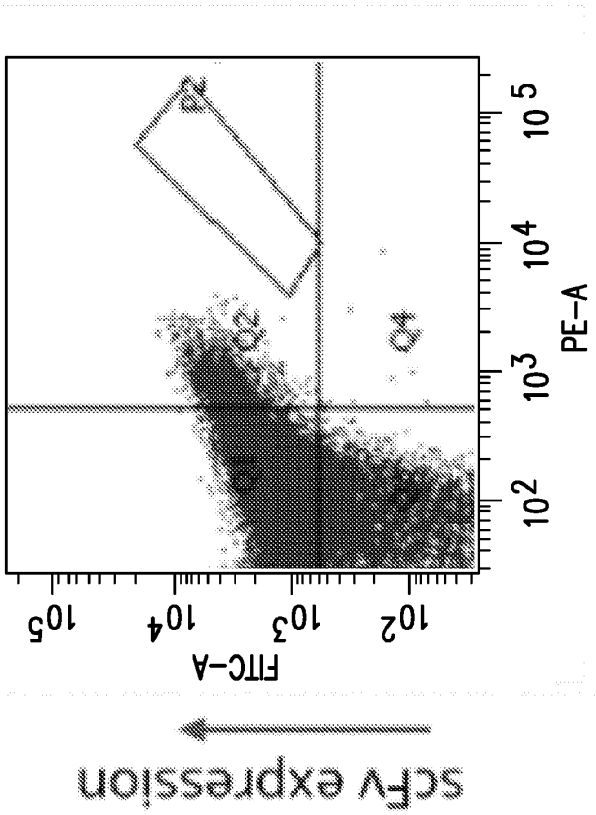

scFv expression
FITC-A
10^2
10^3
10^4
10^5
Q2
Q4
PE-A
10^2
10^3
10^4
10^5
Binding to Clec12a-his

FIG. 105E

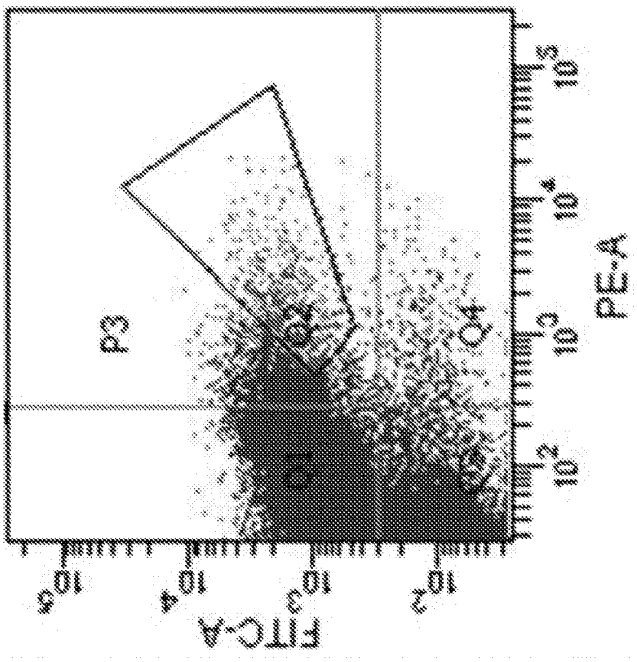


FIG. 106B

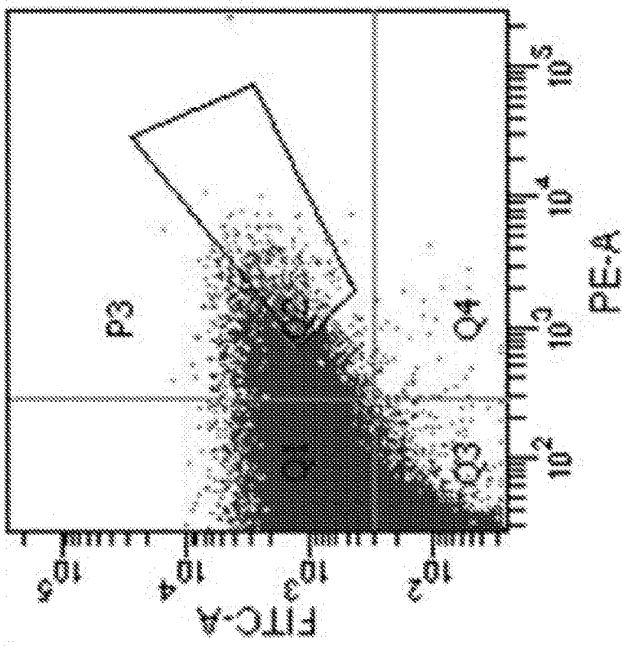


FIG. 106A

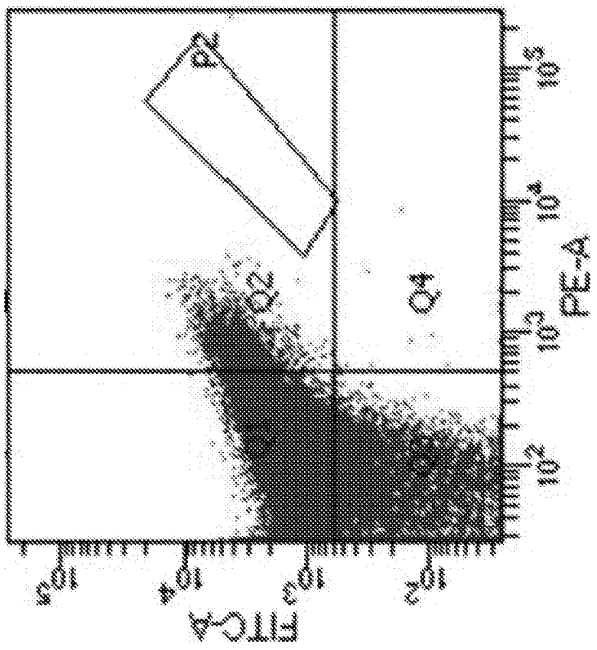


FIG. 106D

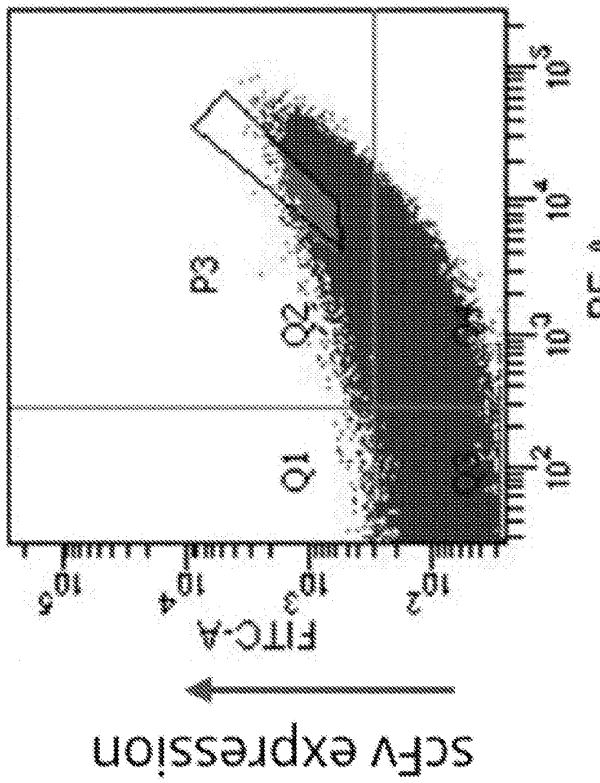


FIG. 106C

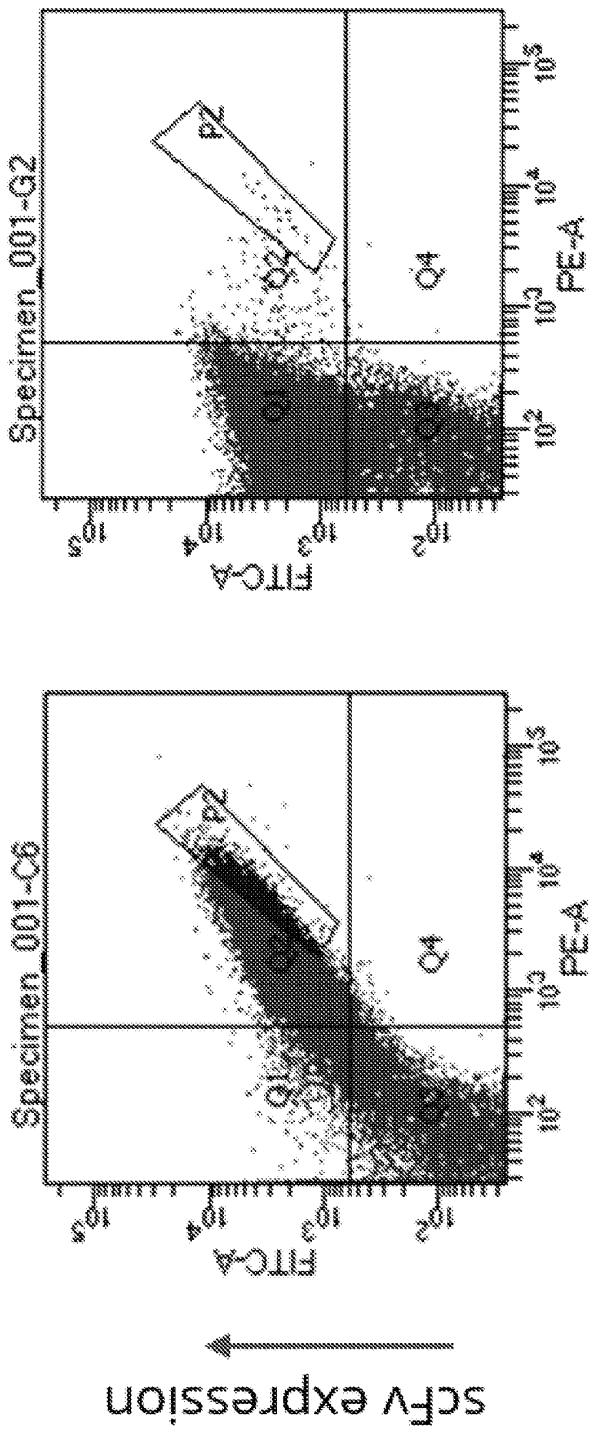

Specimen_001-G2
P2
Q2
Q4
PE-A
FITC-A
Specimen_001-C6
P2
Q4
Q1
PE-A
FITC-A
Binding to Clec12a-his
scFv expression

FIG. 107

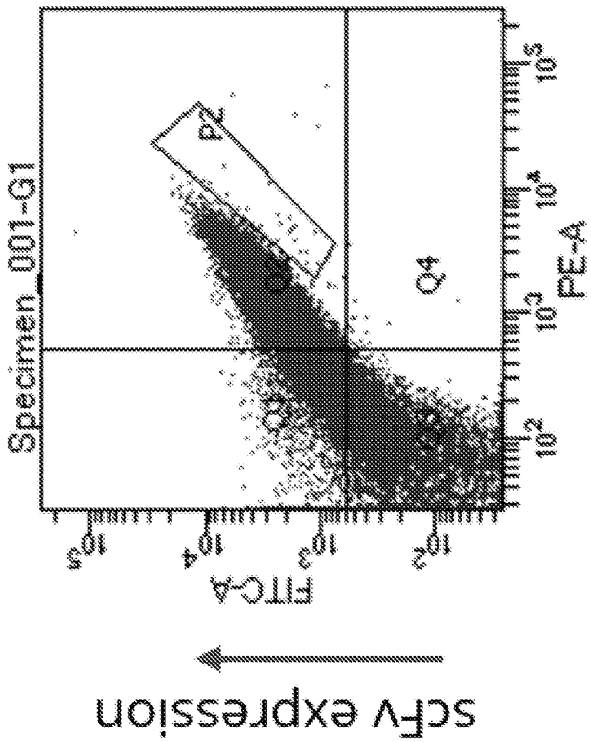


FIG. 107 Continued

	CDRH1	CDRH2	CDRH3
pET1596	GFSLTNY	WSGGK	YDYDDSLDY
Improved affinity clone	GFSLTNY	WVGGA	GDYGDSLDY
AB0237	GFSLTNY	WSGGK	YDYDDSLDY

FIG. 107 Continued

FIG. 108

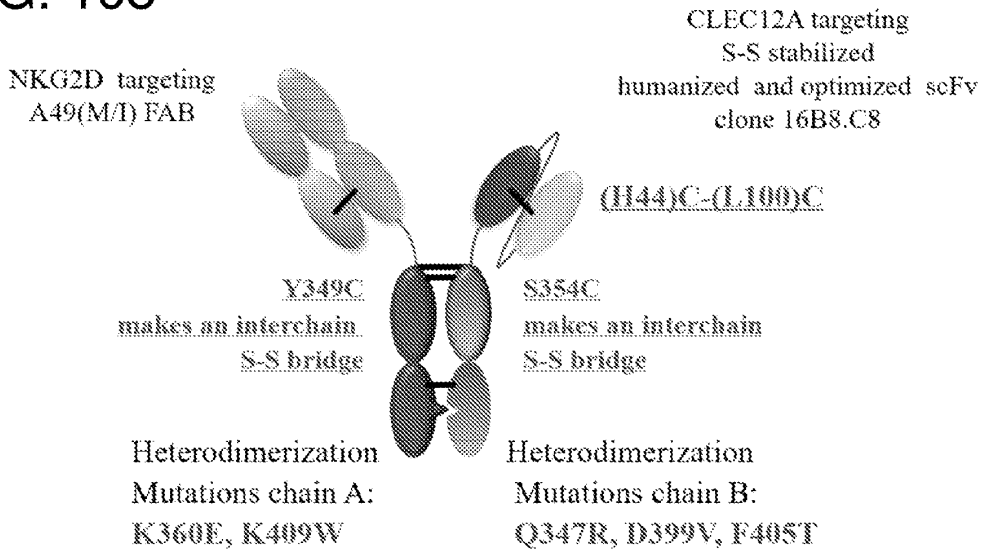

NKG2D targeting
A49(M/I) FAB
CLEC12A targeting
S-S stabilized
humanized and optimized scFv
clone 16B8.C8
(H44)C-(L100)C
Y349C
makes an interchain
S-S bridge
S354C
makes an interchain
S-S bridge
Heterodimerization
Mutations chain A:
K360E, K409W
Heterodimerization
Mutations chain B:
Q347R, D399V, F405T

FIG. 109

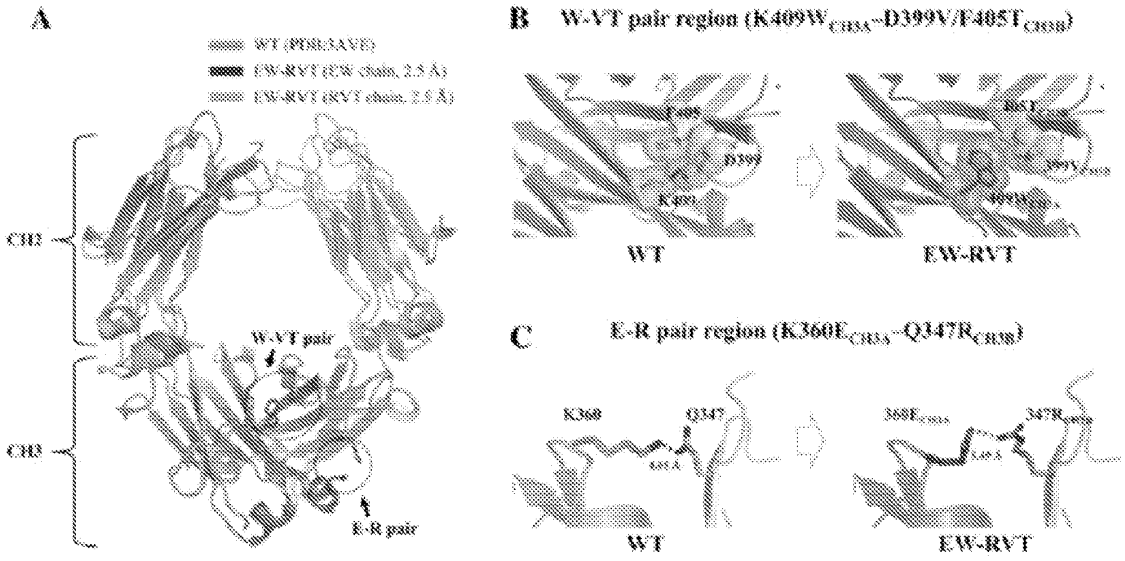
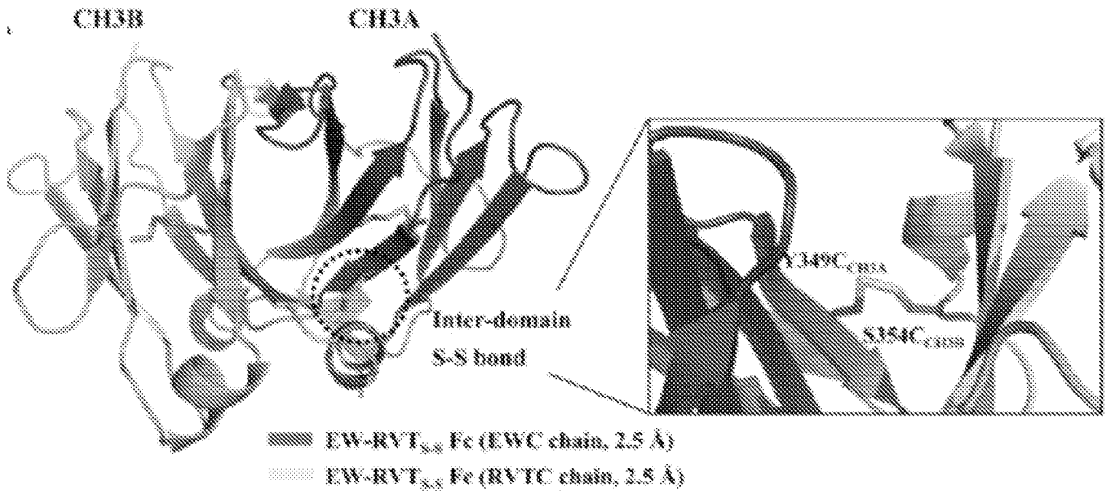


FIG. 110



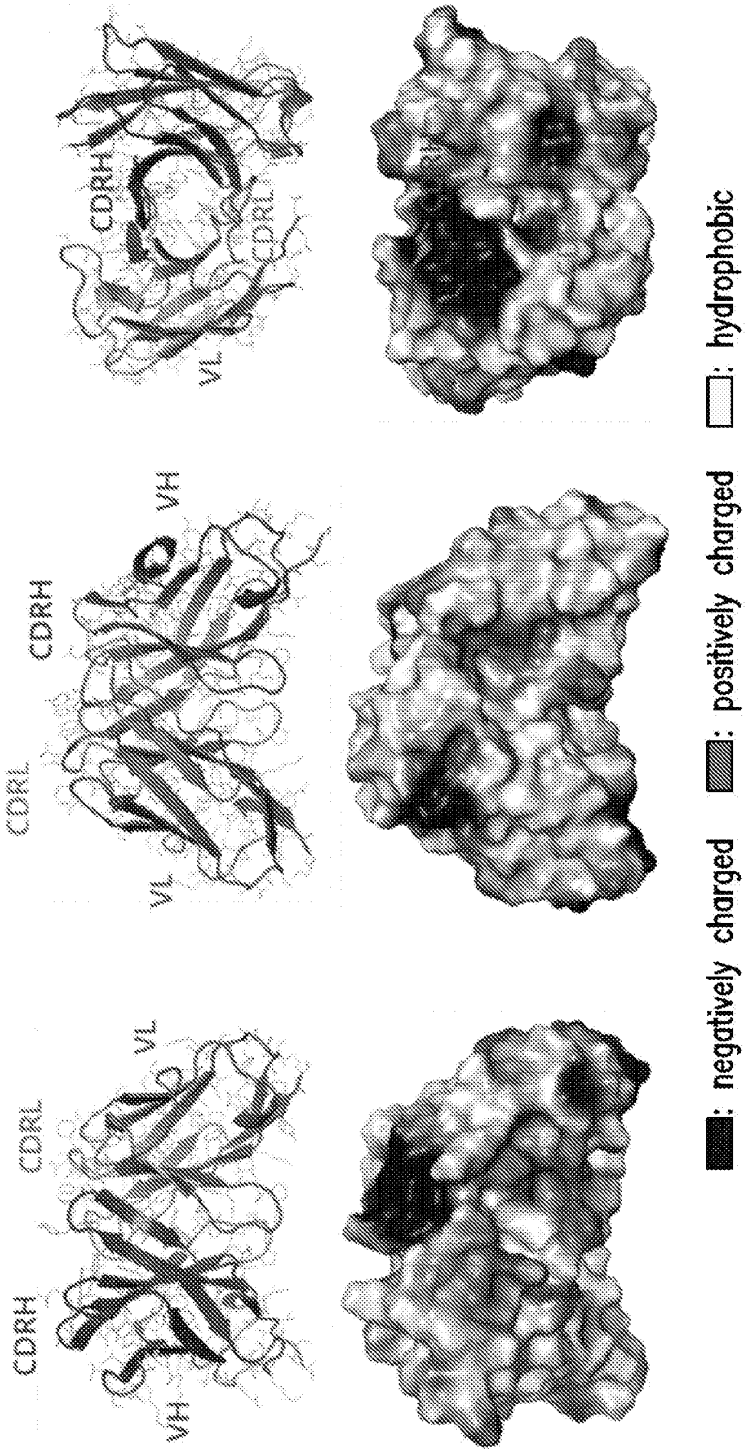

CDRH
CDRL
VL
VH
CDRL
CDRH
VH
VL
CDRH
CDRL
VL
VH
: negatively charged
: positively charged
: hydrophobic

FIG. 111

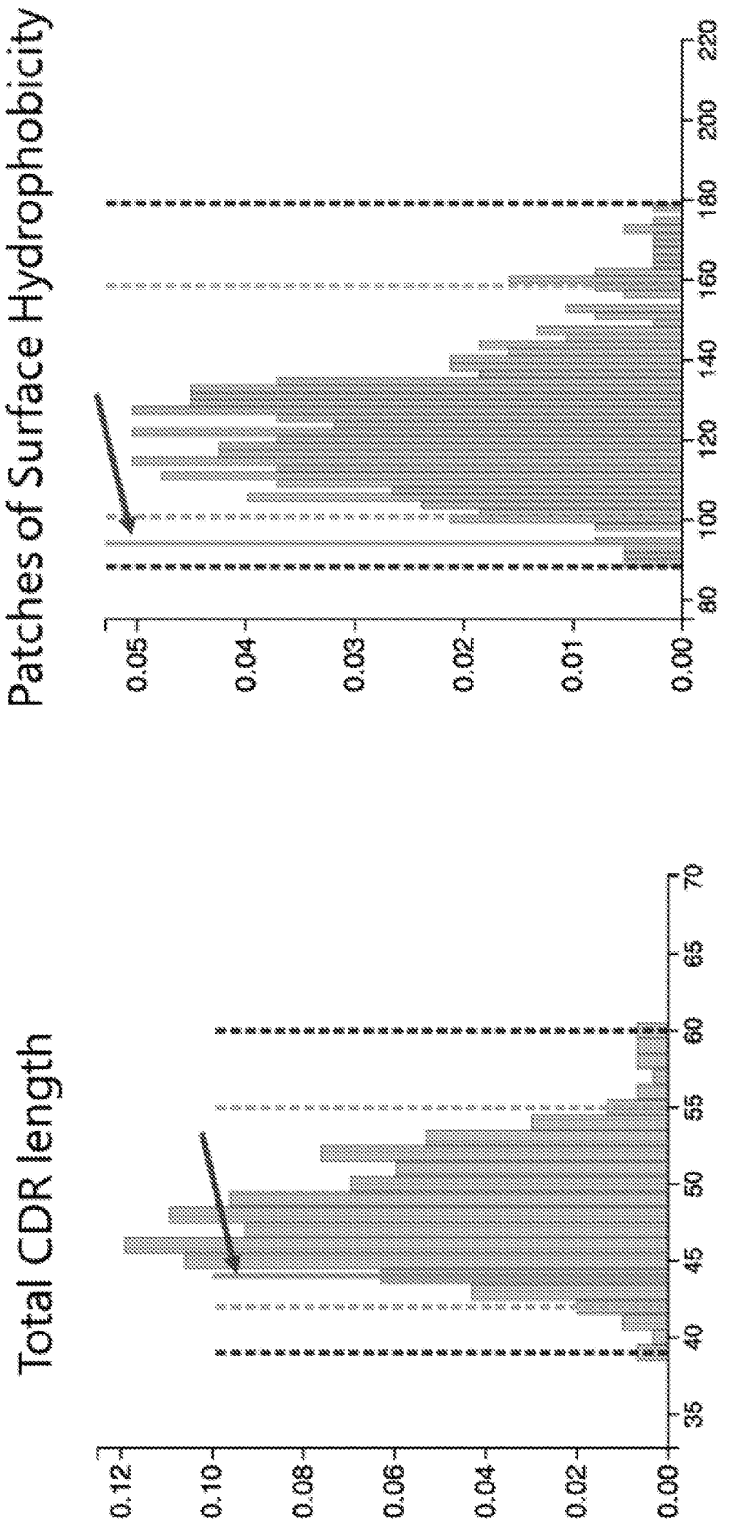

Patches of Surface Hydrophobicity
0.05
0.04
0.03
0.02
0.01
0.00
60
100
120
140
160
180
200
220
Total CDR length
0.12
0.10
0.08
0.06
0.04
0.02
0.00
35
40
45
50
55
60
65
70

FIG. 112

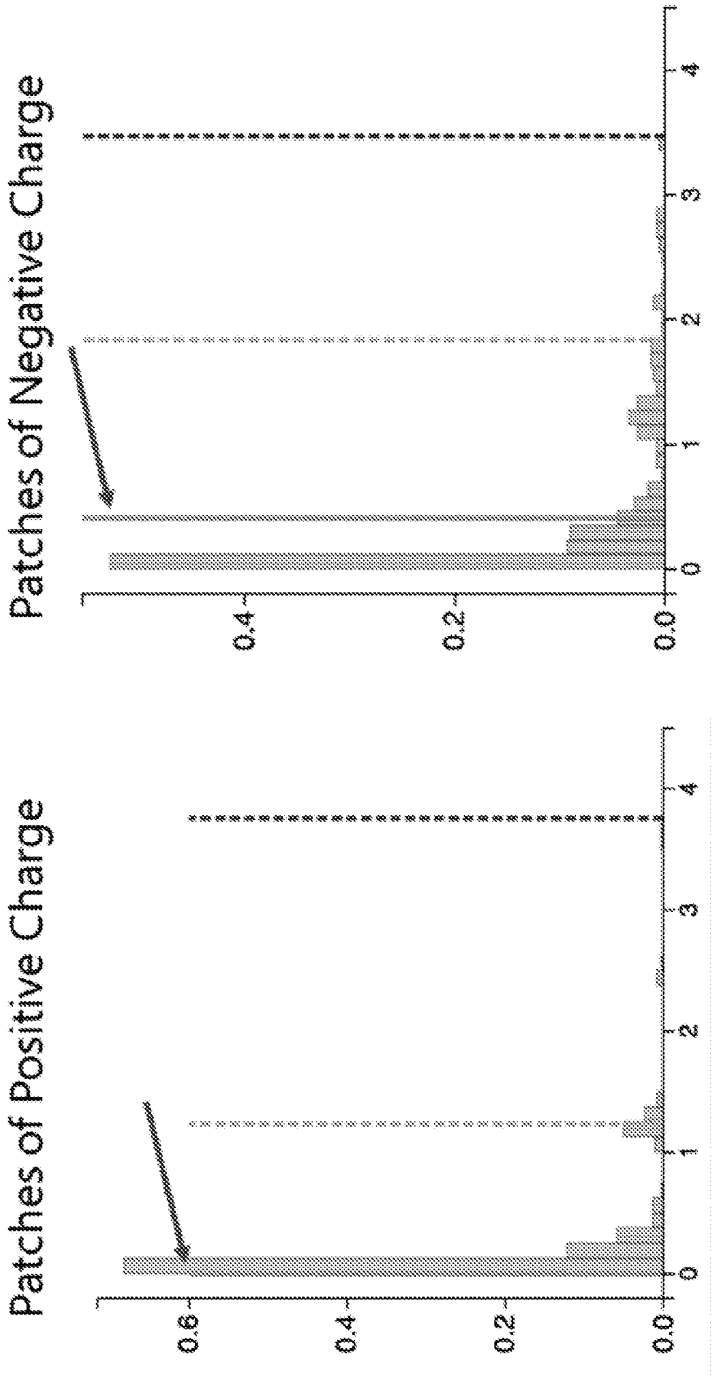

Patches of Negative Charge
0.0
0.2
0.4
0
1
2
3
4
Patches of Positive Charge
0.0
0.2
0.4
0.6
0
1
2
3
4

FIG. 113

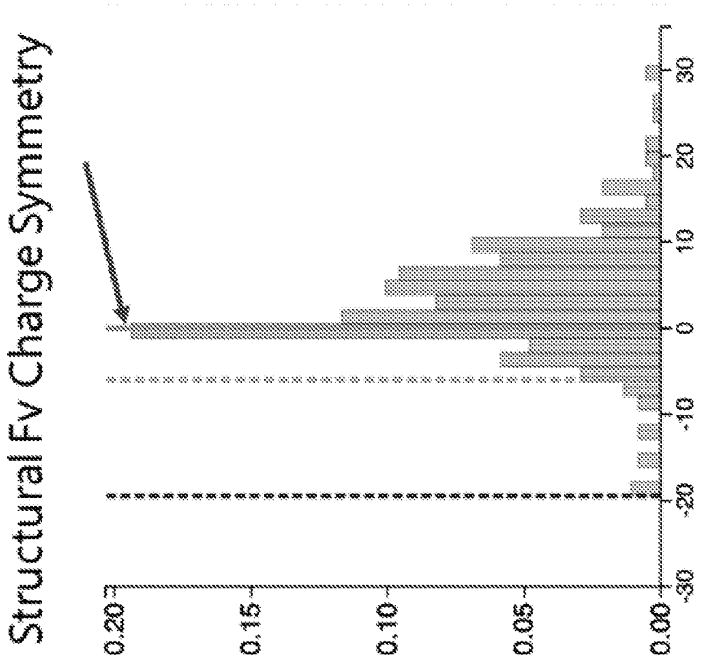


FIG. 113 Continued

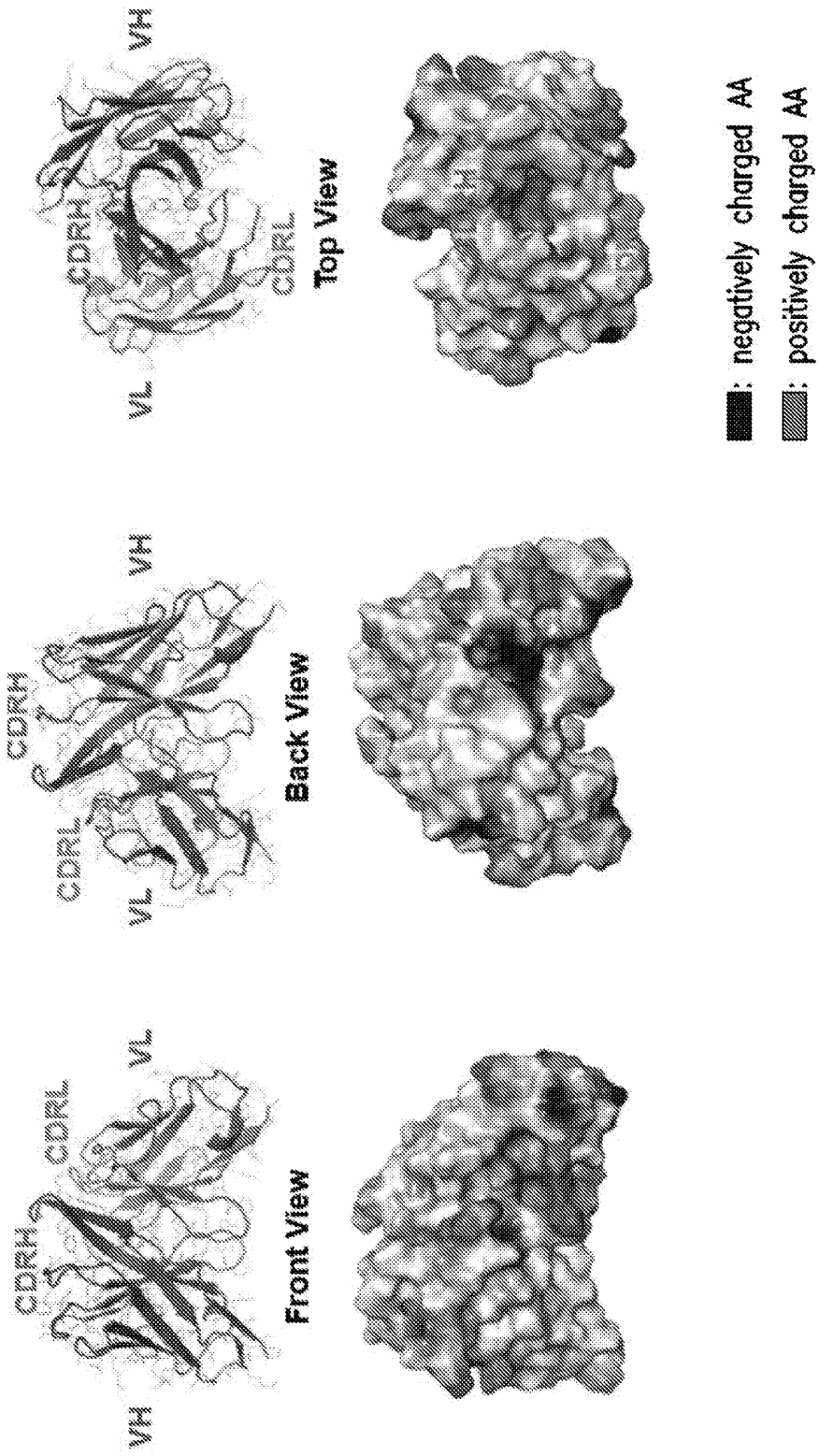

VH
CDRH
CDRL
VL
Top View
: negatively charged AA
: positively charged AA
VH
CDRH
CDRL
VL
Back View
VL
CDRL
CDRH
VH
Front View

FIG. 114

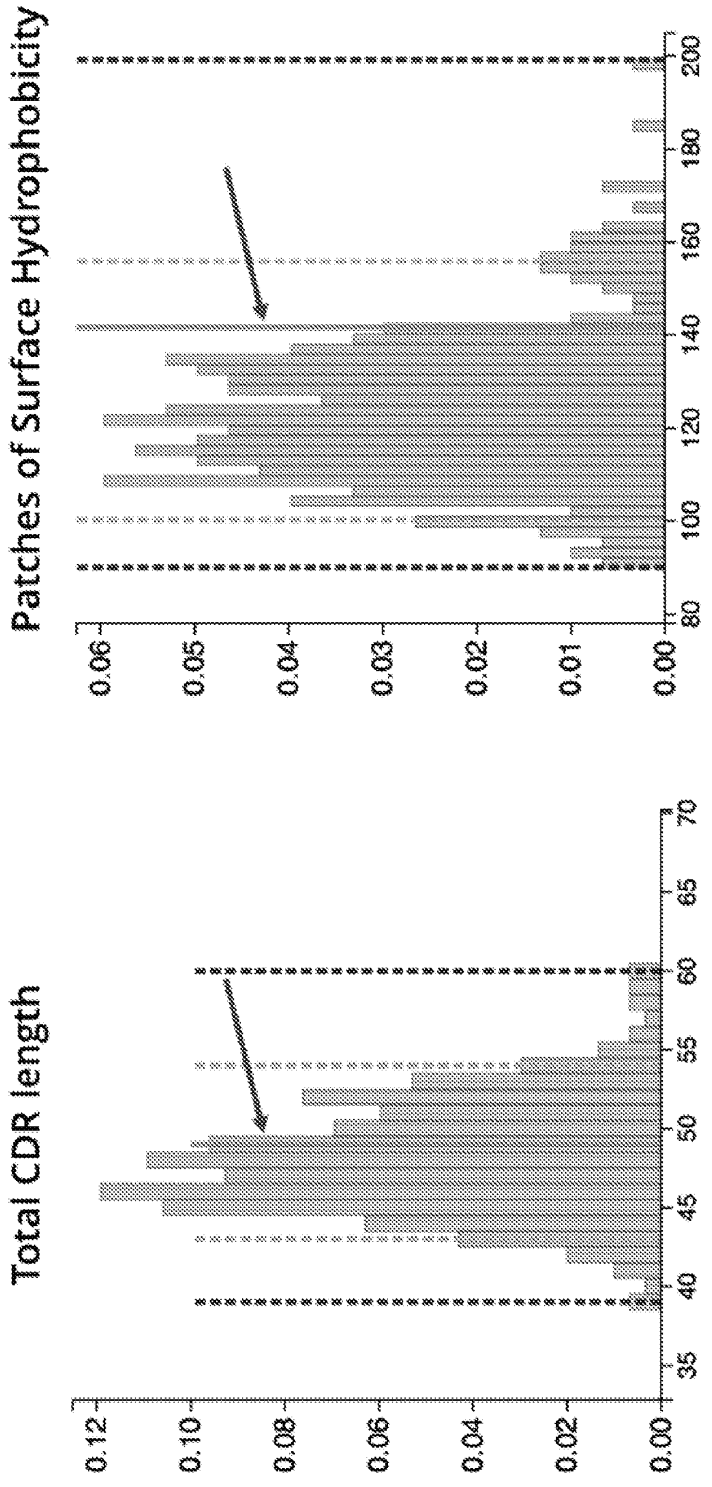

Patches of Surface Hydrophobicity
0.06
0.05
0.04
0.03
0.02
0.01
0.00
80
100
120
140
160
180
200
Total CDR length
0.12
0.10
0.08
0.06
0.04
0.02
0.00
35
40
45
50
55
60
65
70

FIG. 115

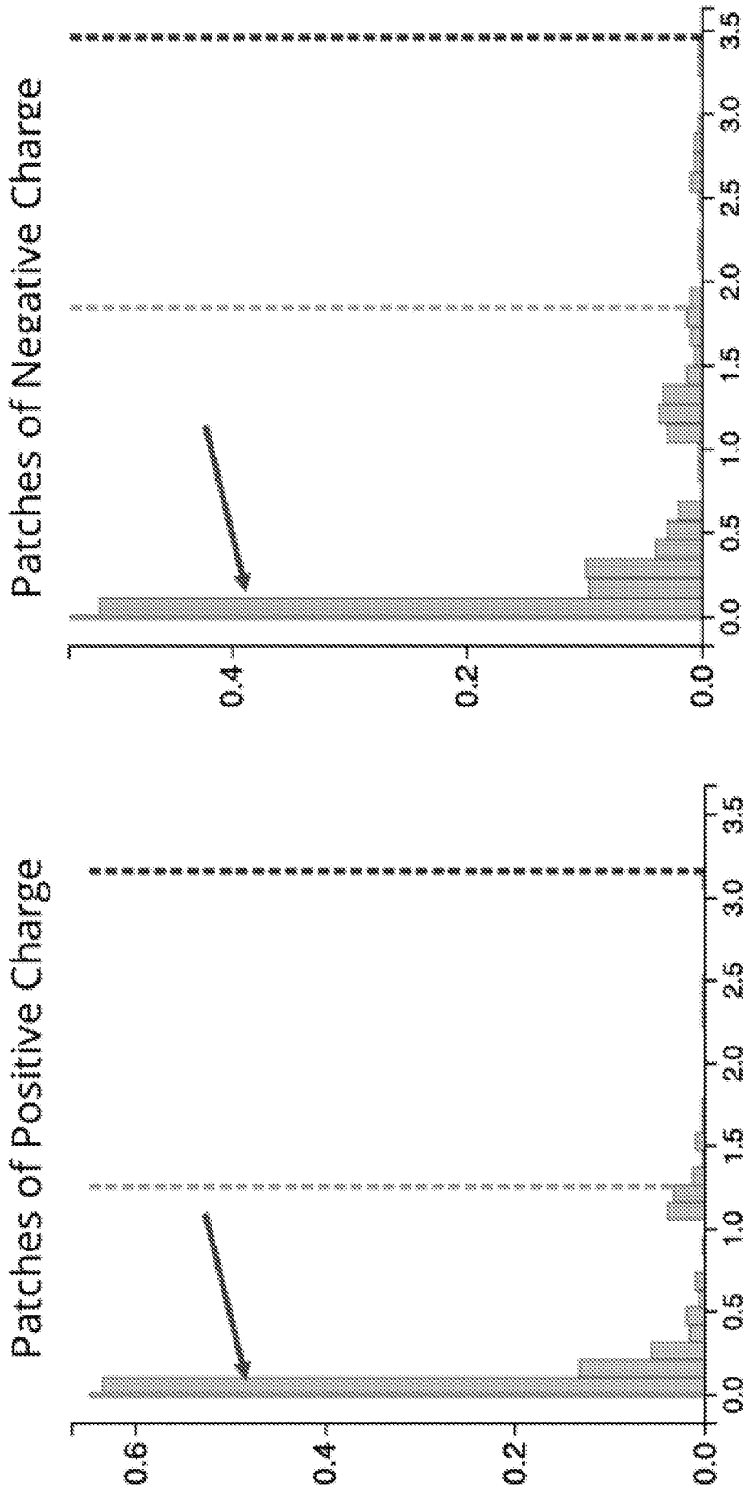

Patches of Negative Charge
0.0
0.2
0.4
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Patches of Positive Charge
0.0
0.2
0.4
0.6
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5

FIG. 116

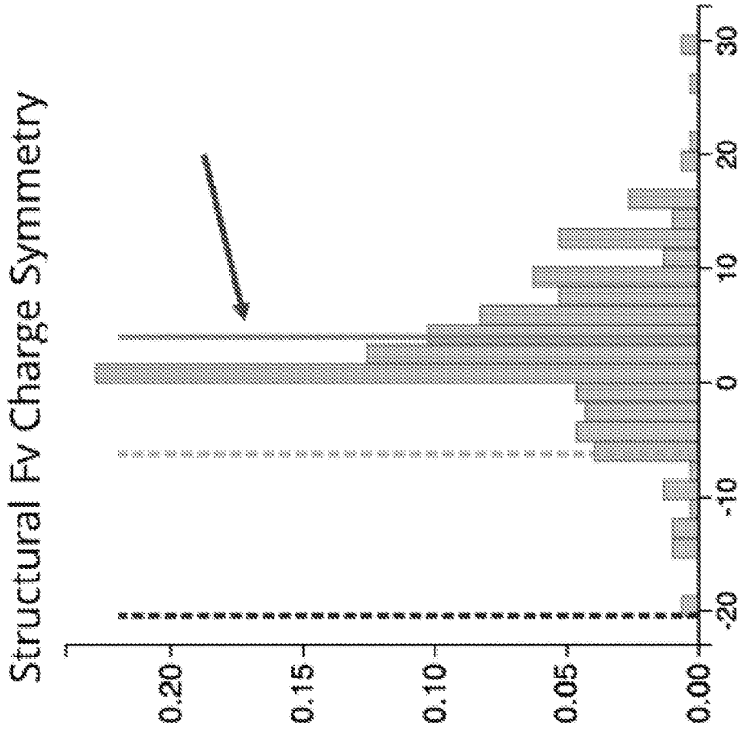
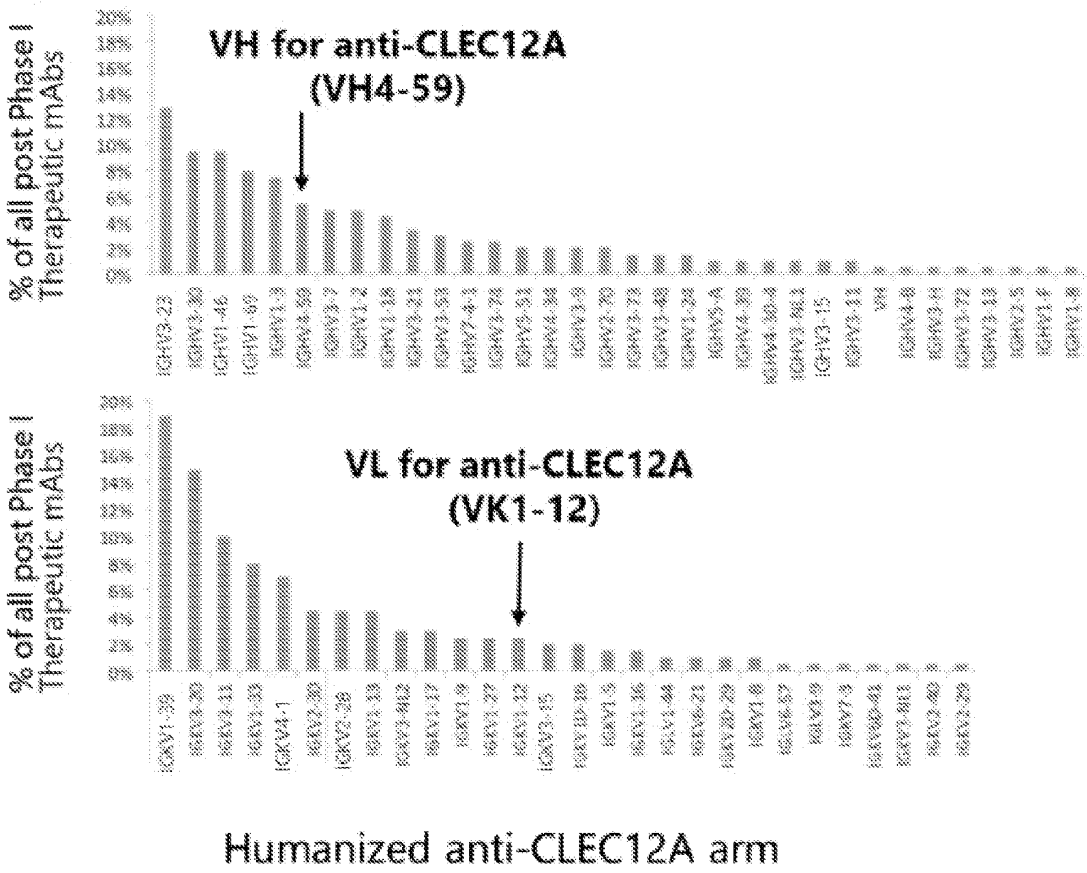


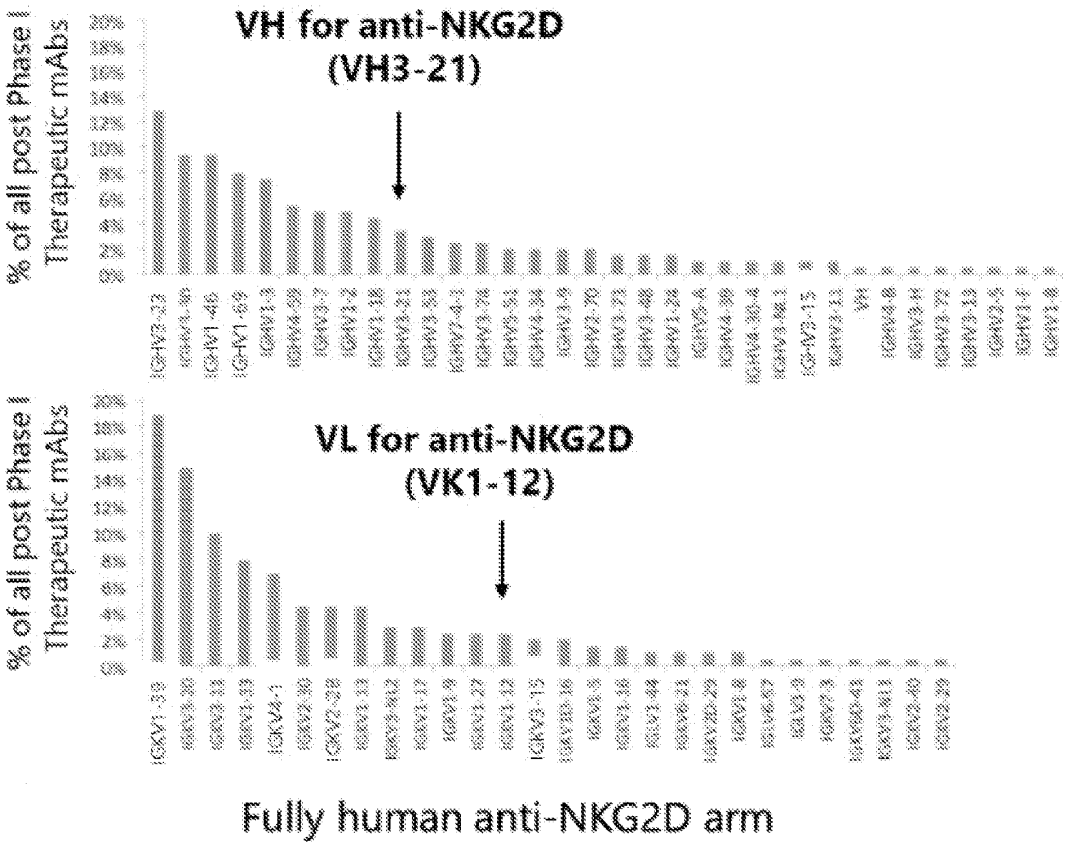
FIG. 116 Continued

FIG. 117



Humanized anti-CLEC12A arm

FIG. 117 continued



A

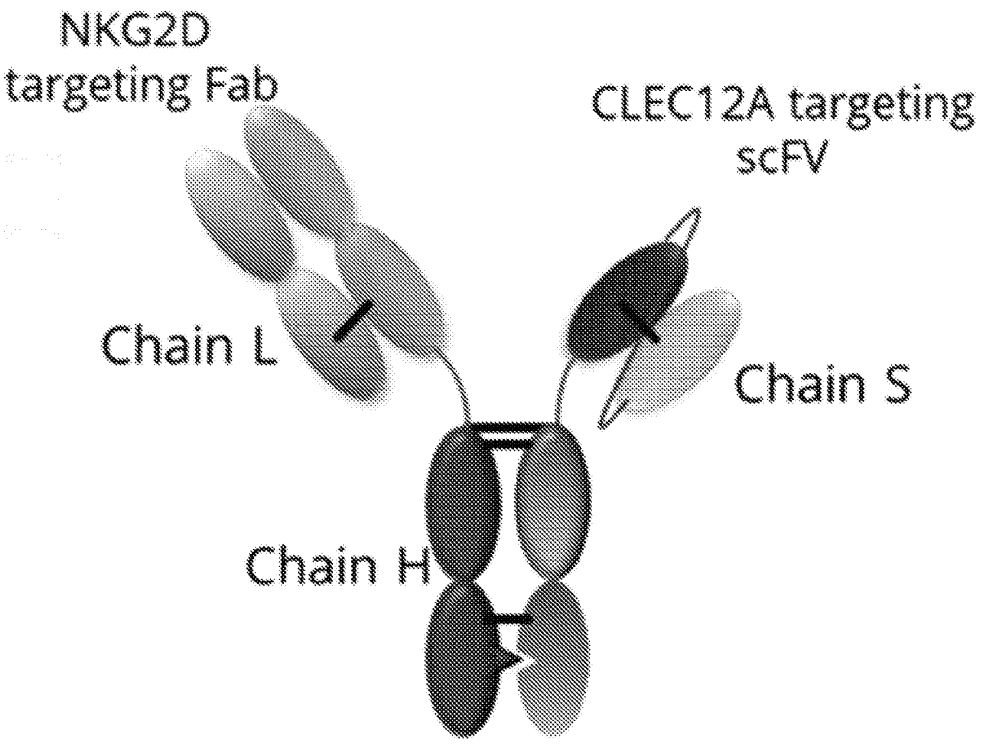

NKG2D
targeting Fab
CLEC12A targeting
scFV
Chain L
Chain S
Chain H

FIG. 118A

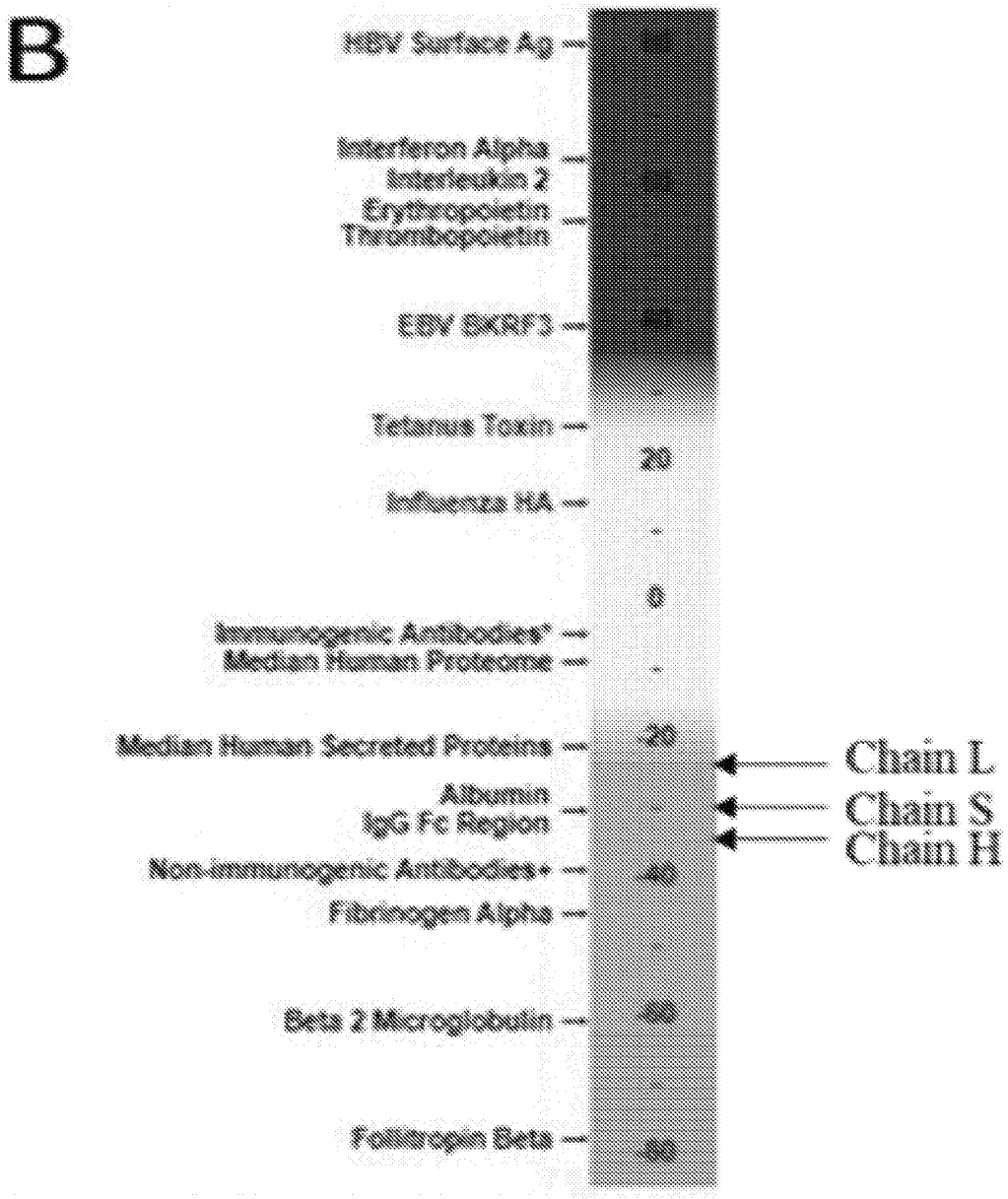
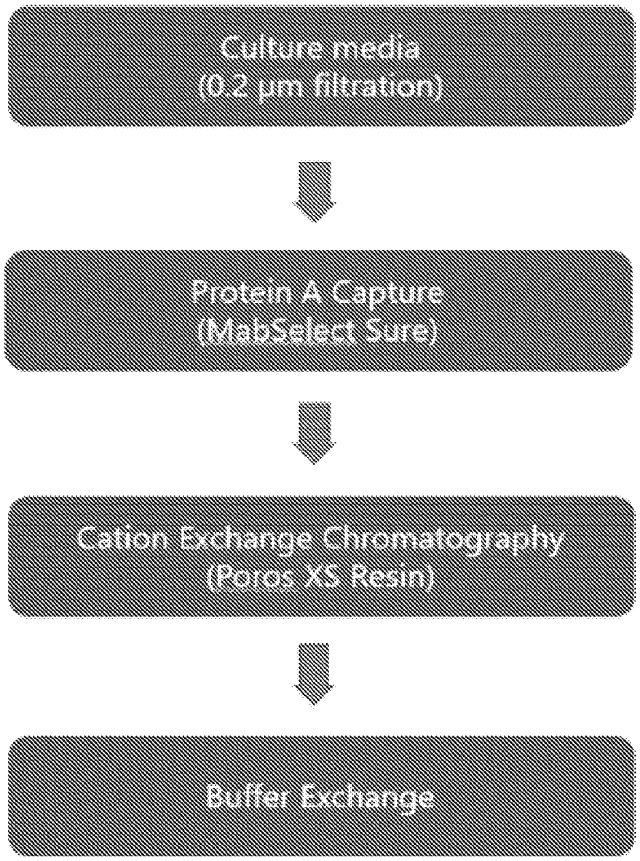

B
HBV Surface Ag
Interferon Alpha
Interleukin 2
Erythropoietin
Thrombopoietin
EBV EKRF3
Tetanus Toxin
Influenza HA
Immunogenic Antibodies*
Median Human Proteome
Median Human Secreted Proteins
Albumin
IgG Fc Region
Non-Immunogenic Antibodies+
Fibrinogen Alpha
Beta 2 Microglobulin
Fibrinogen Beta
80
60
40
20
0
-20
-40
-60
-80
Chain L
Chain S
Chain H

FIG. 118B

FIG. 119


Culture media
(0.2 µm filtration)
Protein A Capture
(MabSelect Sure)
Cation Exchange Chromatography
(Poros XS Resin)
Buffer Exchange

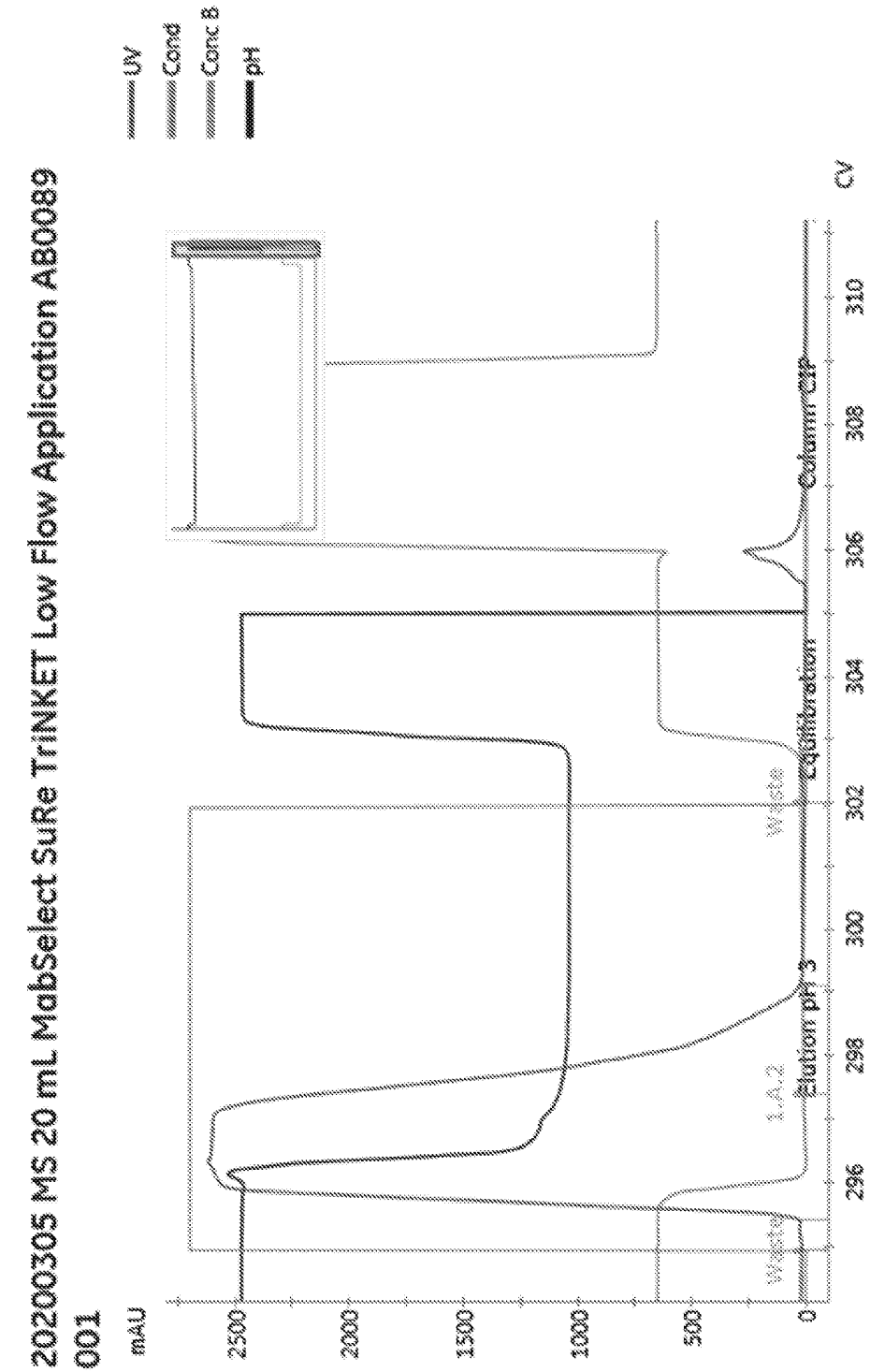

20200305 MS 20 mL MabSelect SuRe TriNKET Low Flow Application AB0089
001
UV
Cond
Conc B
pH
mAU
2500
2000
1500
1000
500
0
CV
296
298
300
302
304
306
308
310
Wash
1.A.2
Elution pH 3
Wash
Equilibration
Column CIP

FIG. 120

FIG. 121

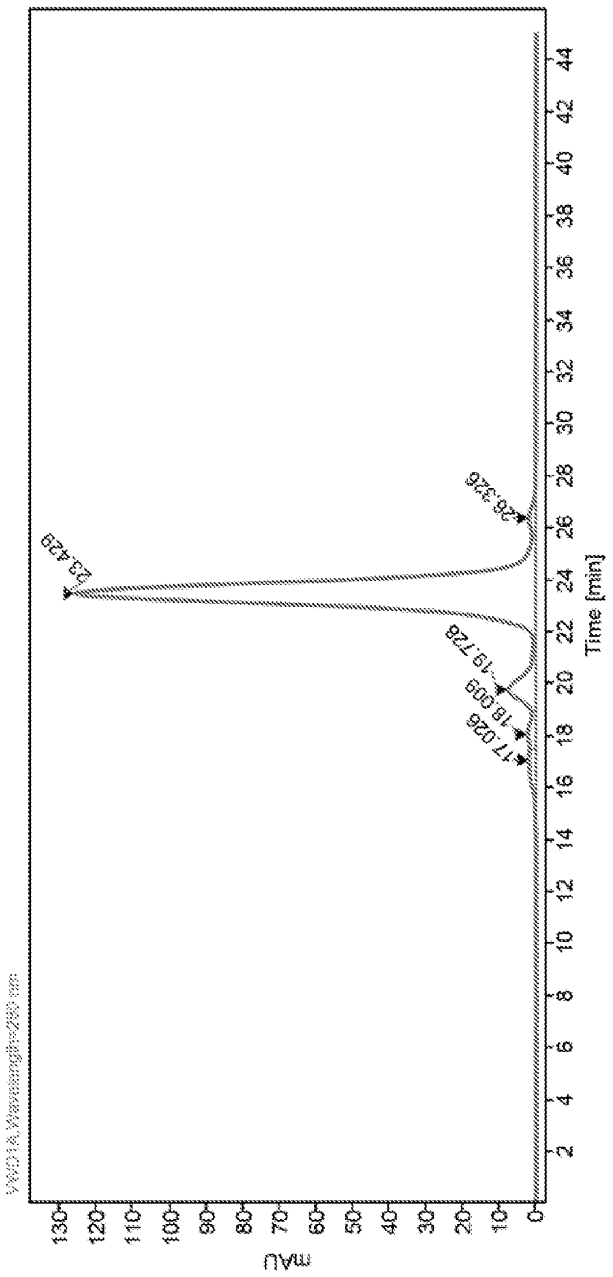
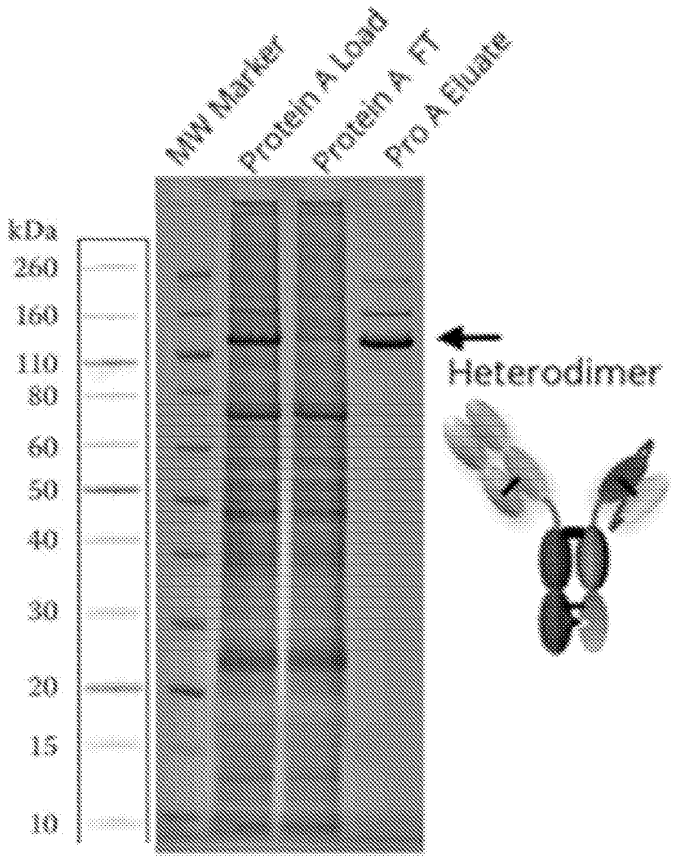


FIG. 122


MW Marker
Protein A Load
Protein A FT
Pro A Eluate
kDa
260
160
110
80
60
50
40
30
20
15
10
Heterodimer

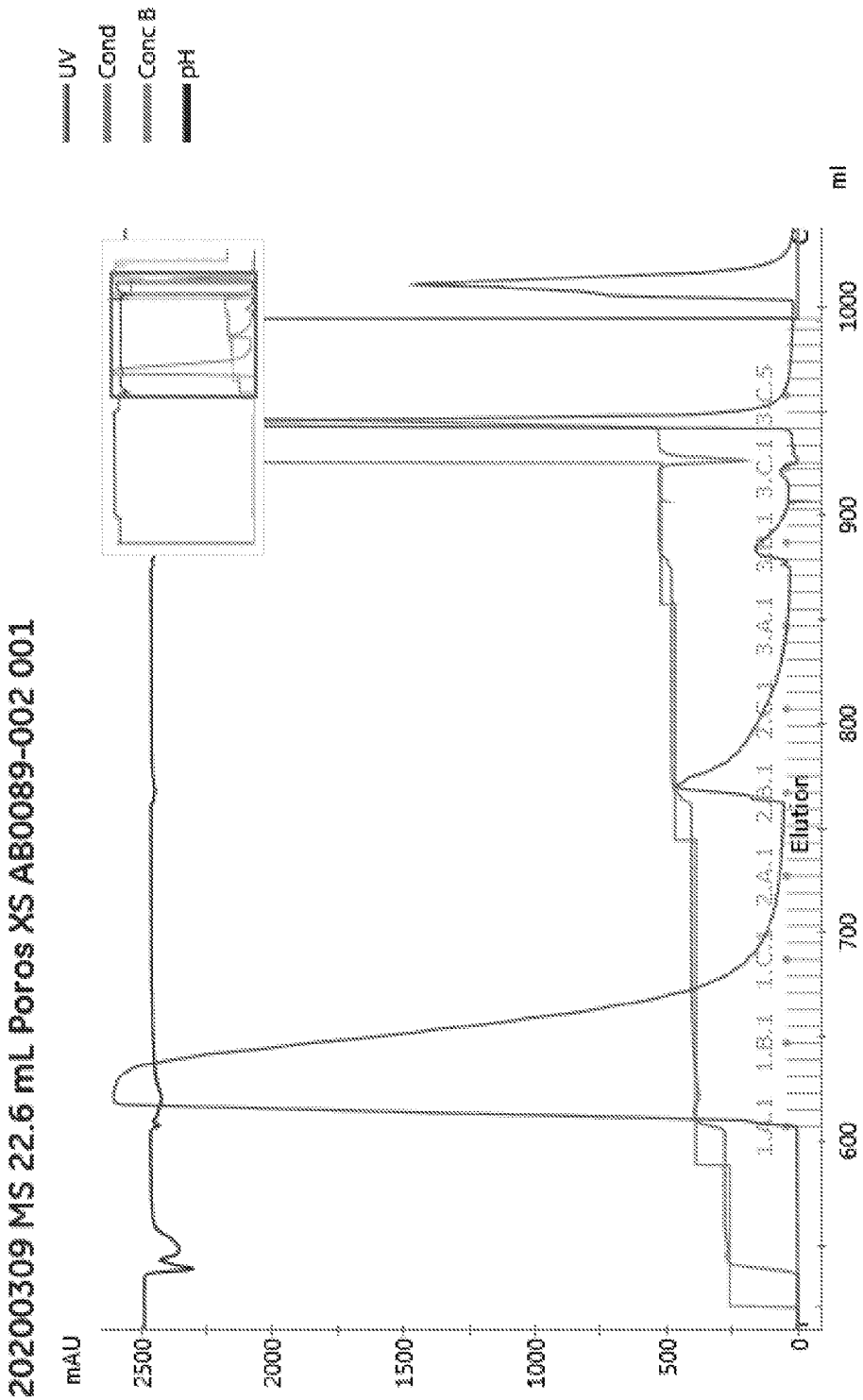

UV
Cond
Conc B
pH
20200309 MS 22.6 mL Poros XS AB0089-002 001
mAU
2500
2000
1500
1000
500
0
ml
600
700
800
900
1000
Elution

FIG. 123

FIG. 124

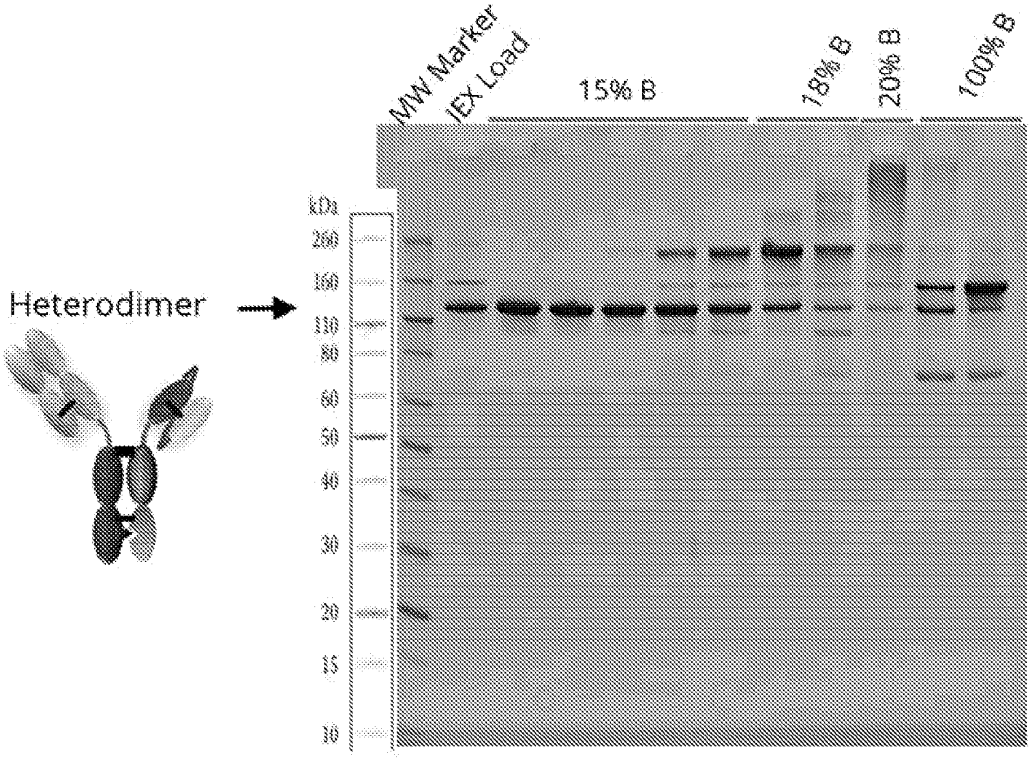
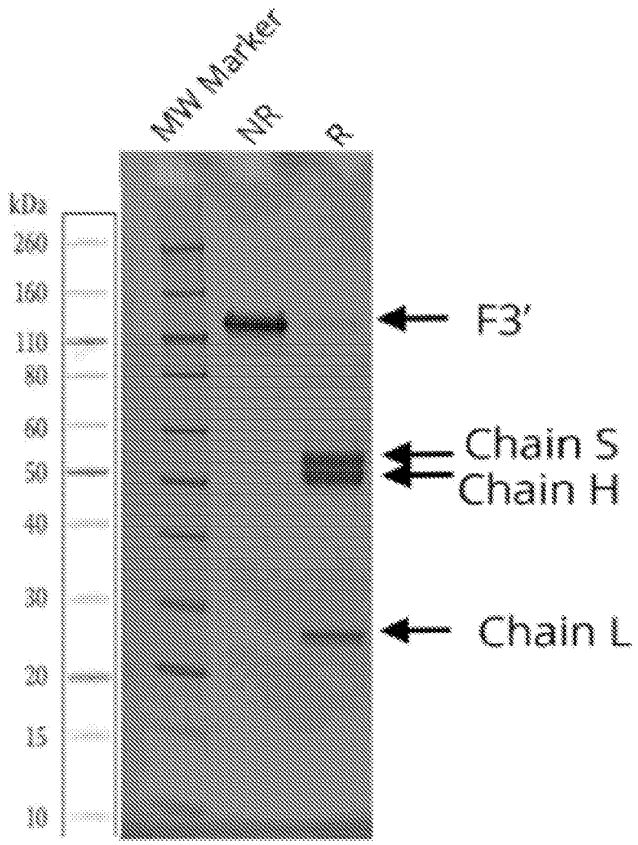

MW Marker
IEX Load
15% B
18% B
20% B
100% B
kDa
260
160
110
80
60
50
40
30
20
15
10
Heterodimer

FIG. 125


MW Marker
NR
R
KDa
260
160
110
80
60
50
40
30
20
15
10
F3'
Chain S
Chain H
Chain L

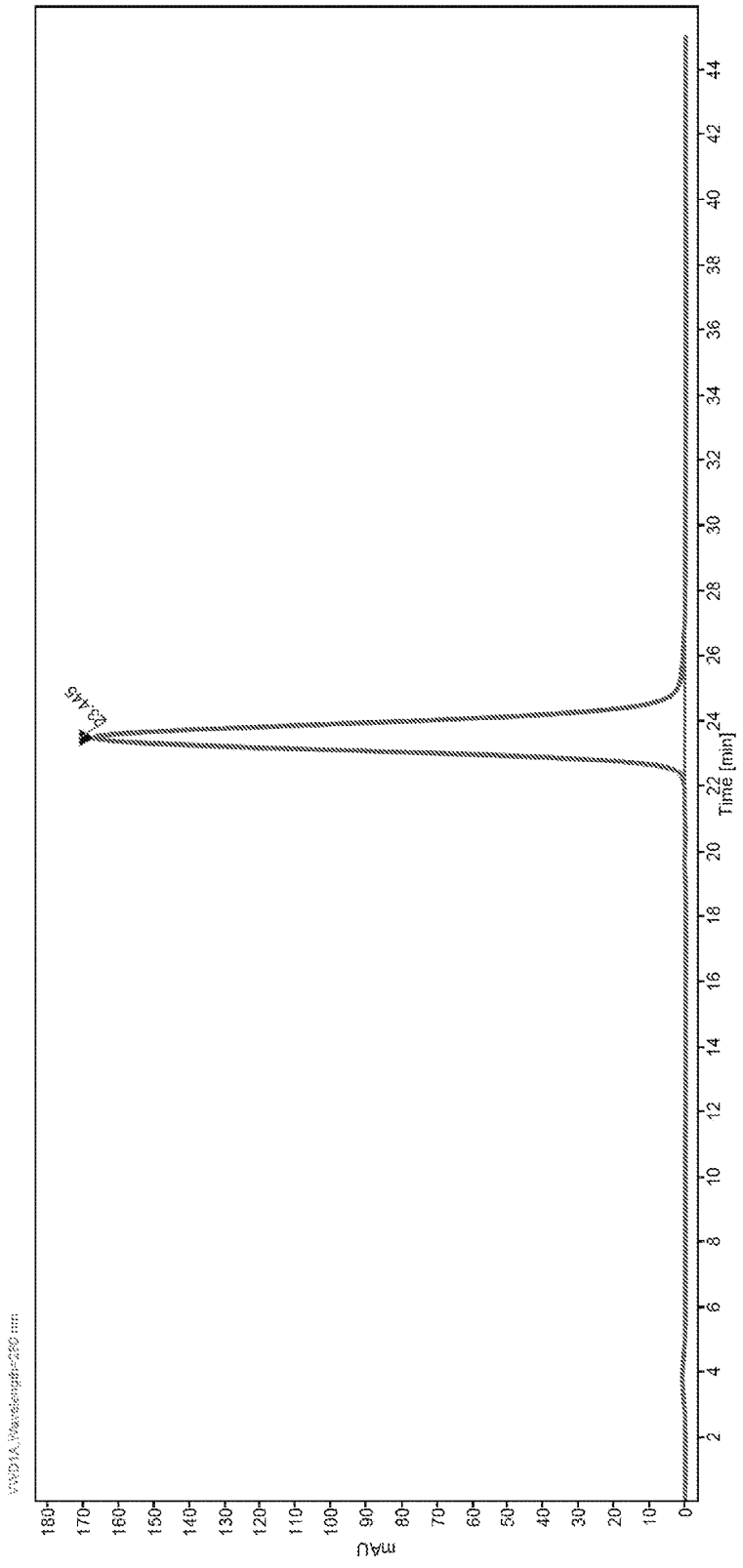
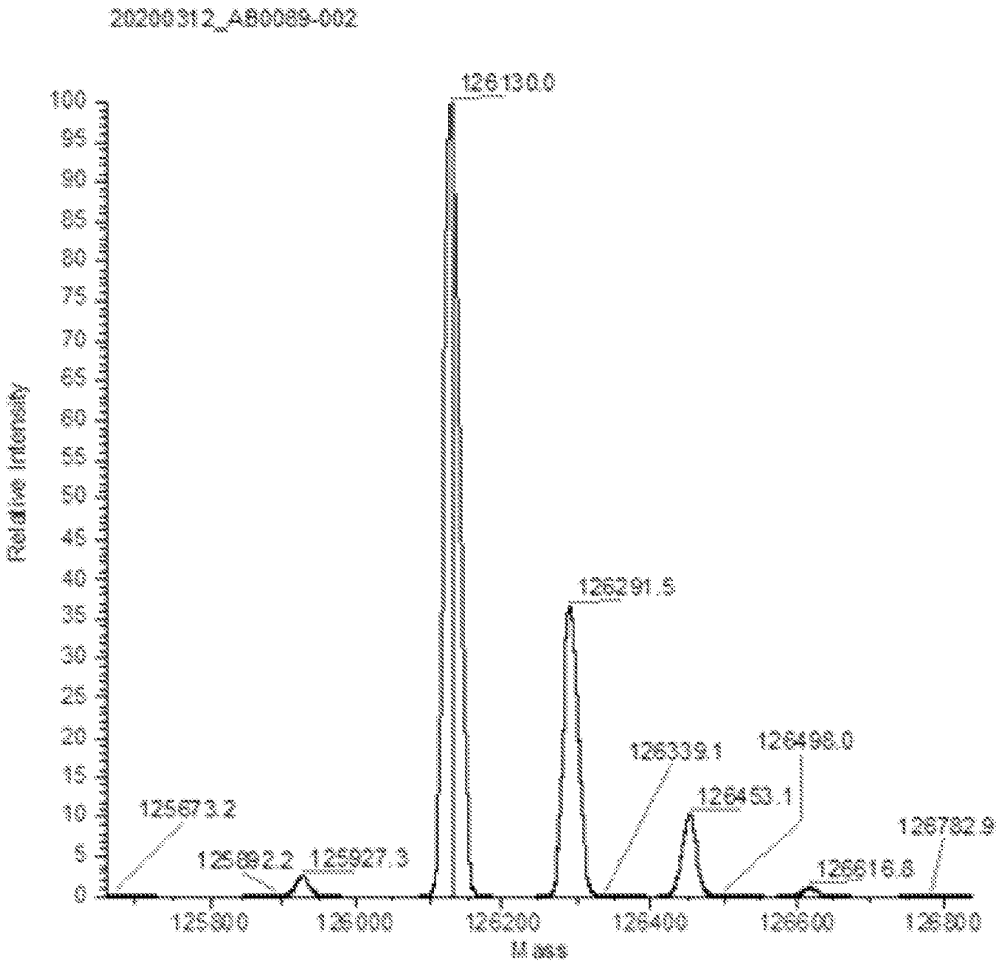


FIG. 126

FIG. 127



intact mass (theoretical)	126,129.1 Da
intact mass (experimental)	126,130.0 Da

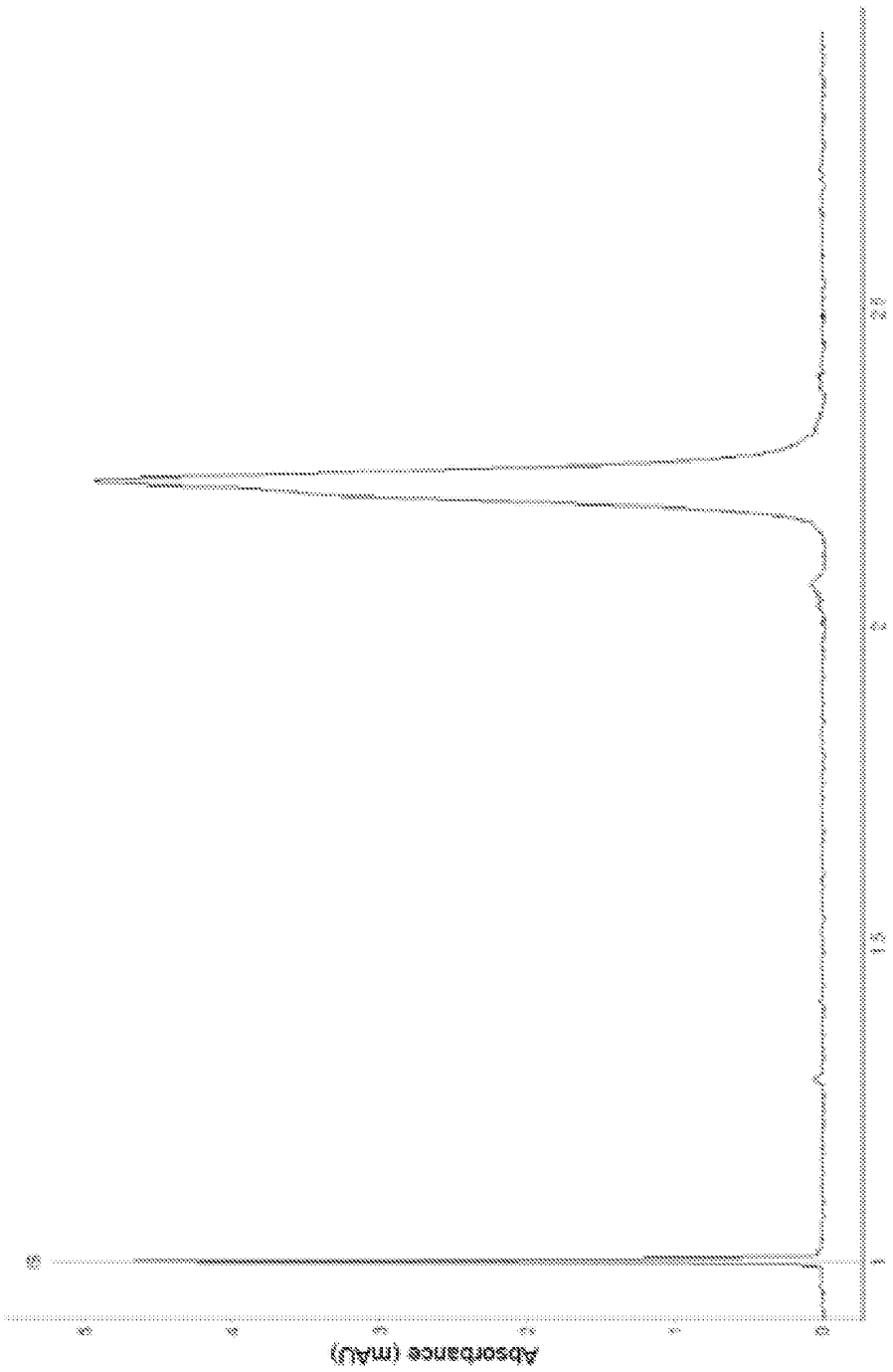


FIG. 128

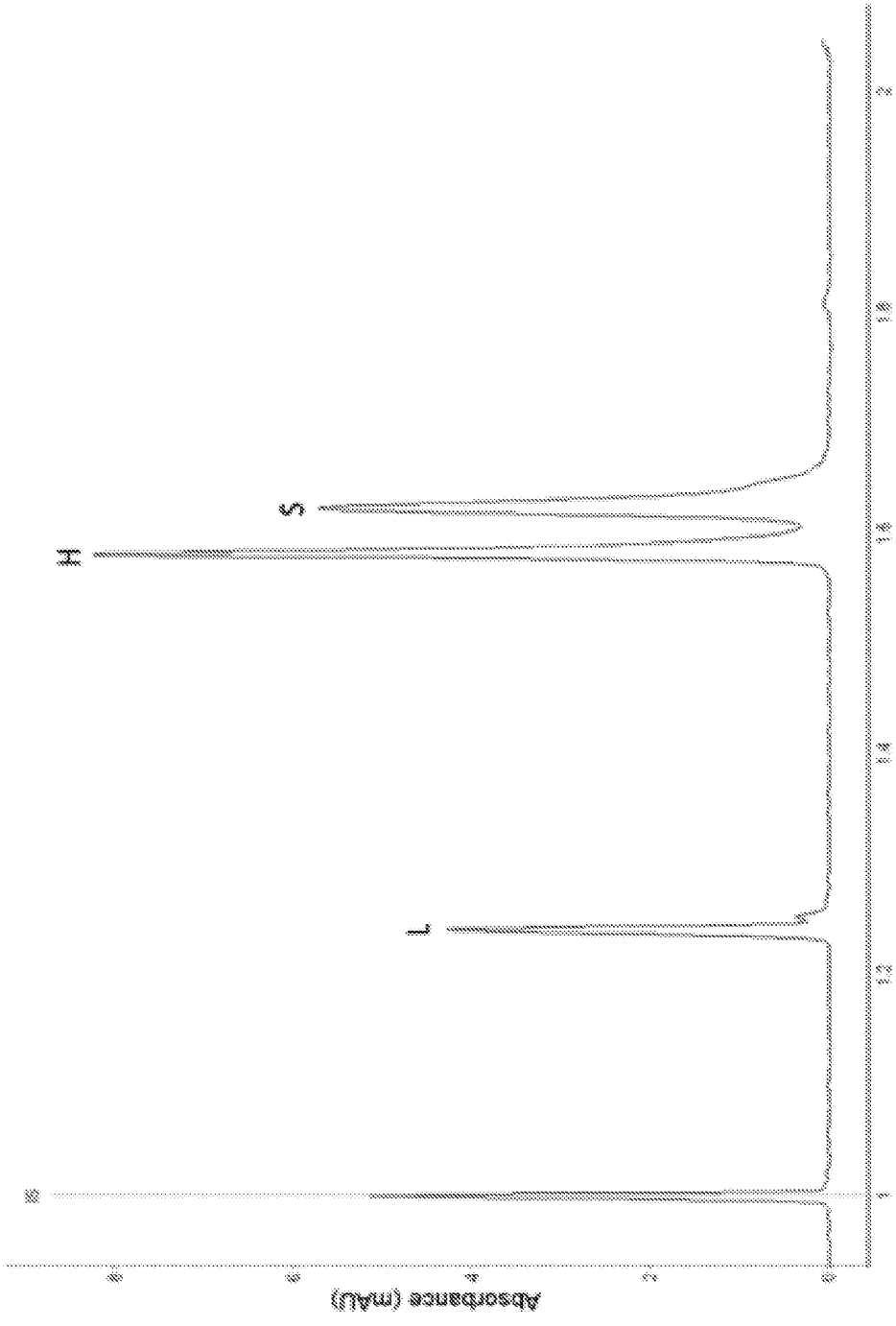


FIG. 128 Continued

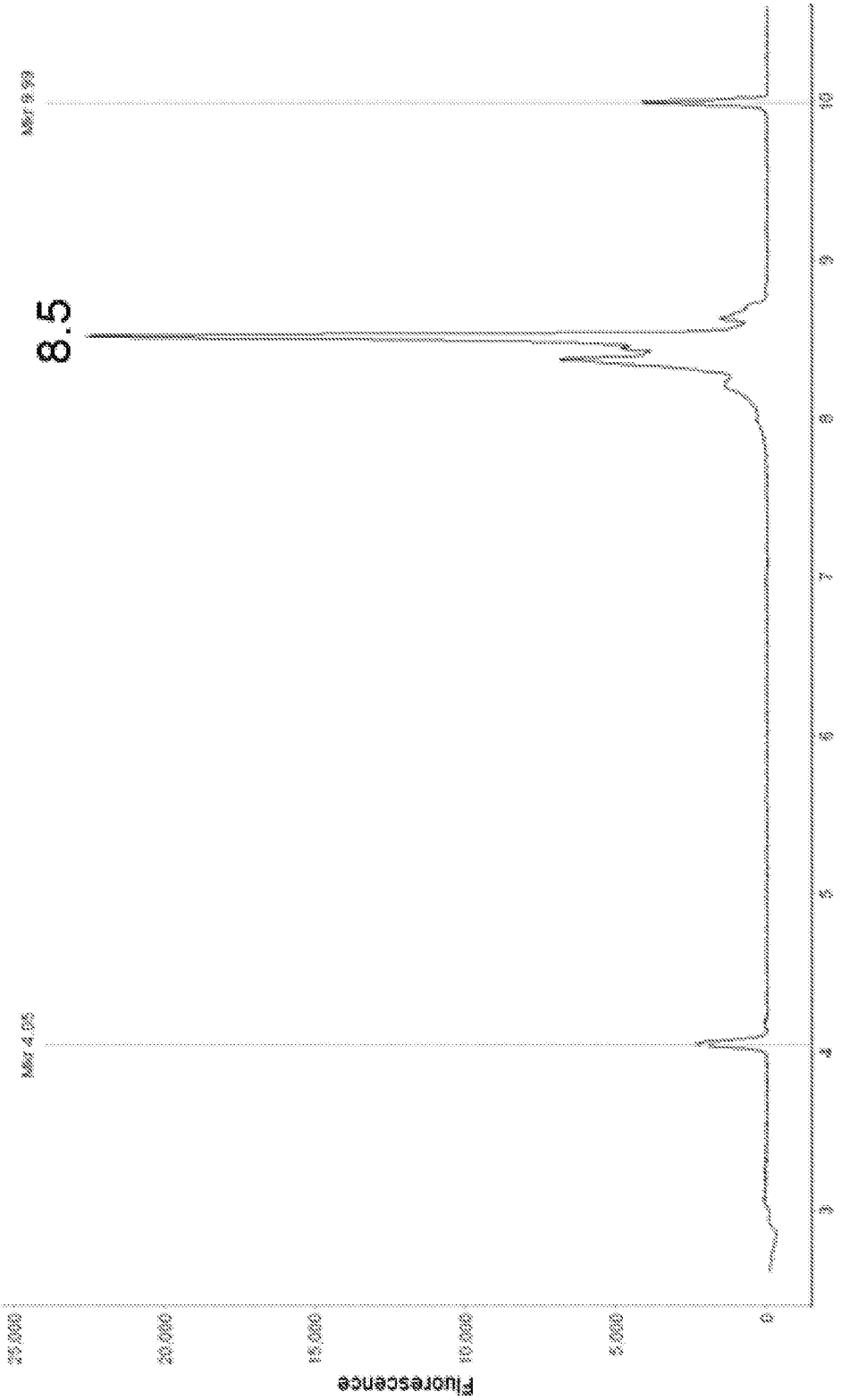

8.5
Mar 9.99
Mar 4.05
Fluorescence
25,000
20,000
15,000
10,000
5,000
0
1
2
3
4
5
6
7
8
9
10
pI

FIG. 129

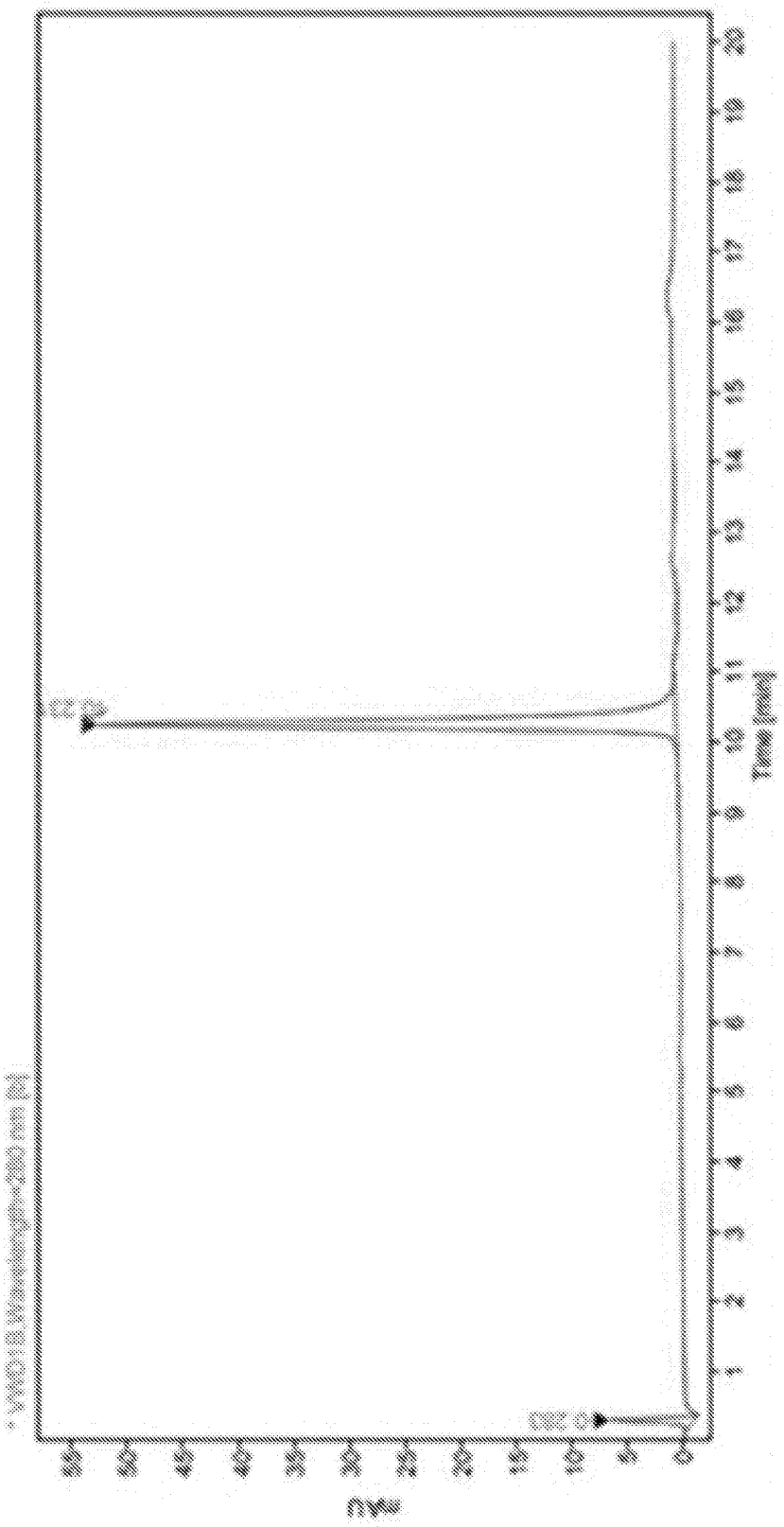


FIG. 130

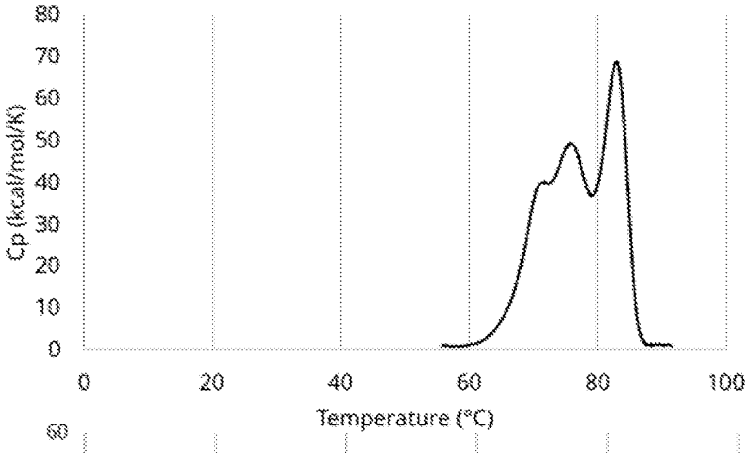


FIG. 131A

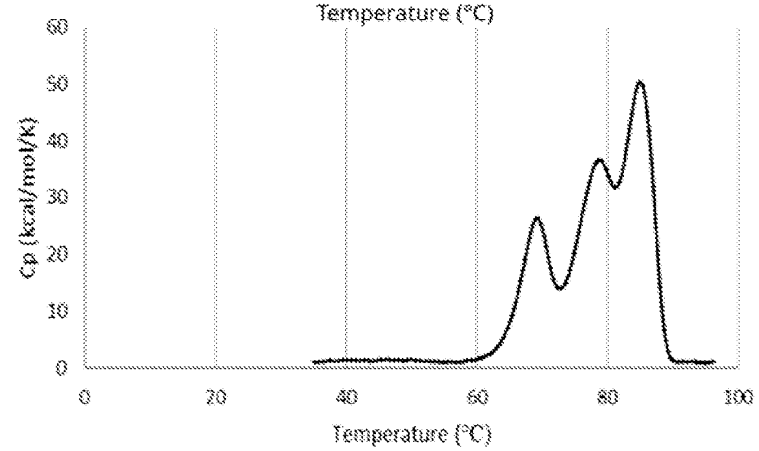


FIG. 131B

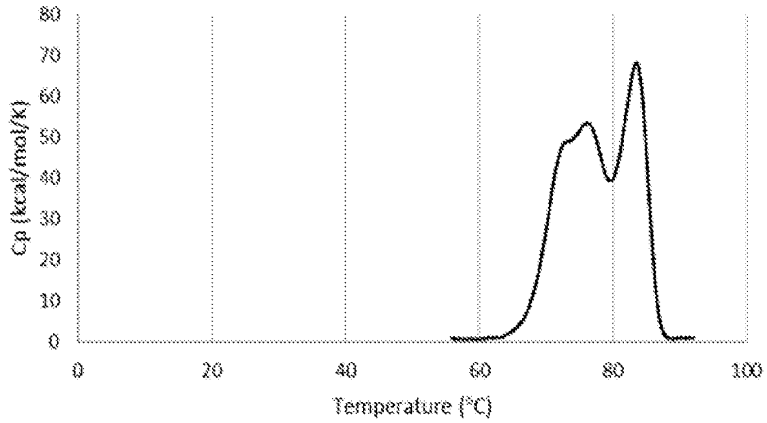
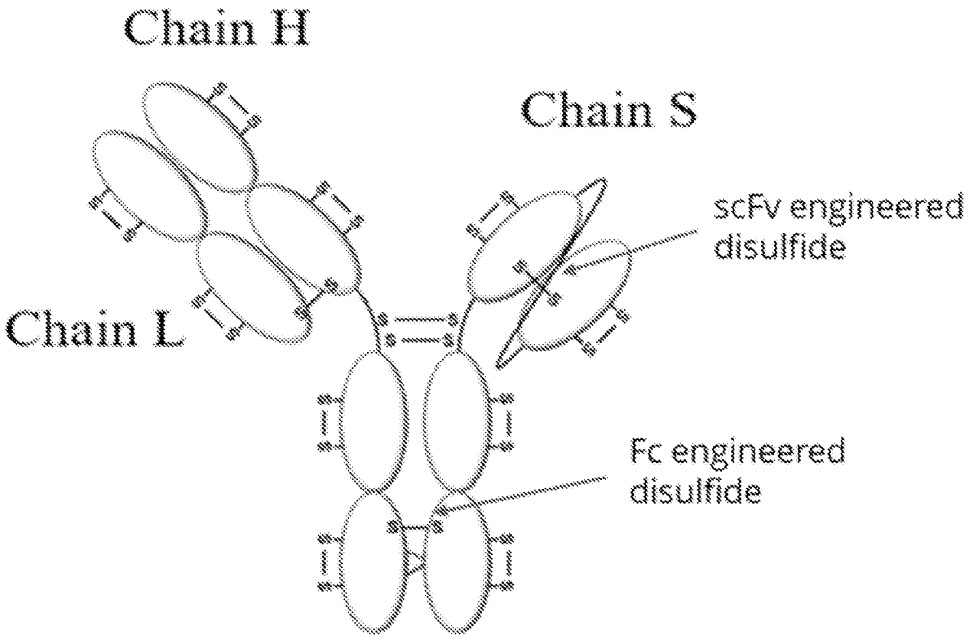


FIG. 131C

FIG. 132


Chain H
Chain S
scFv engineered disulfide
Chain L
Fc engineered disulfide

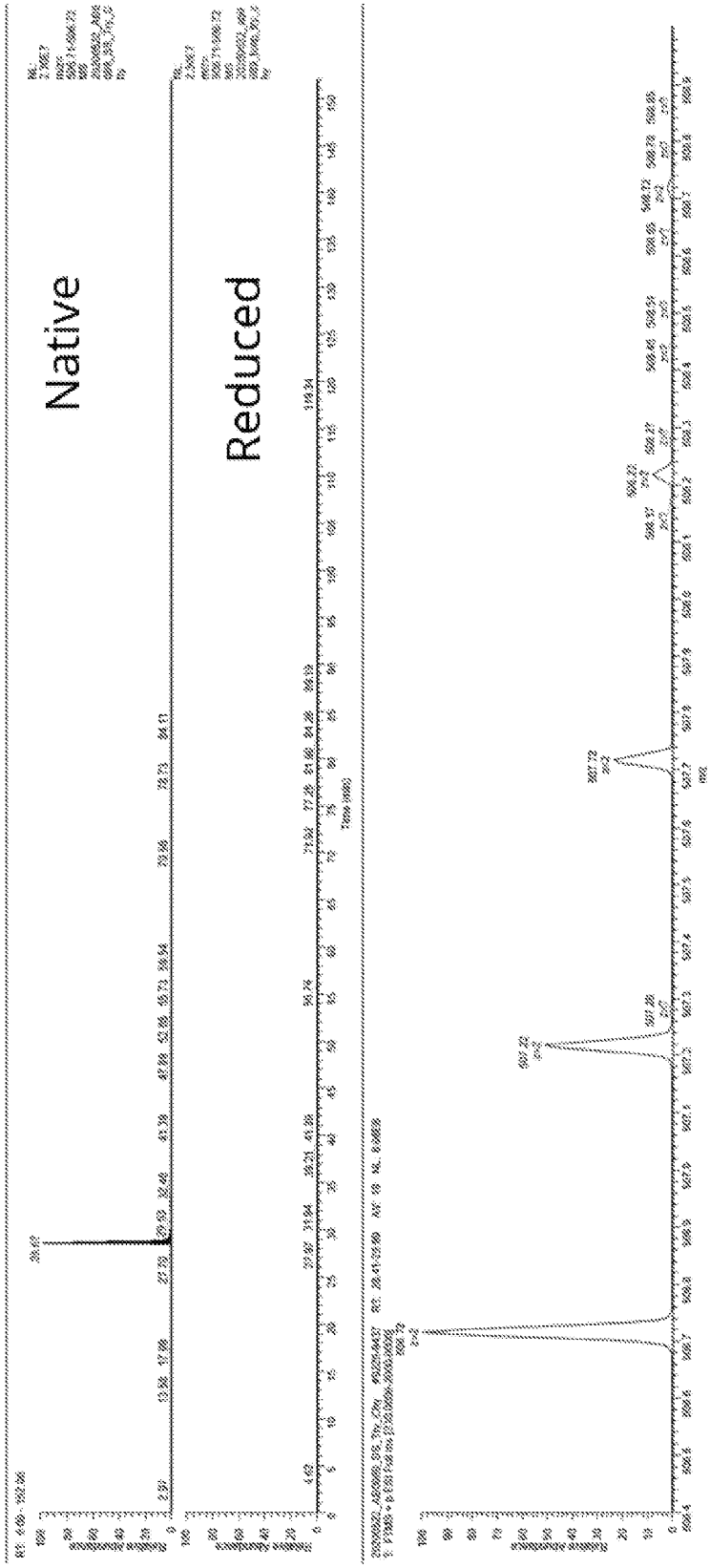


FIG. 133

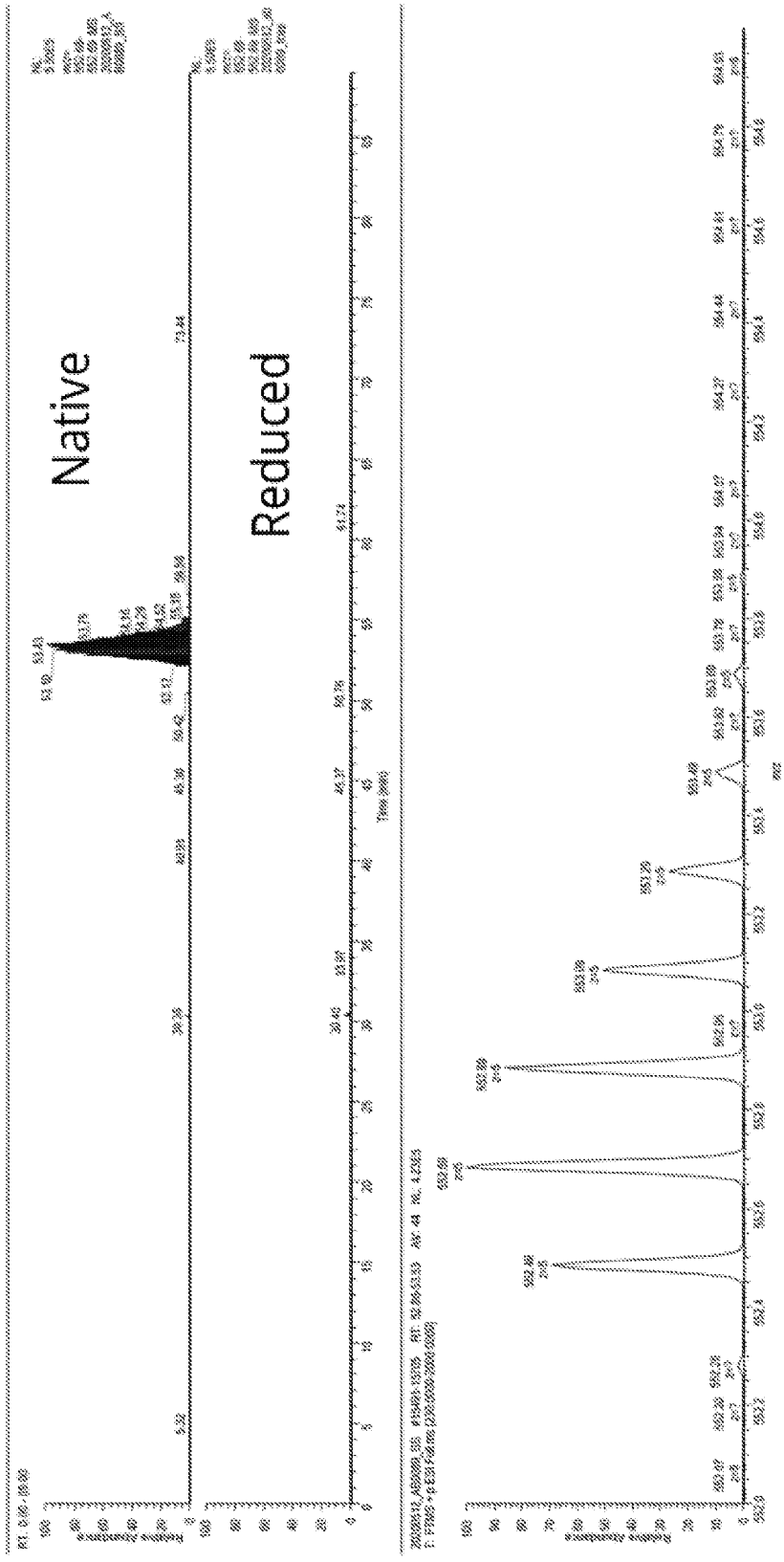
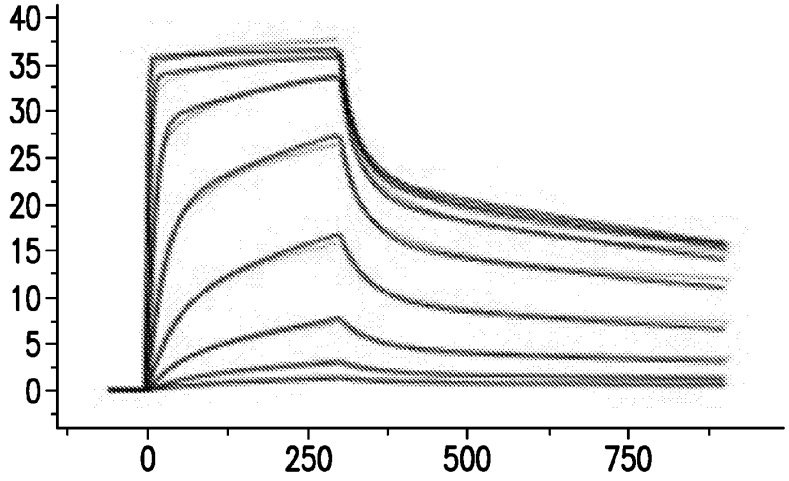


FIG. 134


40
35
30
25
20
15
10
5
0
0
250
500
750
Group 1
hCLEC12A-His; AB0089-002; Two state reaction

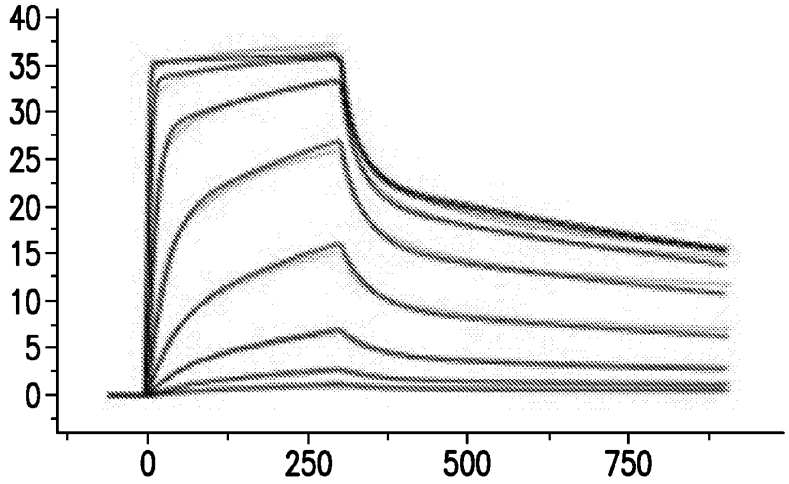
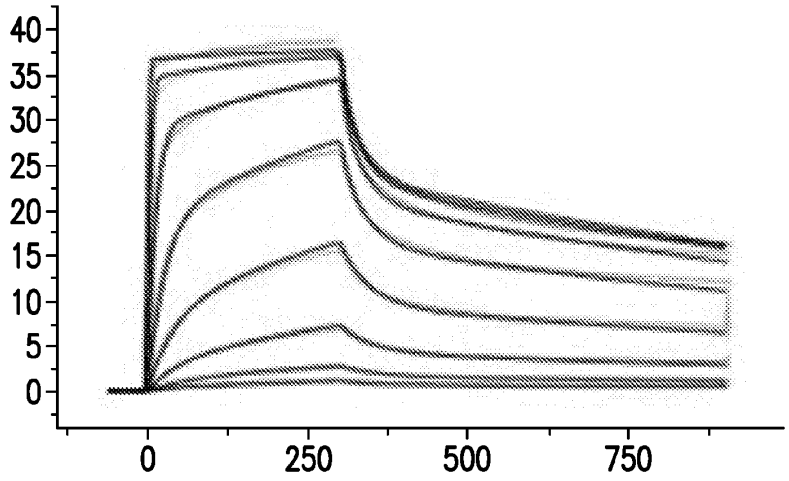

40
35
30
25
20
15
10
5
0
0
250
500
750
Group 2
hCLEC12A-His; AB0089-002; Two state reaction

FIG. 135



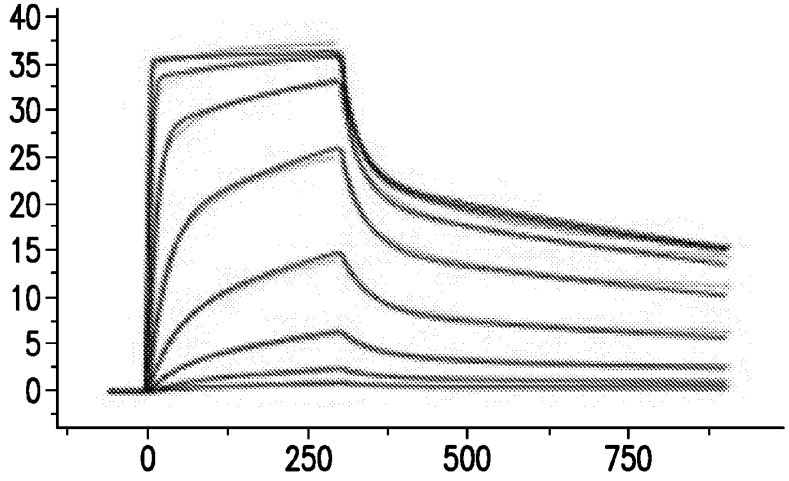
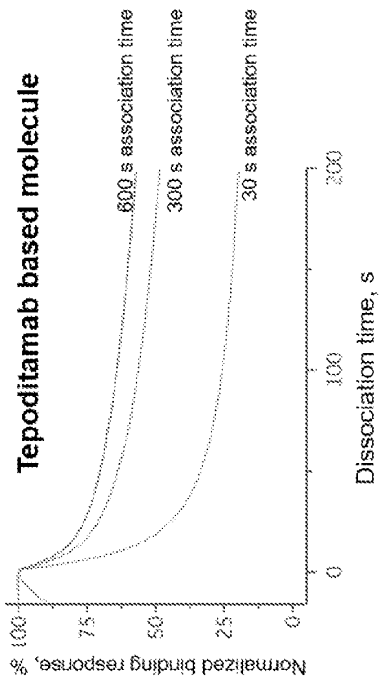


FIG. 135 Continued

FIG. 136


Tepoditamab based molecule
600 s association time
300 s association time
30 s association time
Normalized binding response, %
100
75
50
25
0
0
100
200
300
Dissociation time, s

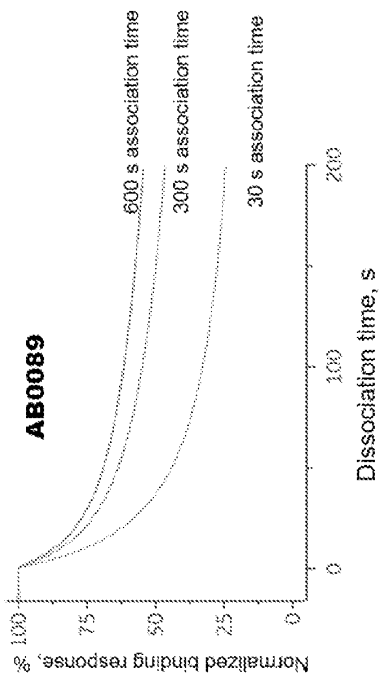
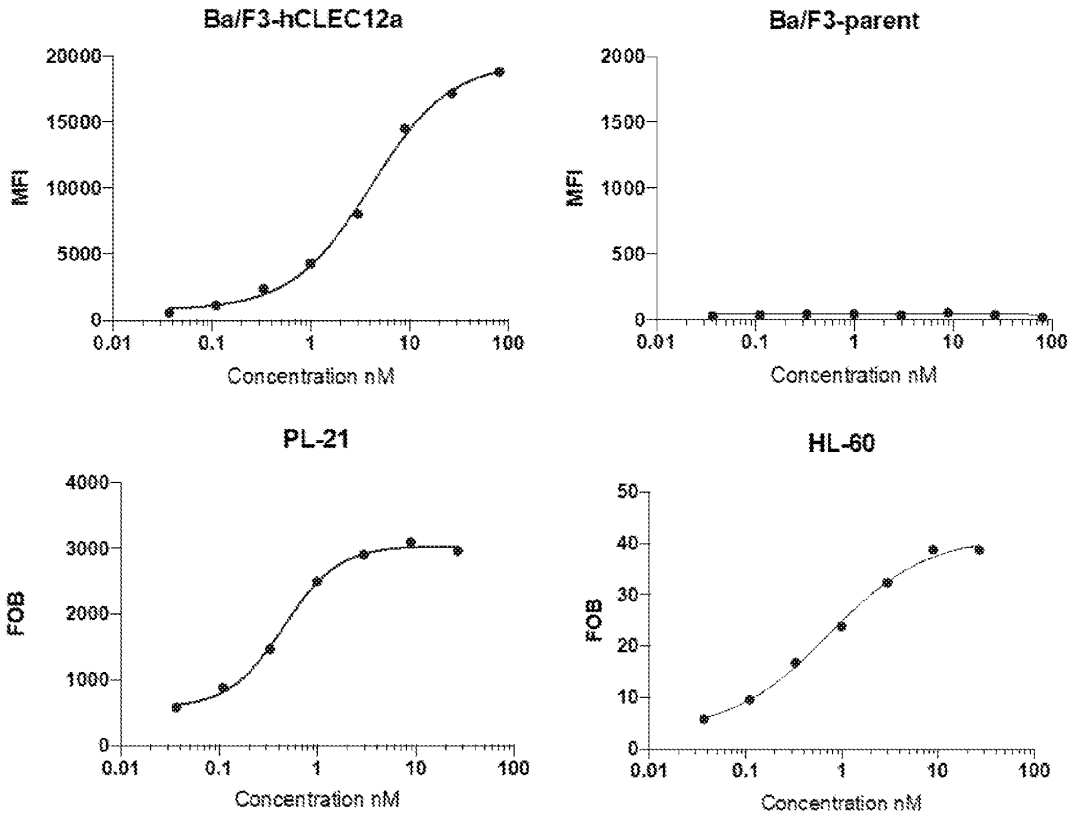
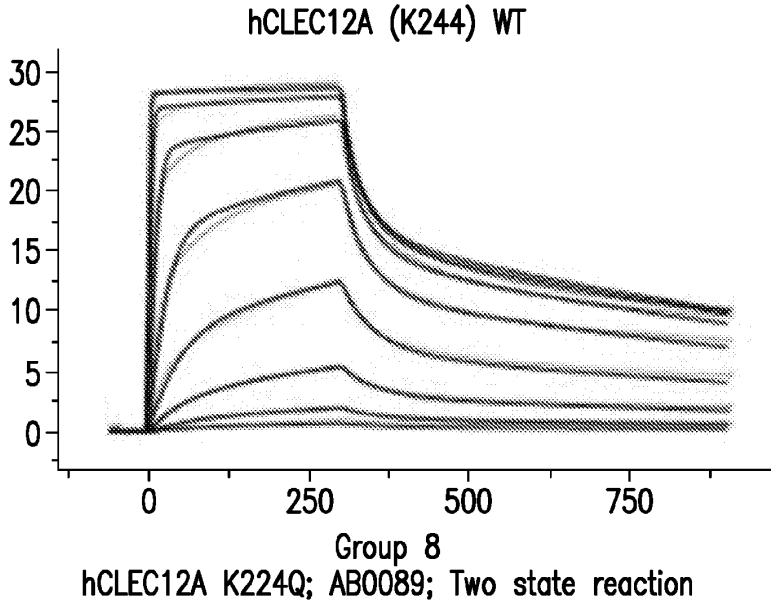

AB0089
600 s association time
300 s association time
30 s association time
Normalized binding response, %
100
75
50
25
0
0
100
200
Dissociation time, s

FIG. 137


Ba/F3-hCLEC12a
MFI
20000
15000
10000
5000
0
0.01
0.1
1
10
100
Concentration nM
Ba/F3-parent
MFI
2000
1500
1000
500
0
0.01
0.1
1
10
100
Concentration nM
PL-21
FOB
4000
3000
2000
1000
0
0.01
0.1
1
10
100
Concentration nM
HL-60
FOB
50
40
30
20
10
0
0.01
0.1
1
10
100
Concentration nM


hCLEC12A (K244) WT
30
25
20
15
10
5
0
0
250
500
750
Group 8
hCLEC12A K224Q; AB0089; Two state reaction

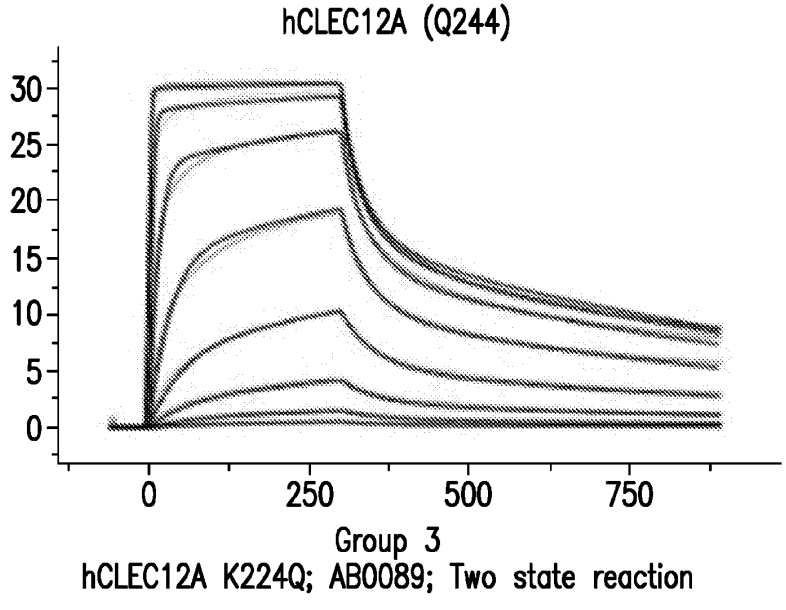

hCLEC12A (Q244)
30
25
20
15
10
5
0
0
250
500
750
Group 3
hCLEC12A K224Q; AB0089; Two state reaction

FIG. 138

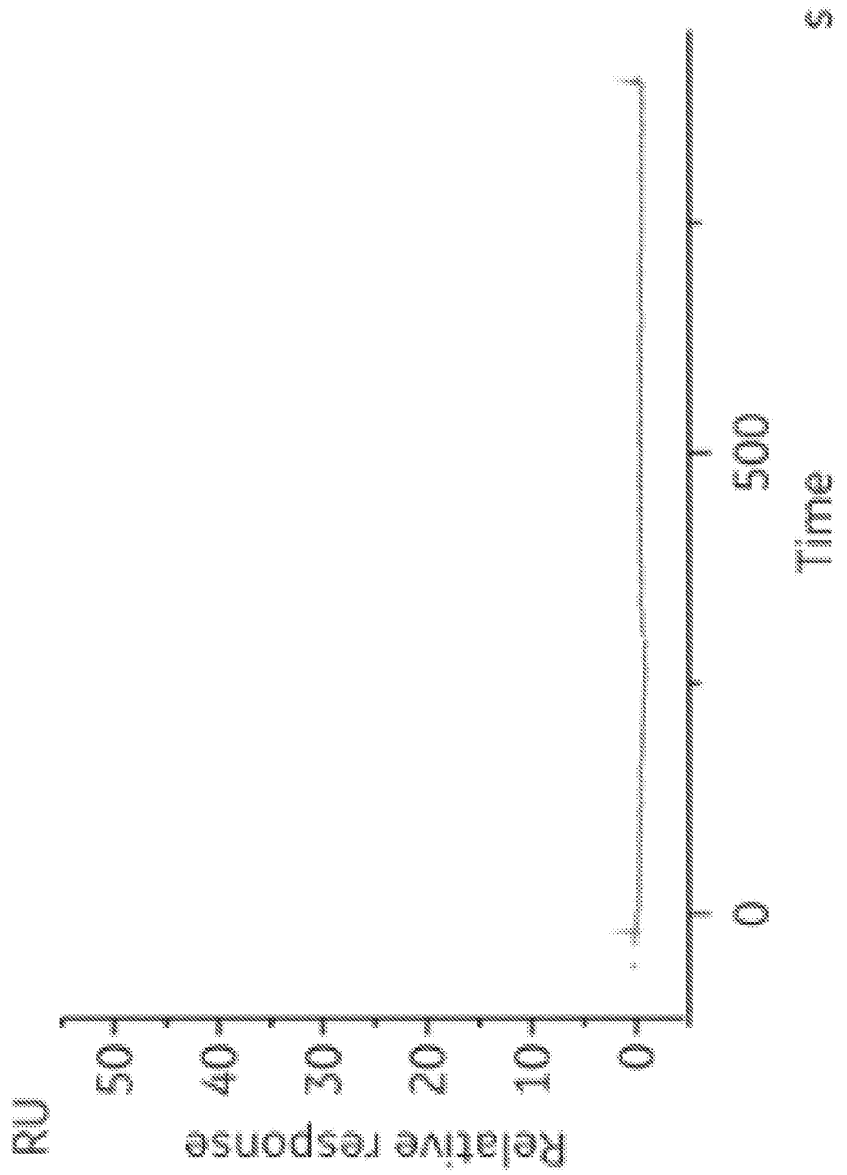
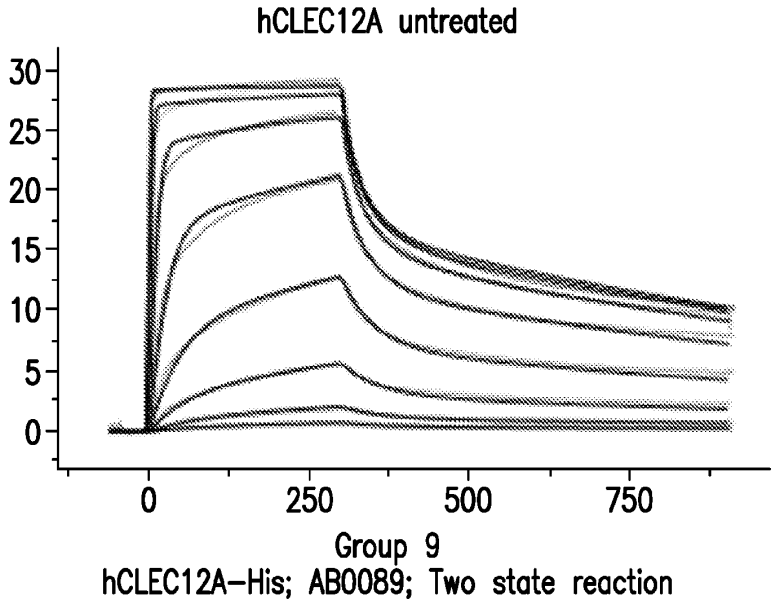


FIG. 138 Continued


hCLEC12A untreated
30
25
20
15
10
5
0
0
250
500
750
Group 9
hCLEC12A–His; AB0089; Two state reaction

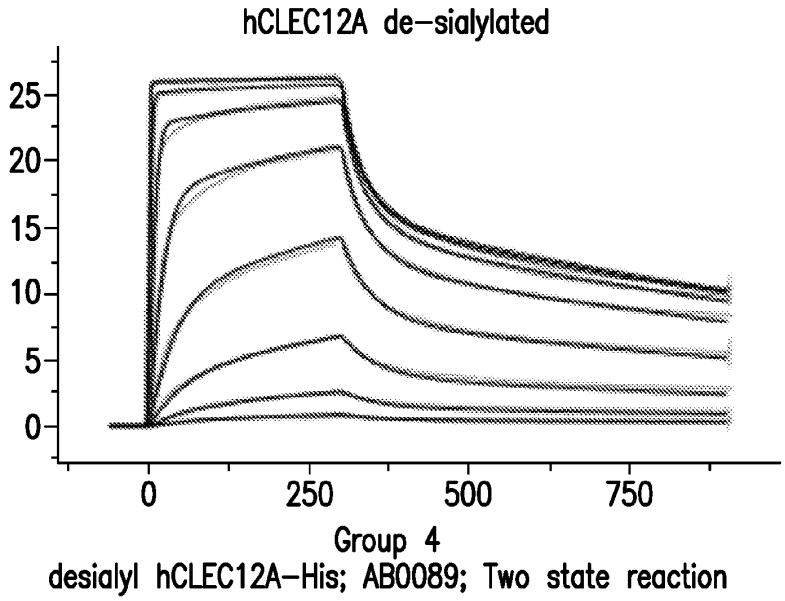

hCLEC12A de–sialylated
25
20
15
10
5
0
0
250
500
750
Group 4
desialyl hCLEC12A–His; AB0089; Two state reaction

FIG. 139

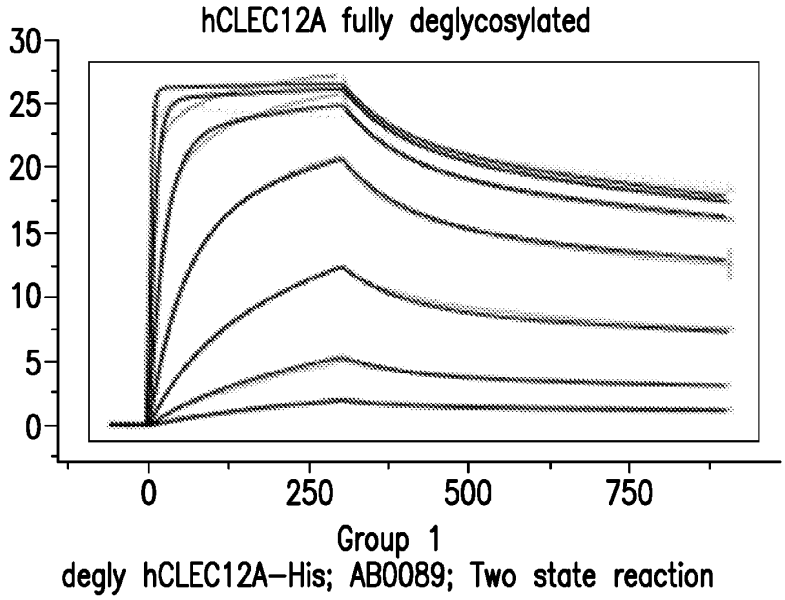
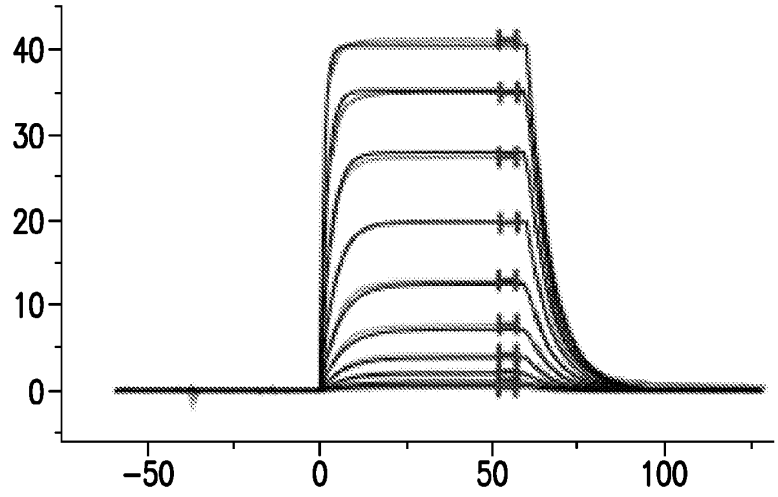
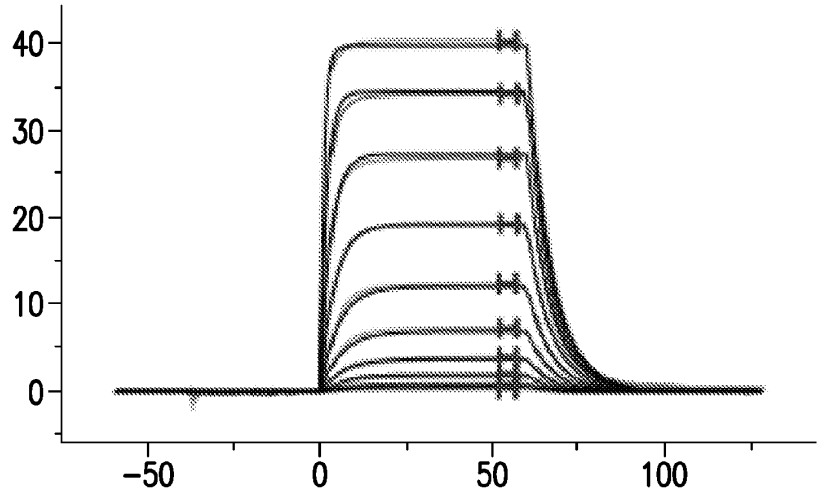


FIG. 139 Continued

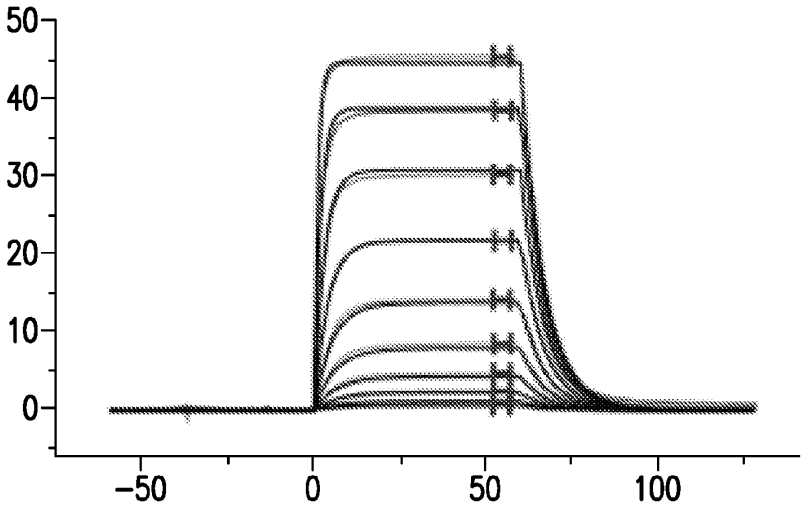


Group 16
AB0089-002; mFc-hNKG2D; 1:1 binding

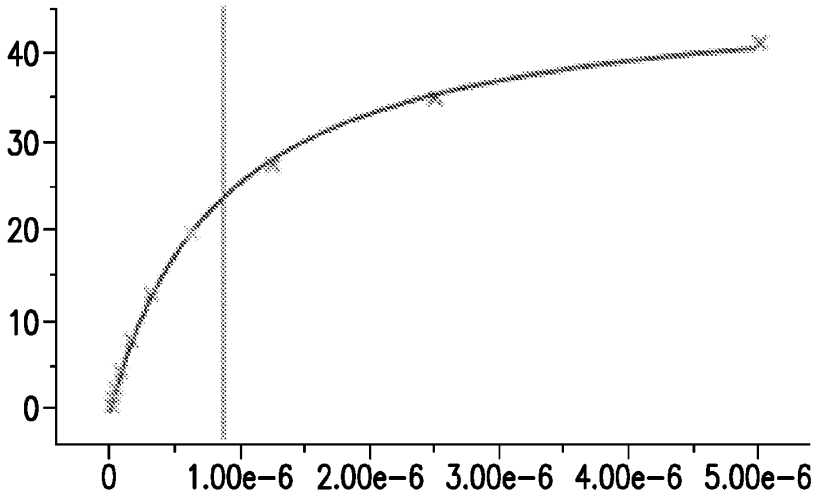


Group 17
AB0089-002; mFc-hNKG2D; 1:1 binding

FIG. 140

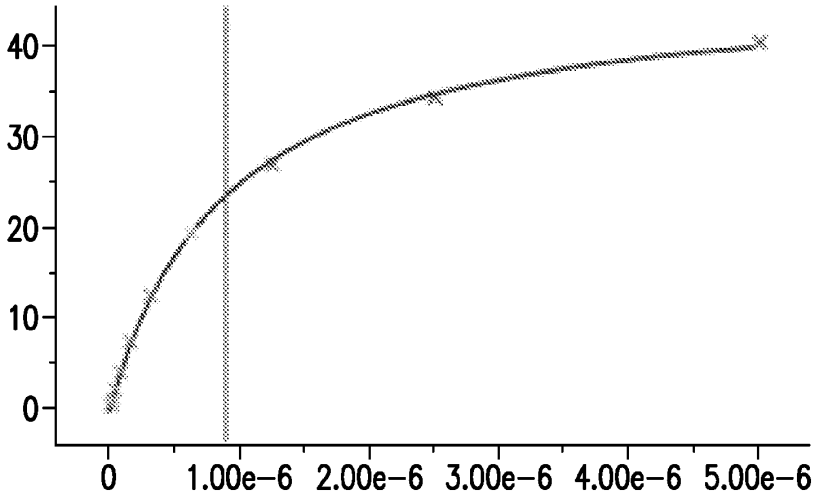


Group 18
AB0089−002; mFc−hNKG2D; 1:1 binding

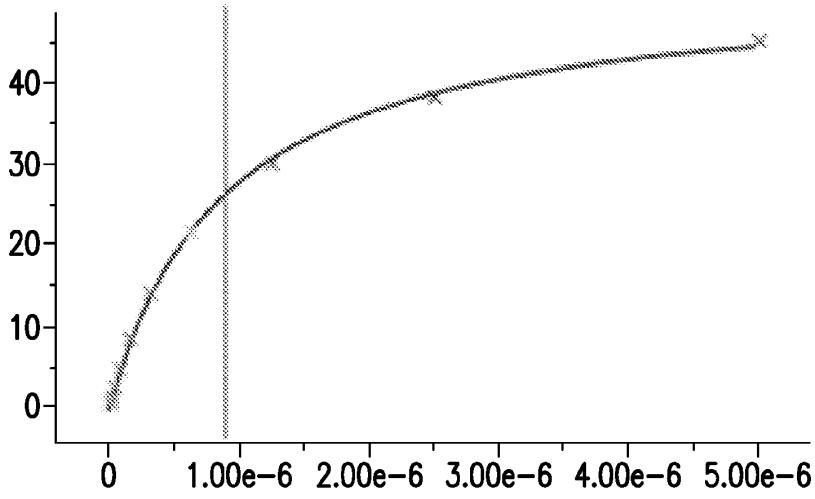


Group 16
AB0089−002; mFc−hNKG2D; Steady state affinity

FIG. 140 Continued

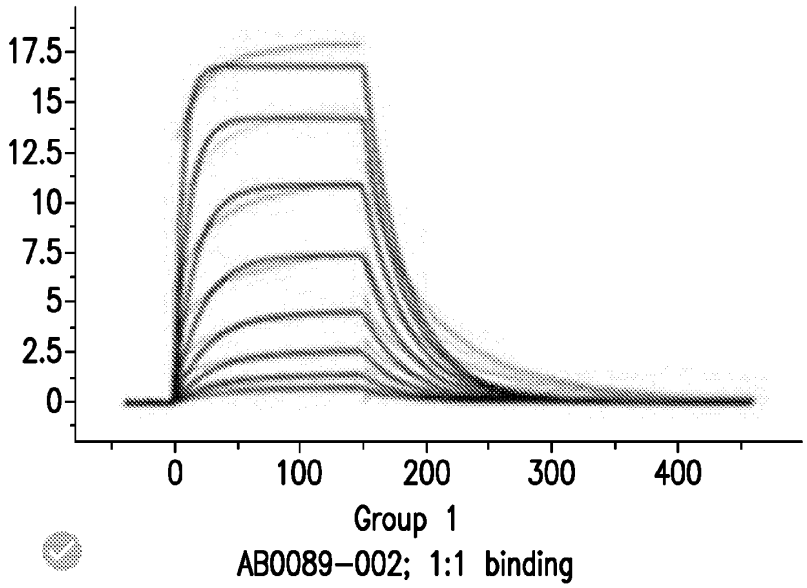


Group 17
AB0089-002; mFc-hNKG2D; Steady state affinity



Group 18
AB0089-002; mFc-hNKG2D; Steady state affinity

FIG. 140 Continued


17.5
15
12.5
10
7.5
5
2.5
0
0
100
200
300
400
Group 1
AB0089-002; 1:1 binding

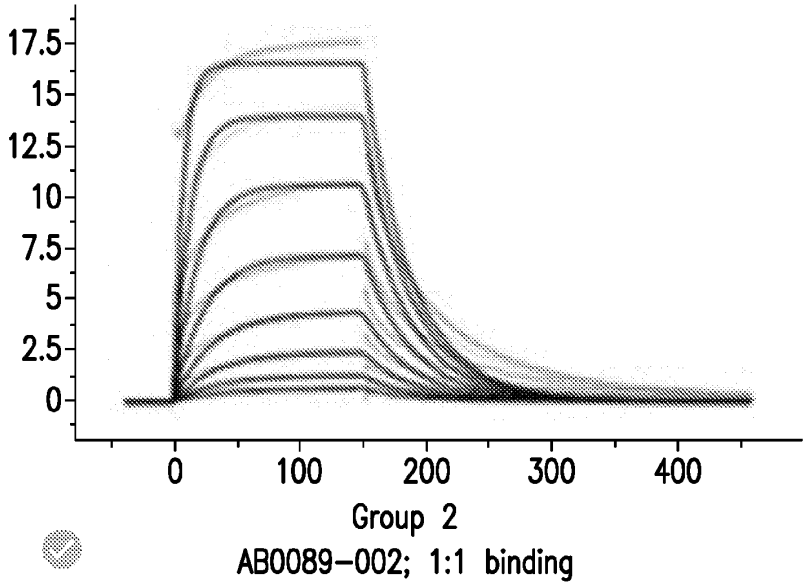
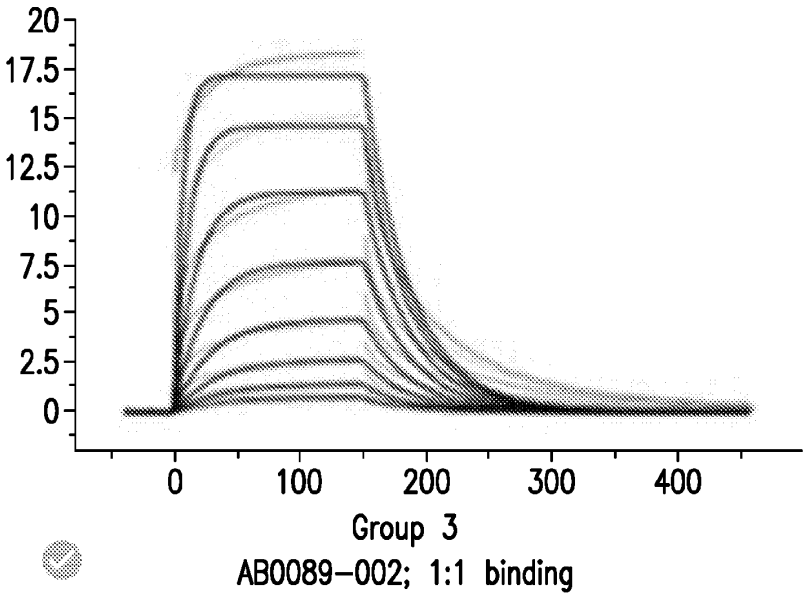

17.5
15
12.5
10
7.5
5
2.5
0
0
100
200
300
400
Group 2
AB0089-002; 1:1 binding

FIG. 141



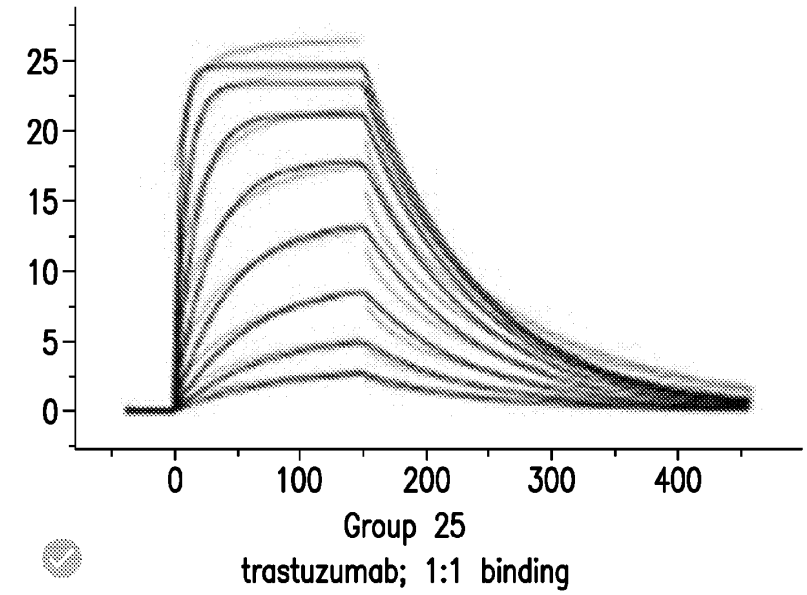
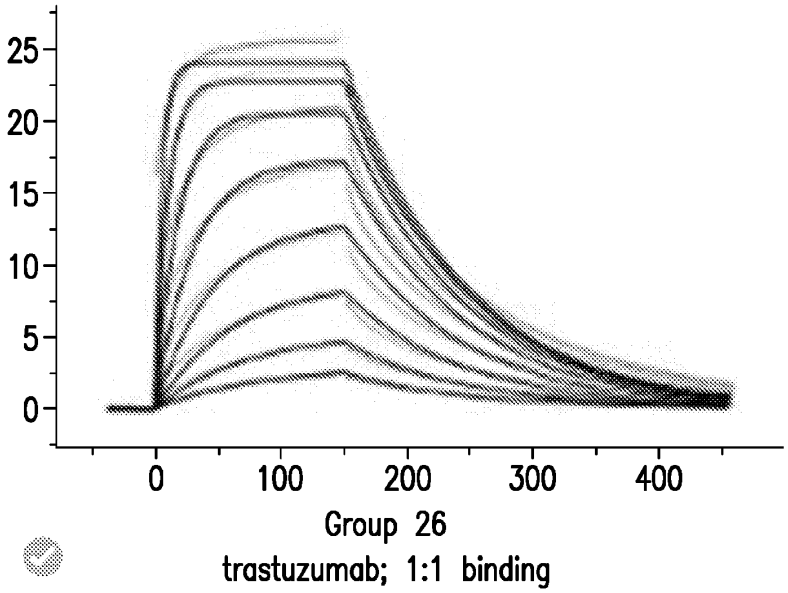


FIG. 141 Continued



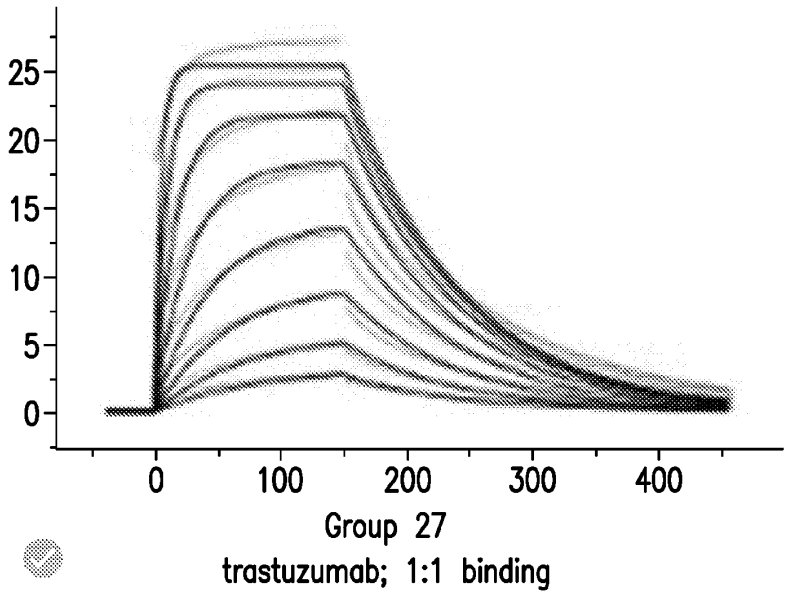
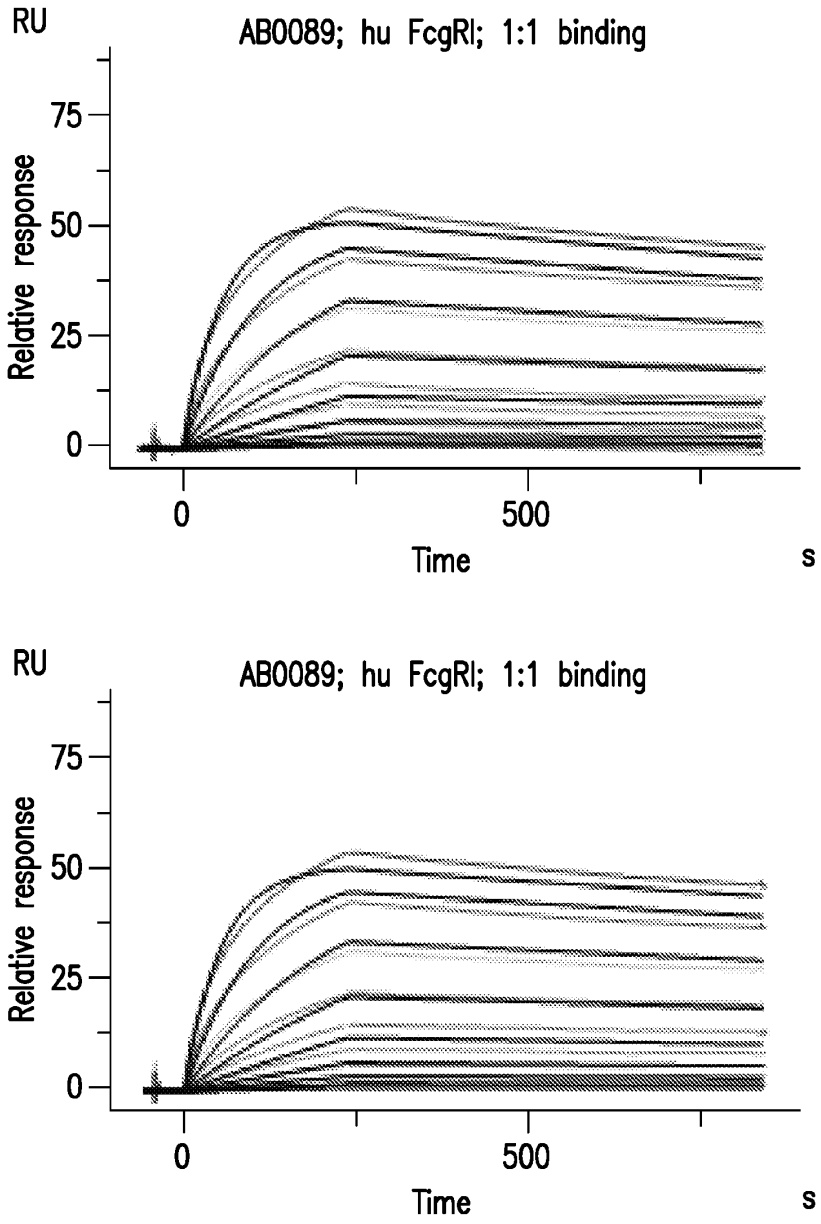


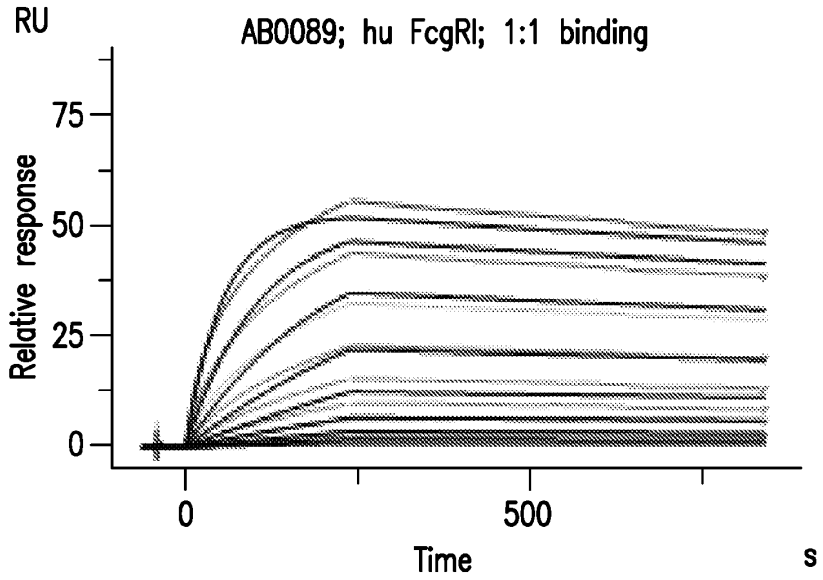
FIG. 141 Continued

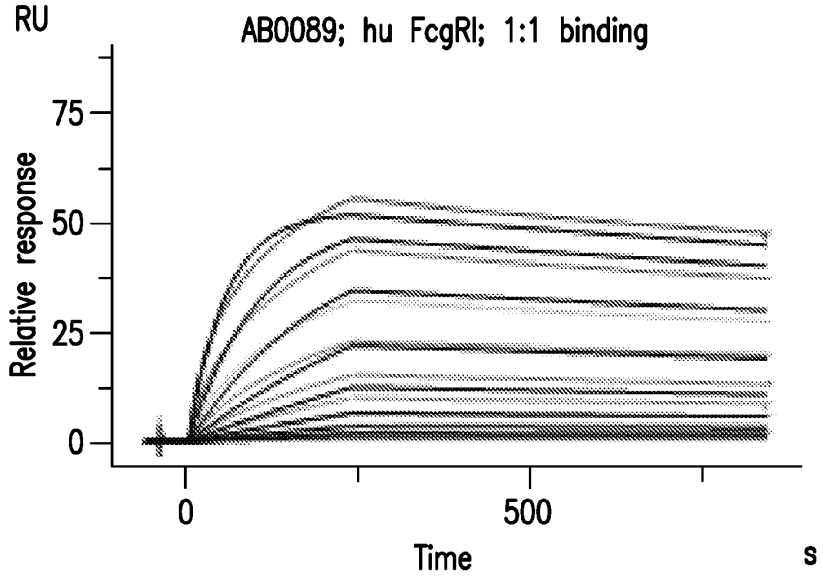


AB0089

$K_D = 5.1 \pm 1.0$ nM

FIG. 142

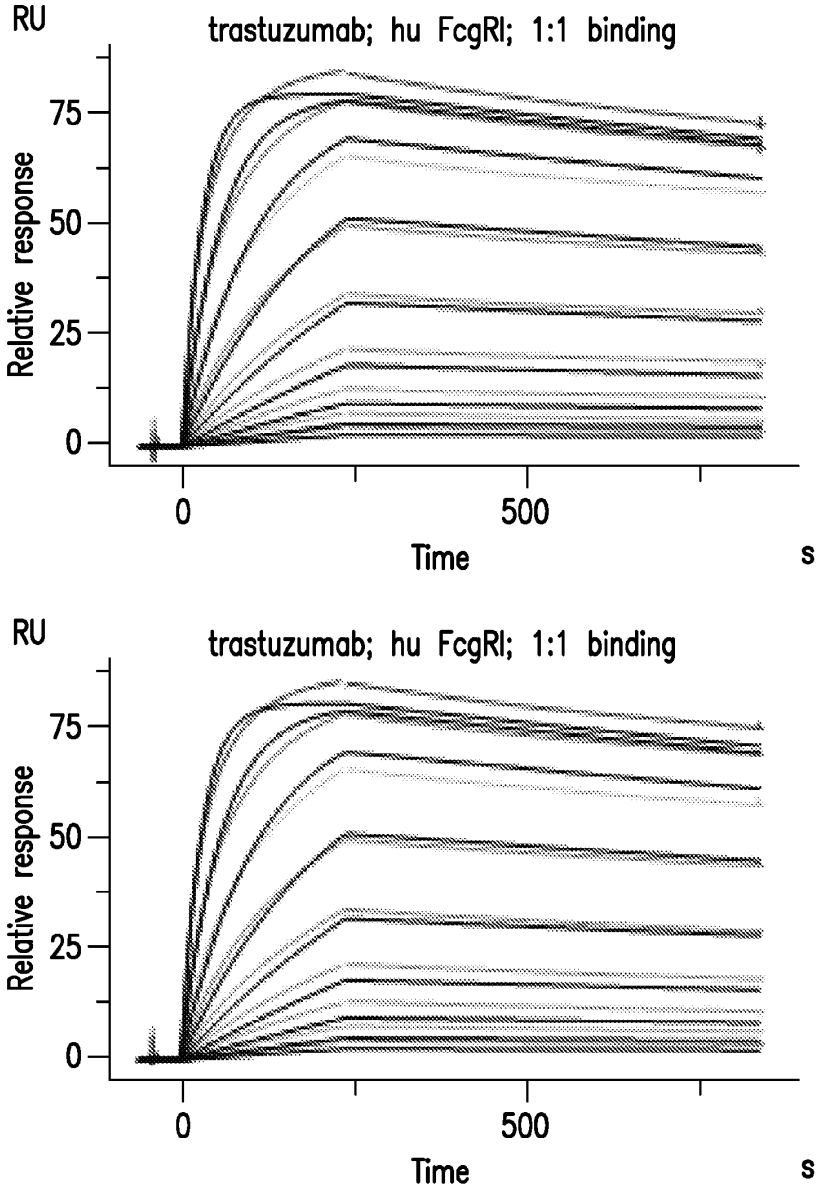




AB0089

$K_D = 5.1 \pm 1.0$ nM

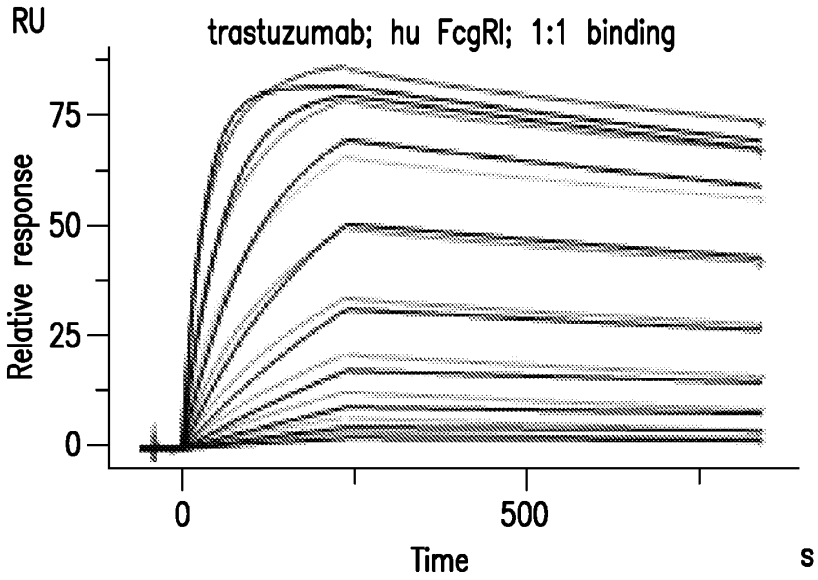
FIG. 142 Continued

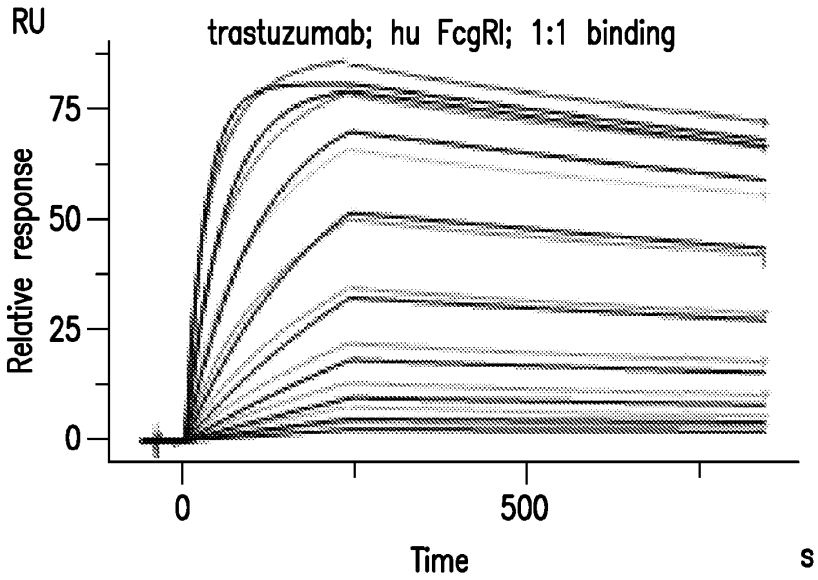


trastuzumab

$K_D = 2.9 \pm 0.4$ nM

FIG. 142 Continued

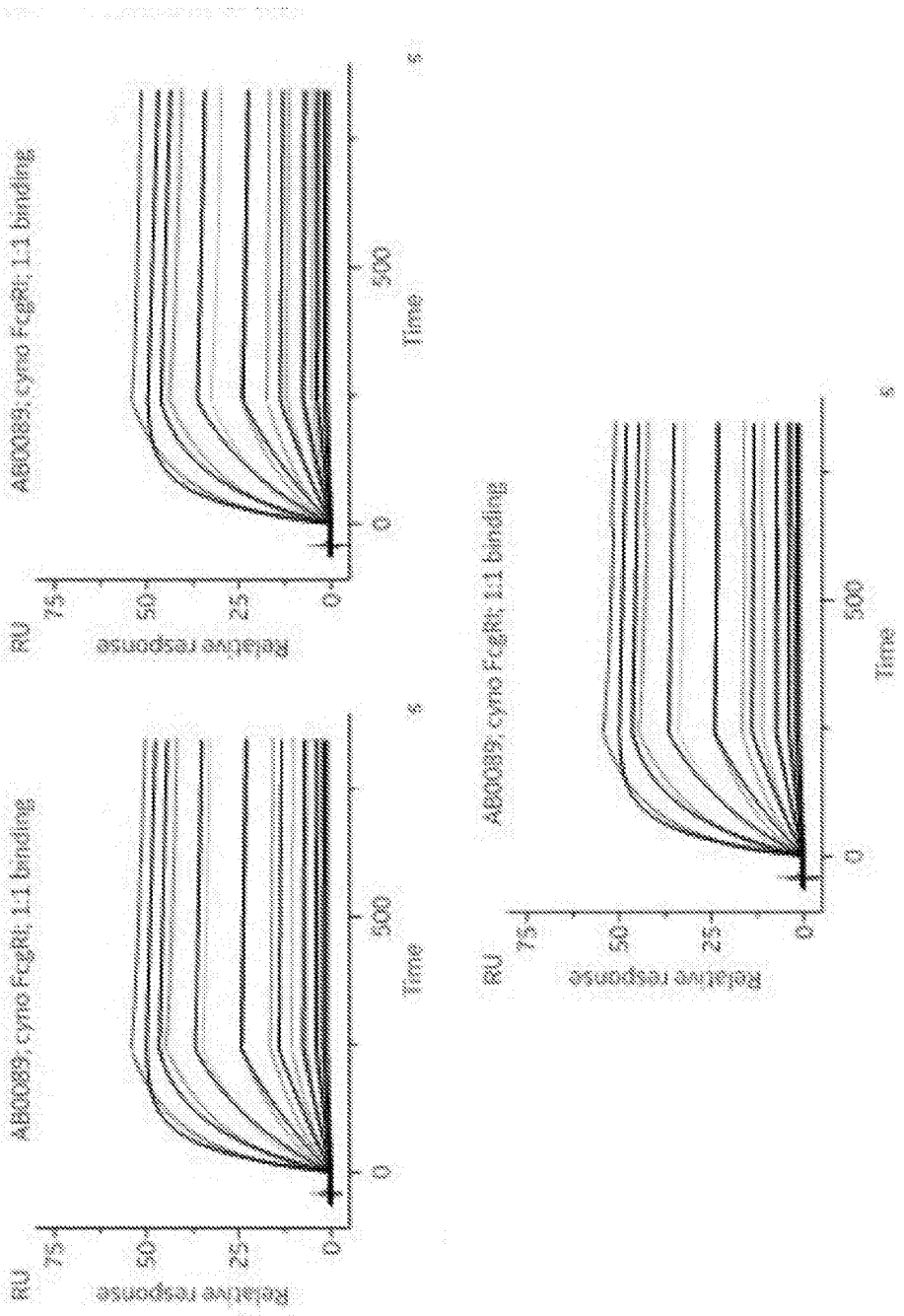




trastuzumab

$K_D = 2.9 \pm 0.4$ nM

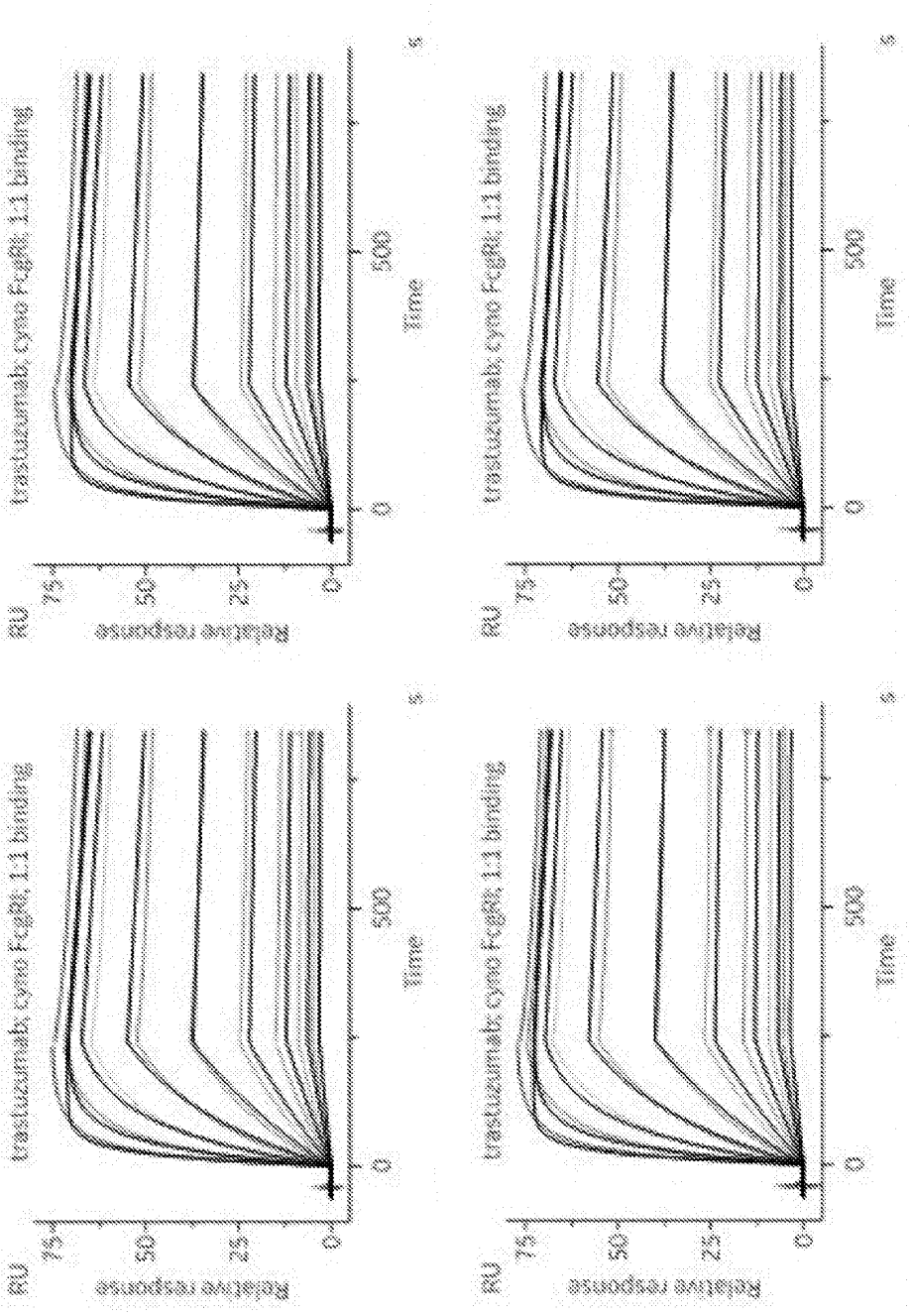
FIG. 142 Continued



AB0089

K_D = 1.5 ± 0.3 nM

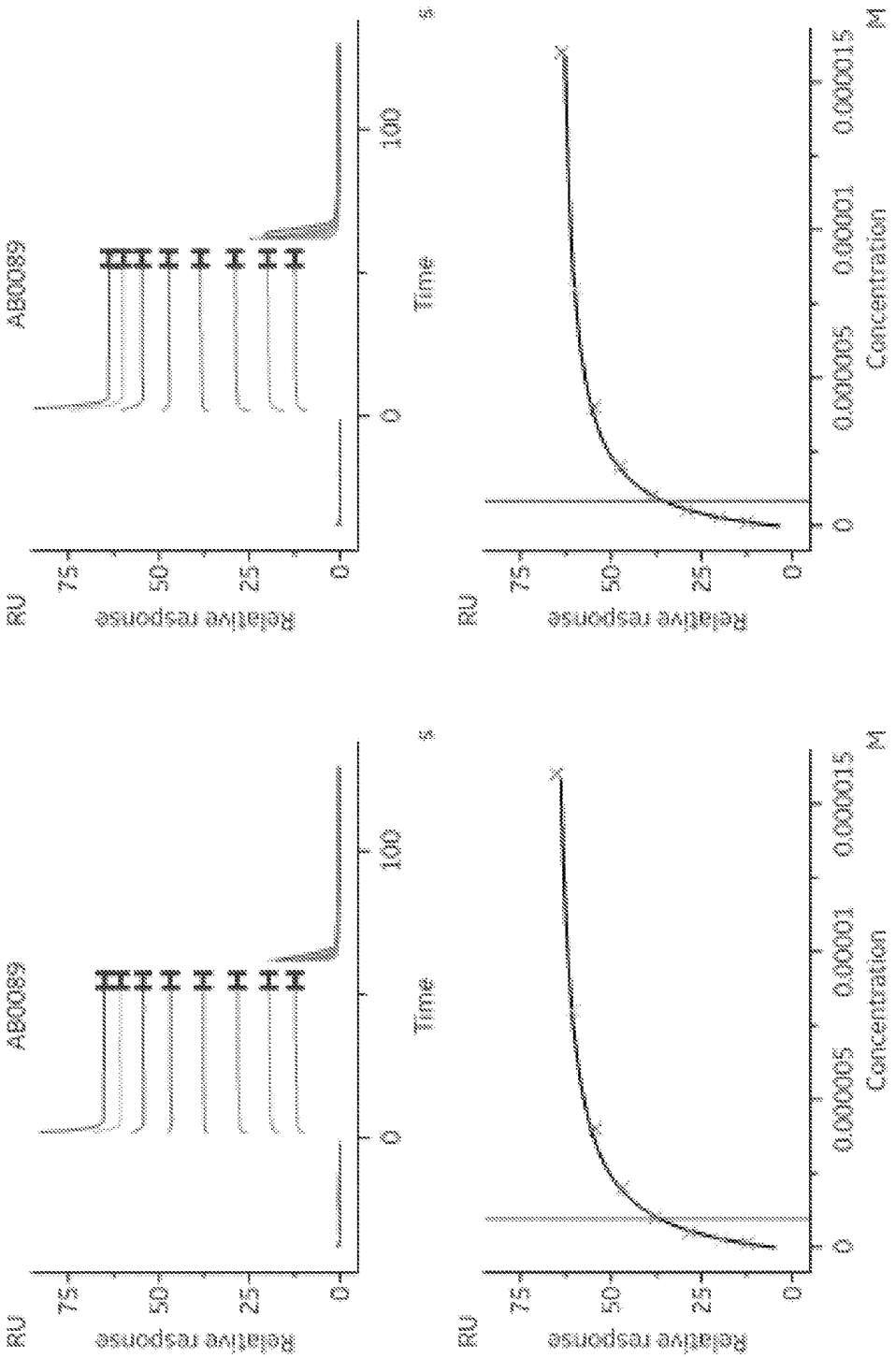
FIG. 143



trastuzumab

K_D = 1.0 ± 0.1 nM

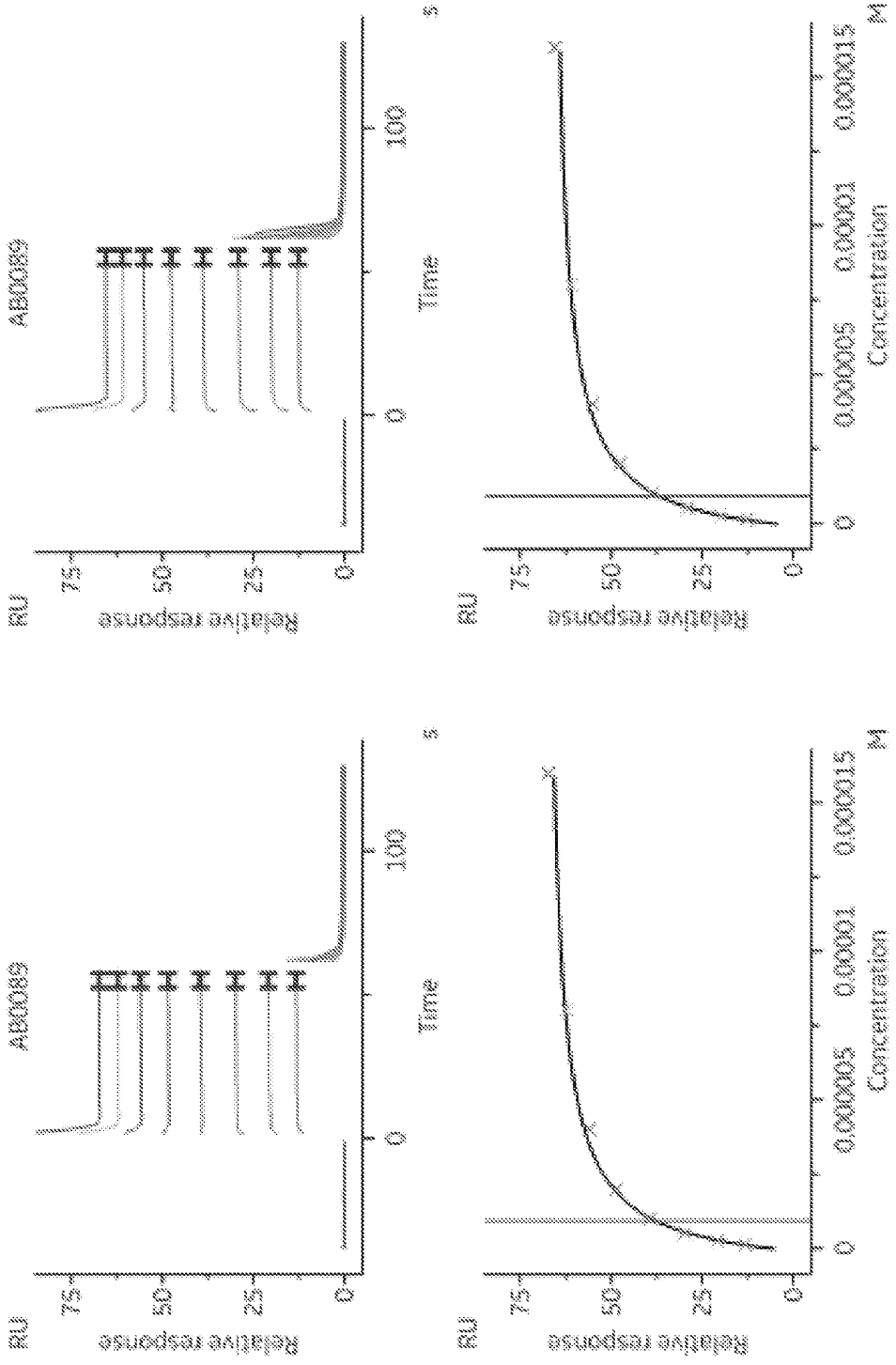
FIG. 143 Continued



AB0089

K_D = 900.4 ± 57.1 nM

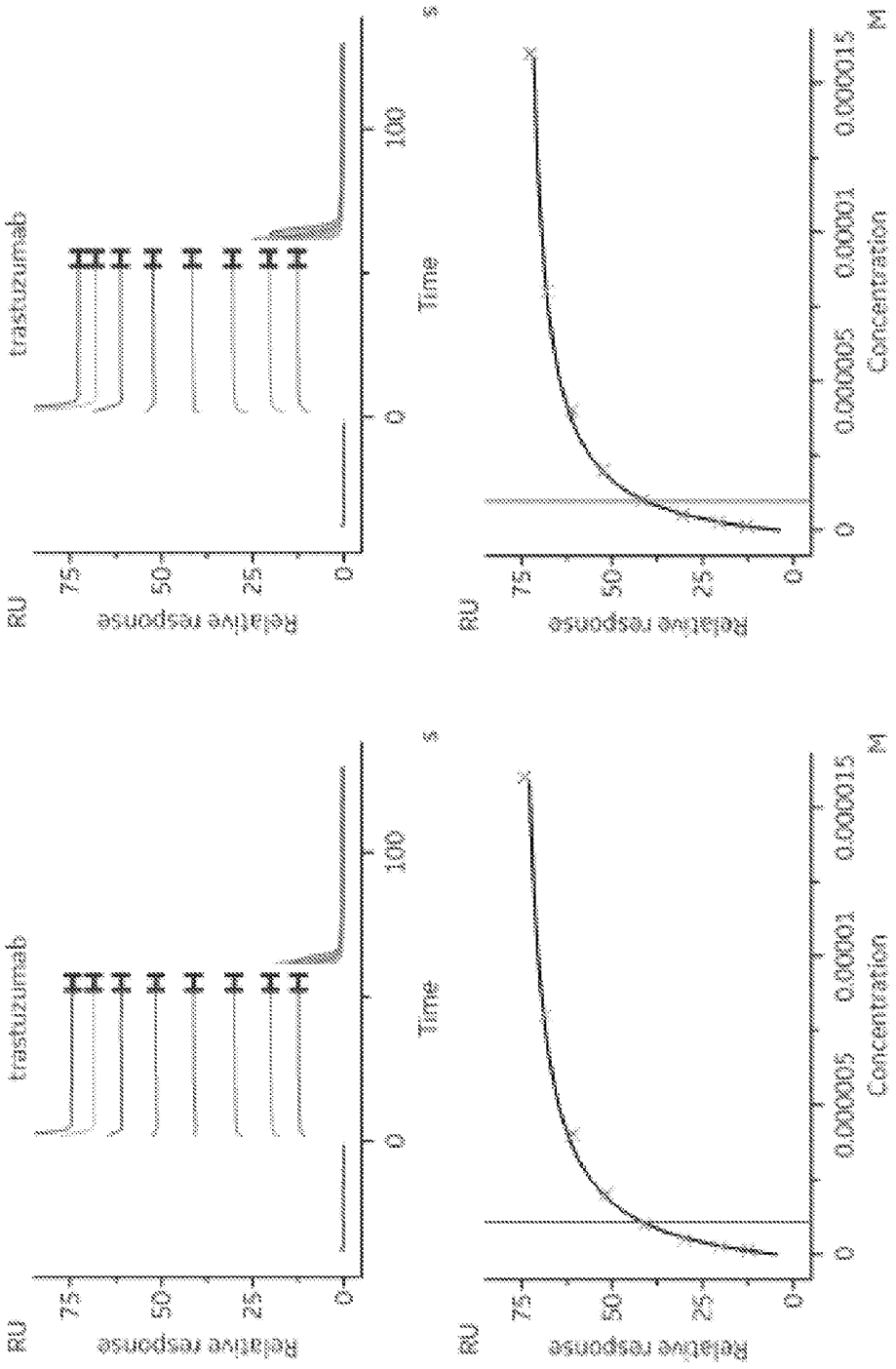
FIG. 143 Continued



AB0089

K_D = 900.4 ± 57.1 nM

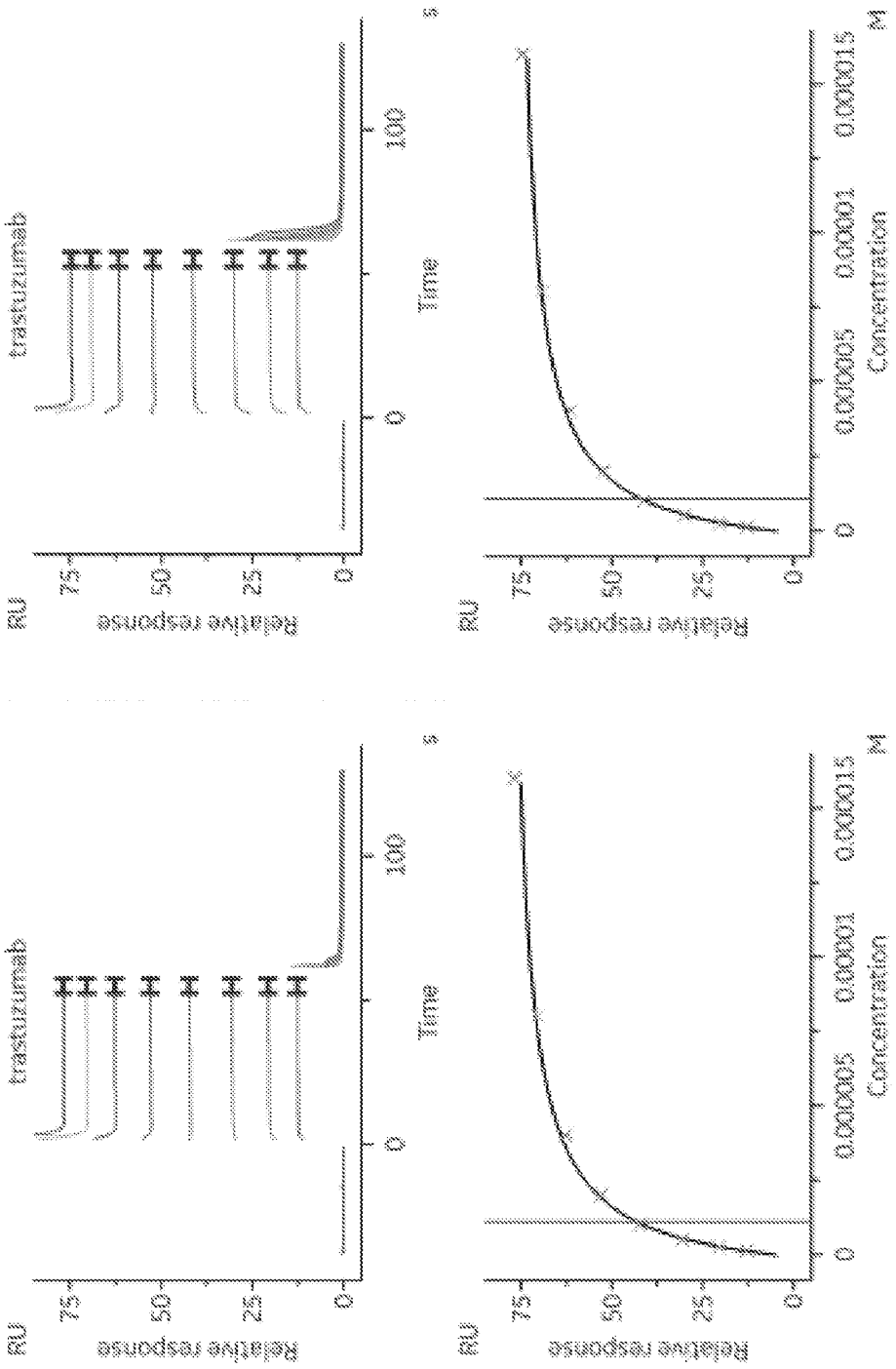
FIG. 143 Continued



trastuzumab

K_D = 1045.1 ± 62.2 nM

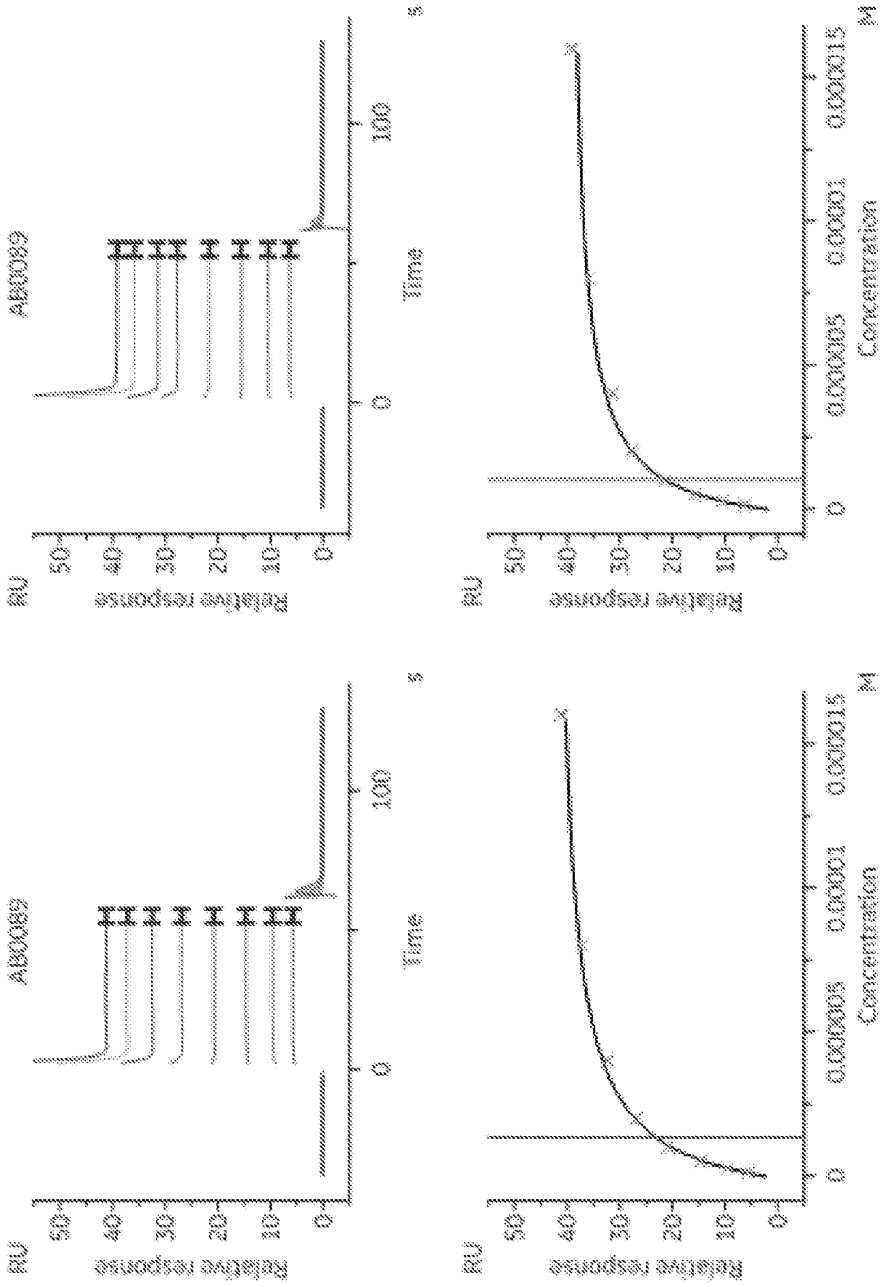
FIG. 144



trastuzumab

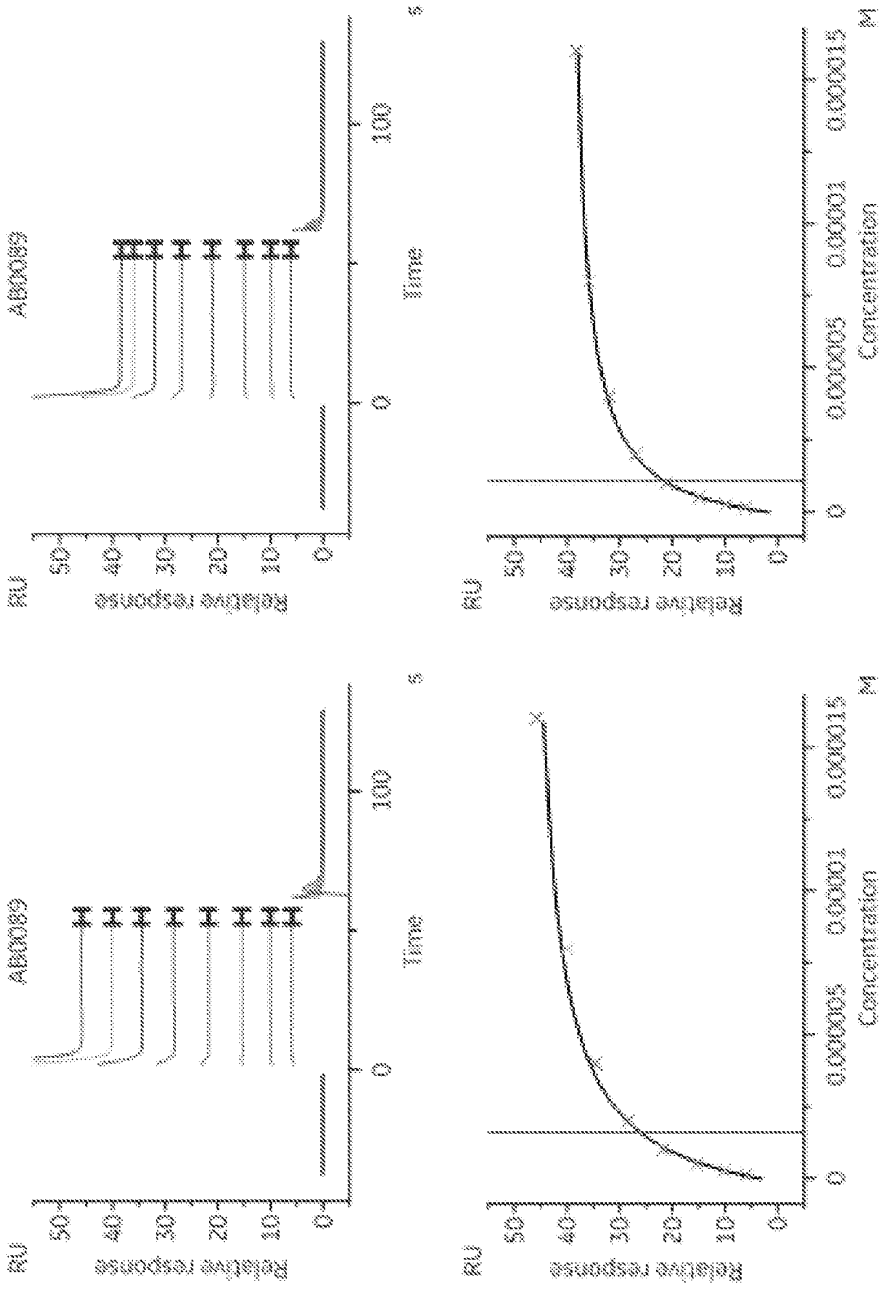
K_D = 1045.1 ± 62.2 nM

FIG. 144 Continued



AB0089

K_D = 1253.3 ± 269.2 nM FIG. 145



AB0089

K_D = 1253.3 ± 269.2 nM FIG. 145 Continued

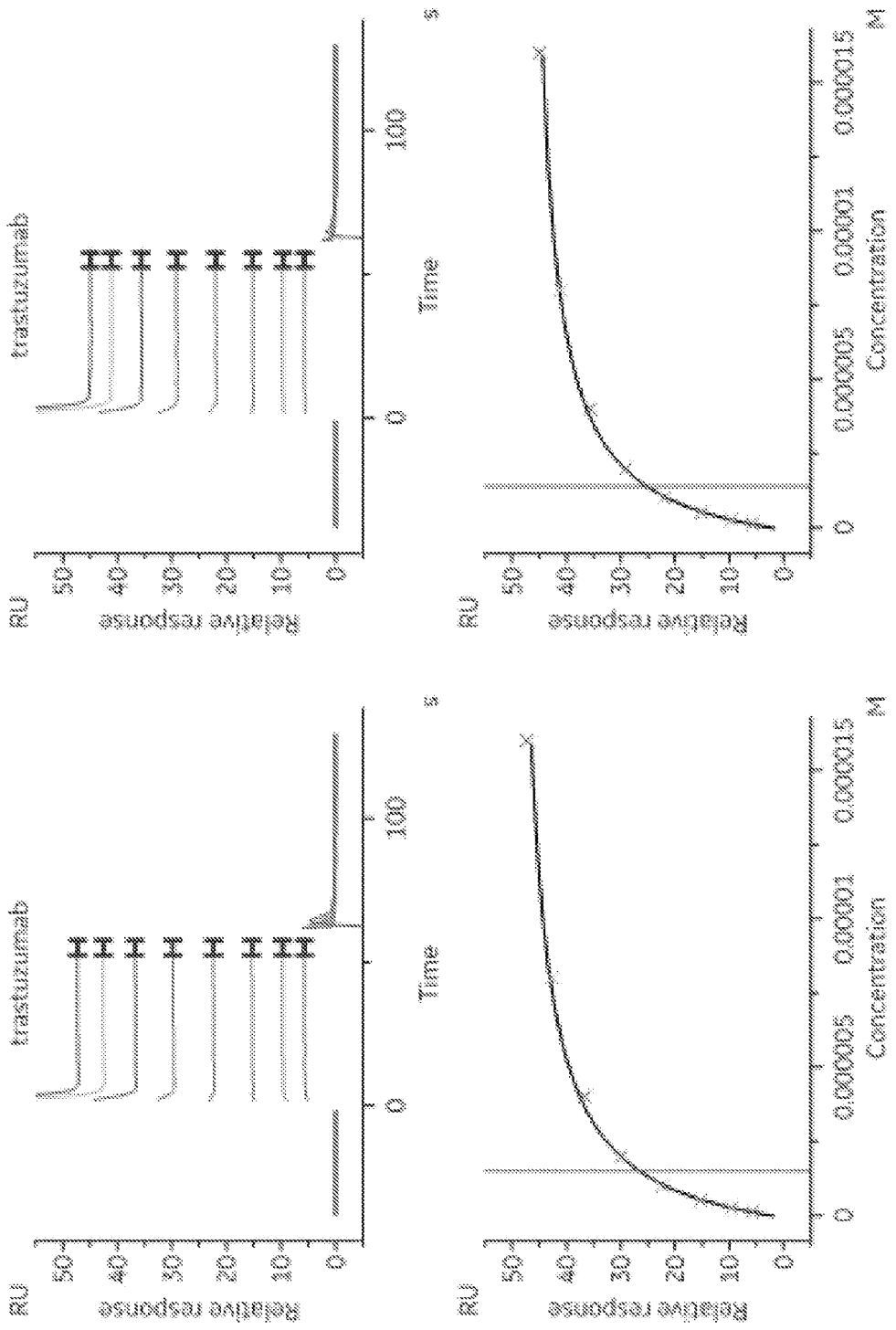


FIG. 145 Continued

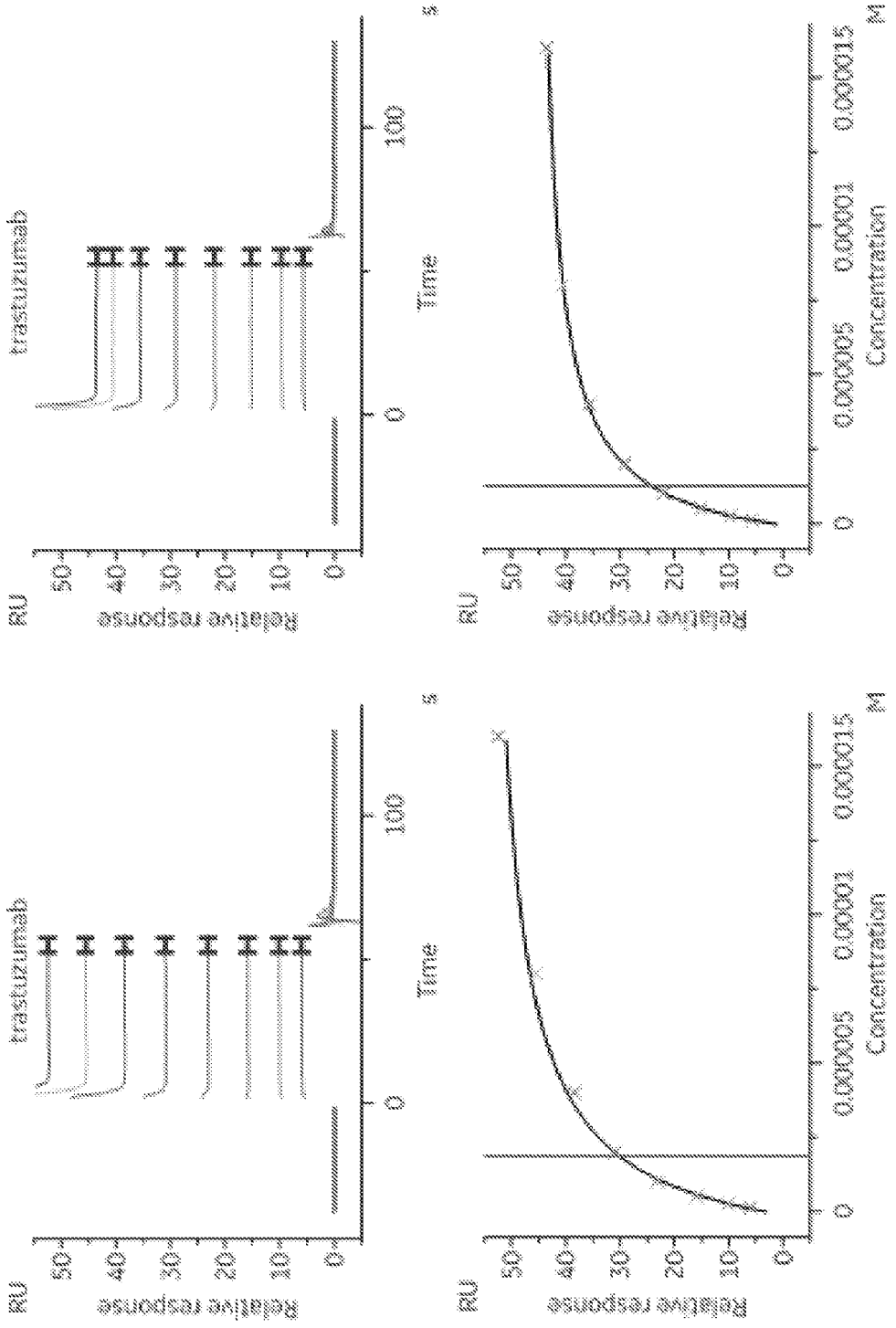
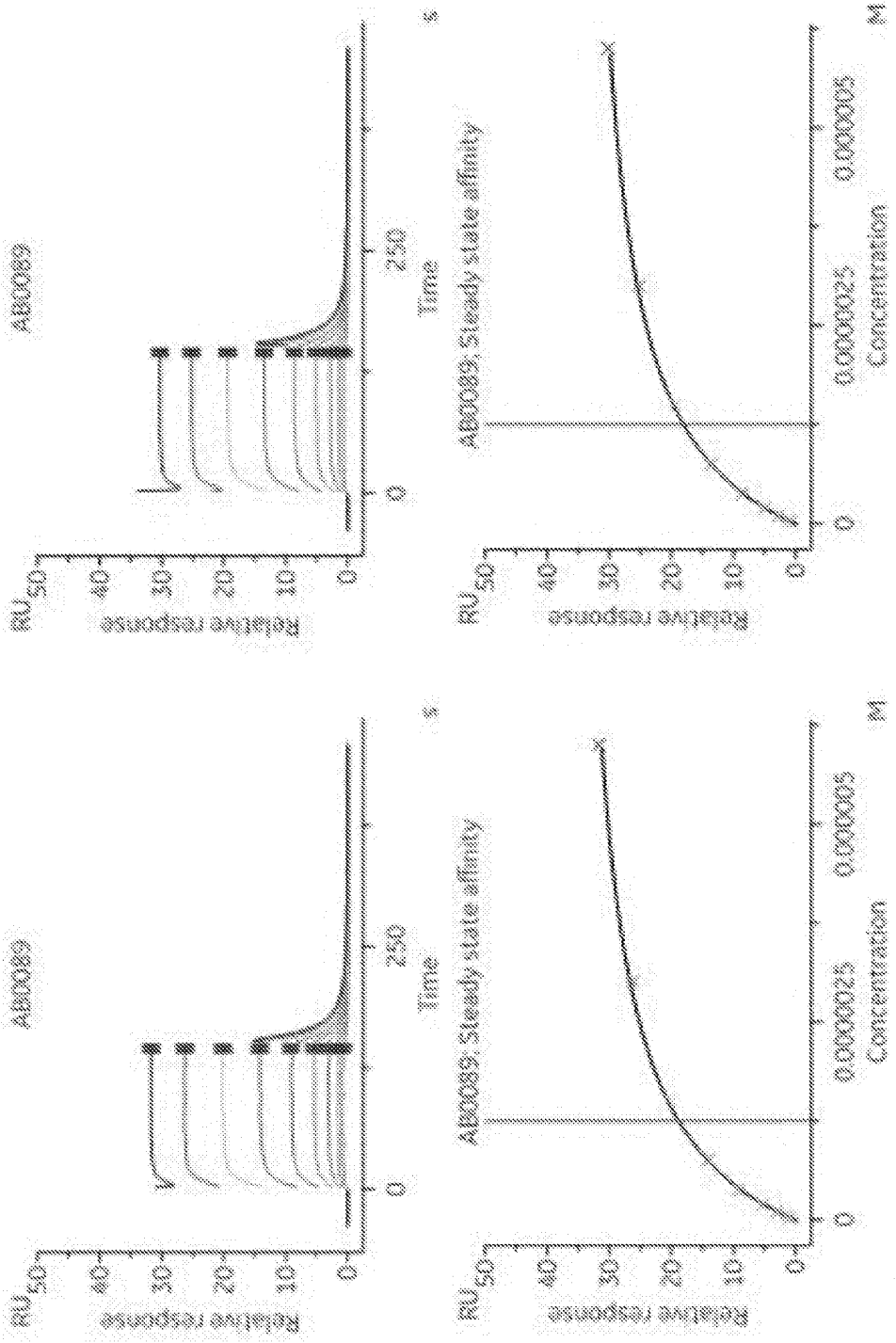


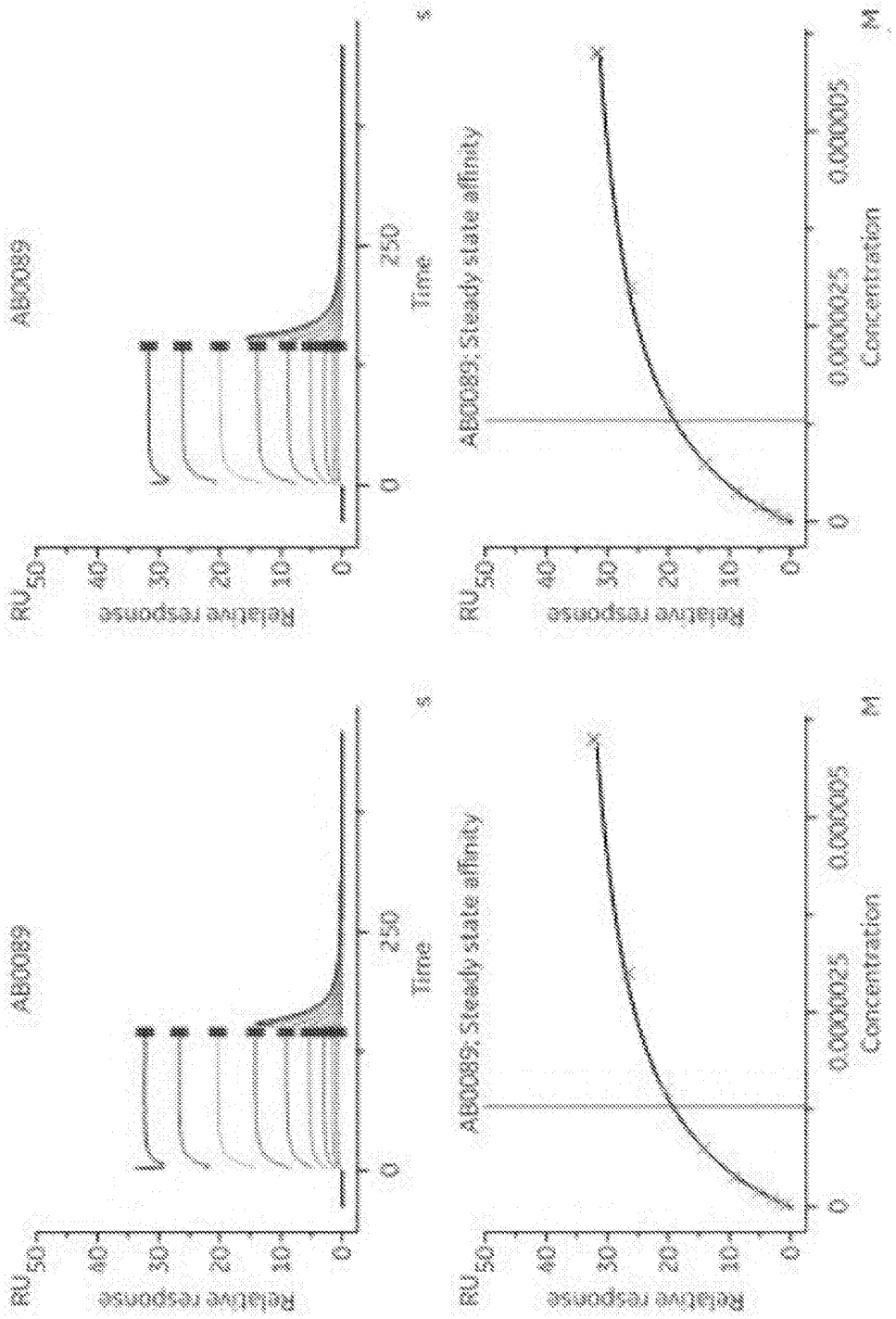
FIG. 145 Continued



AB0089

K_D = 1271.0 ± 24.6 nM

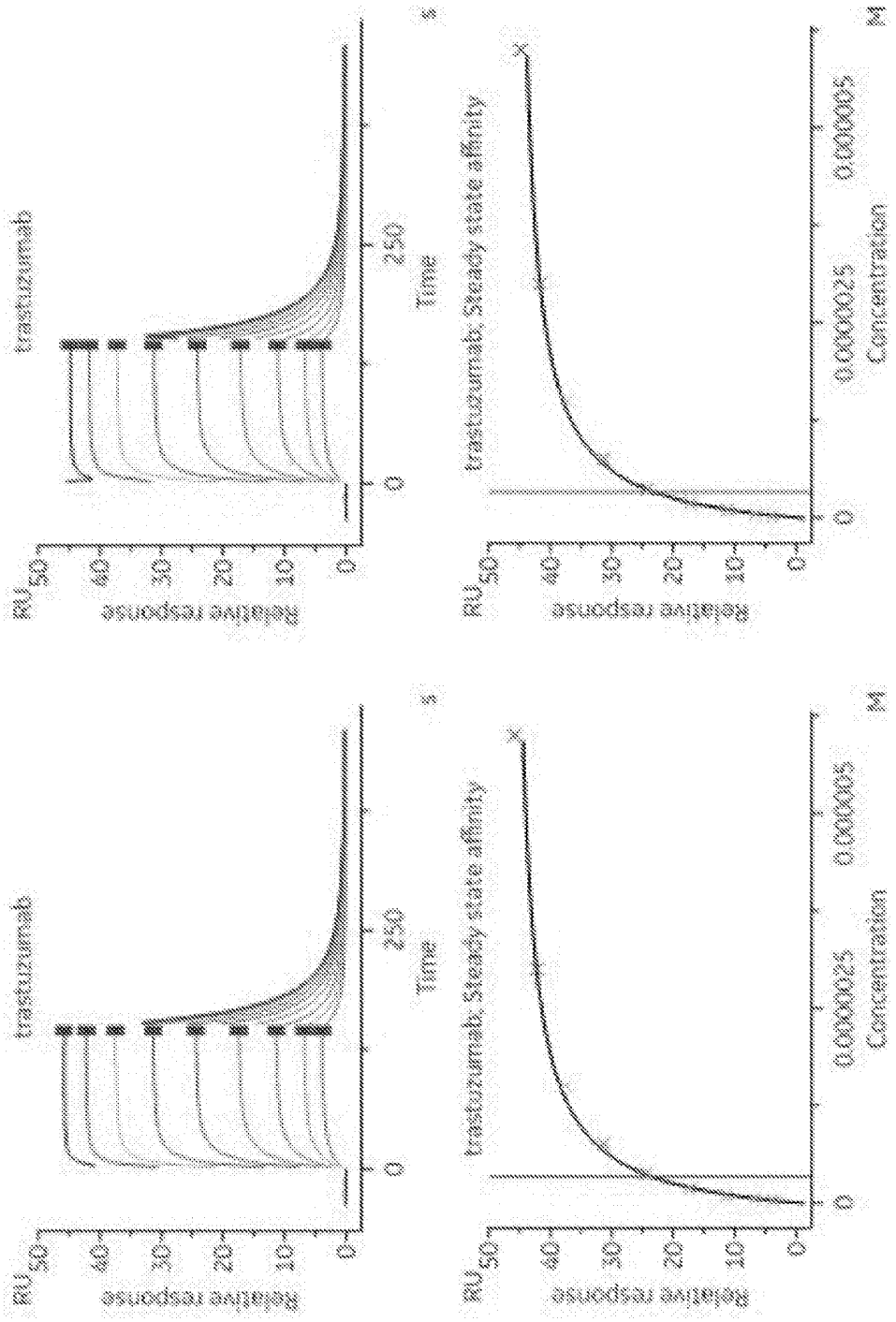
FIG. 146



AB0089

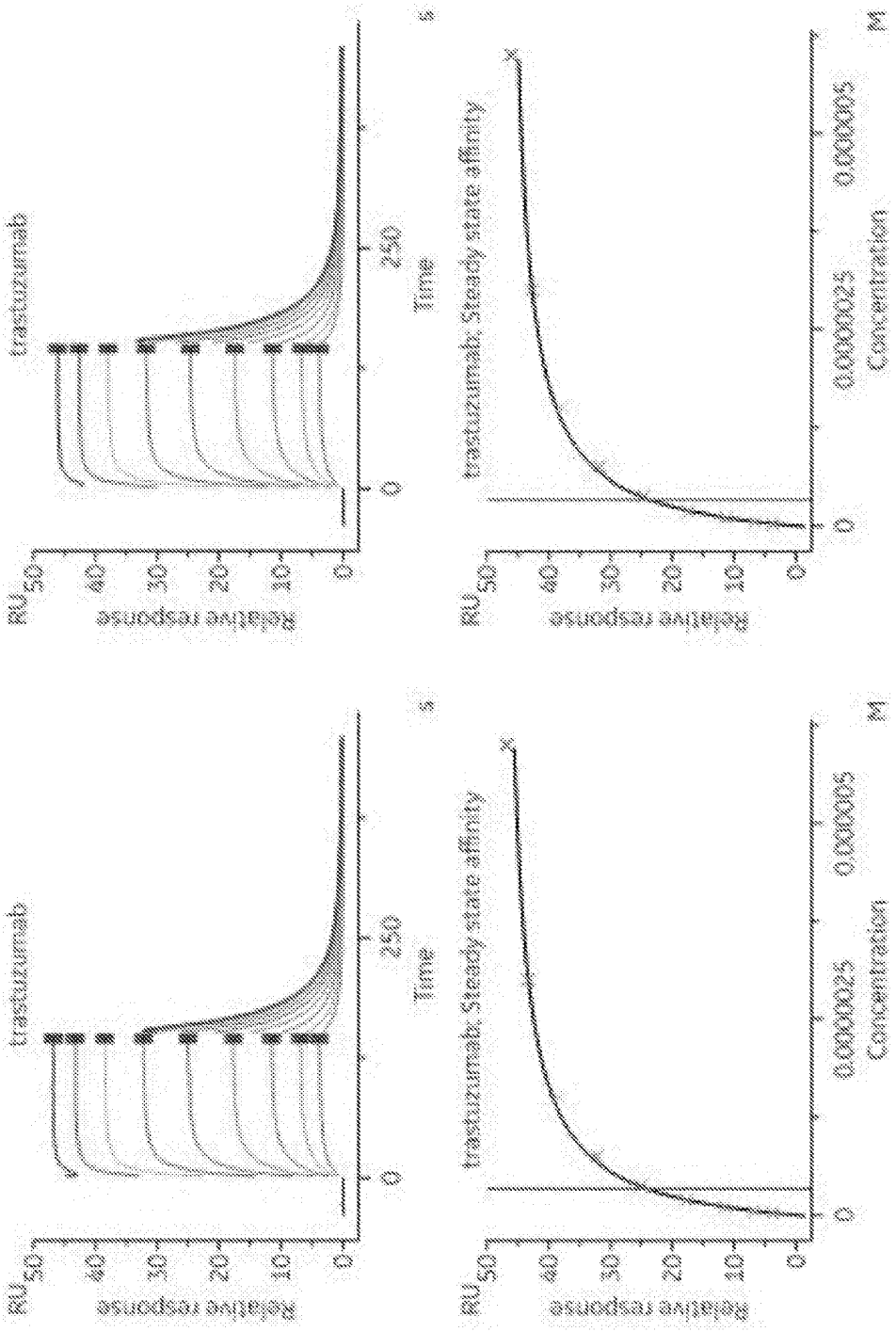
K_D = 1271.0 ± 24.6 nM

FIG. 146 Continued



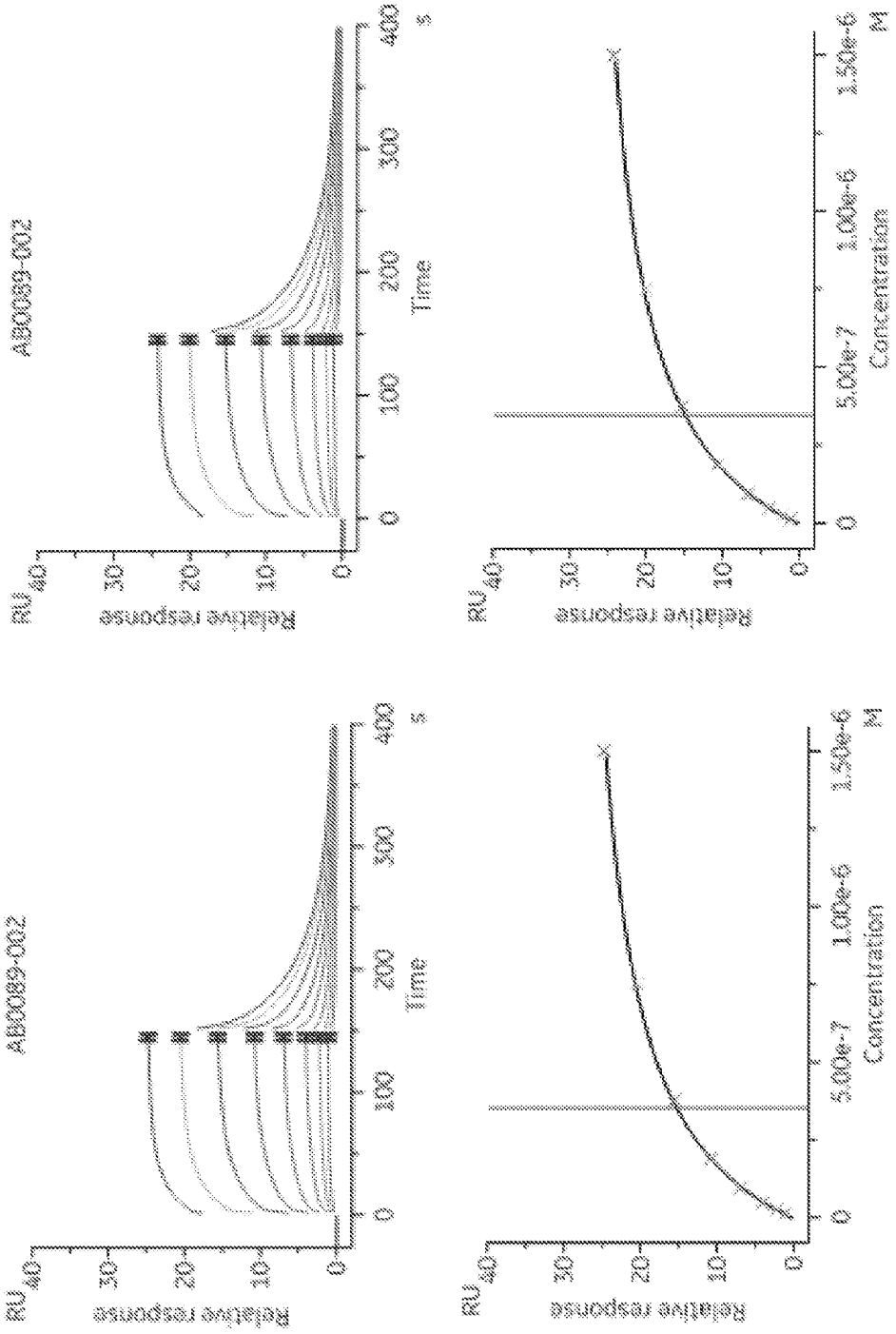
trastuzumab
K_D = 331.4 ± 3.3 nM

FIG. 146 Continued



trastuzumab
K_D = 331.4 ± 3.3 nM

FIG. 146 Continued



AB0089

K_D = 348.8 ± 2.4 nM

FIG. 147

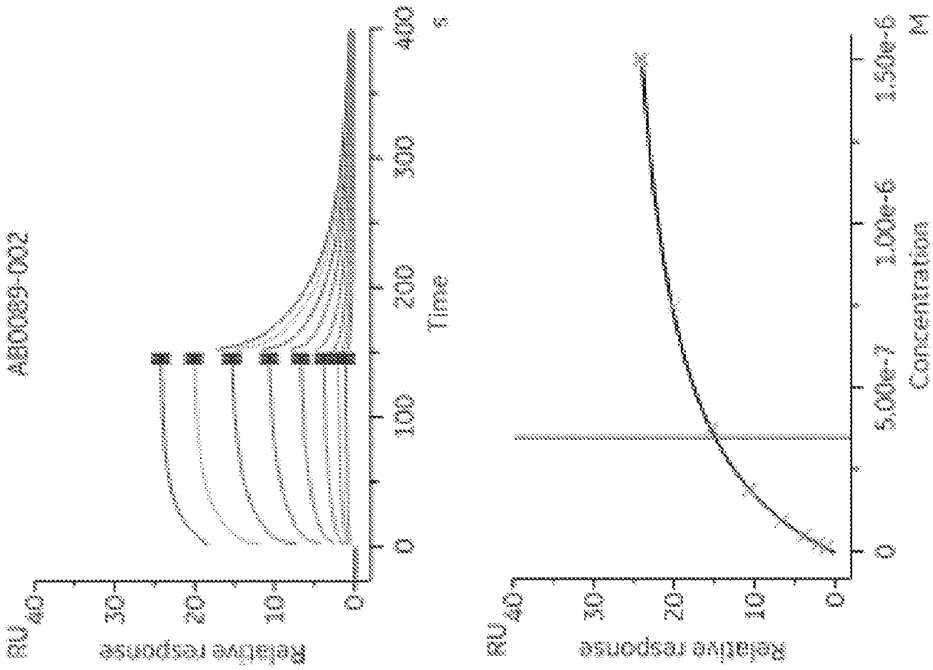
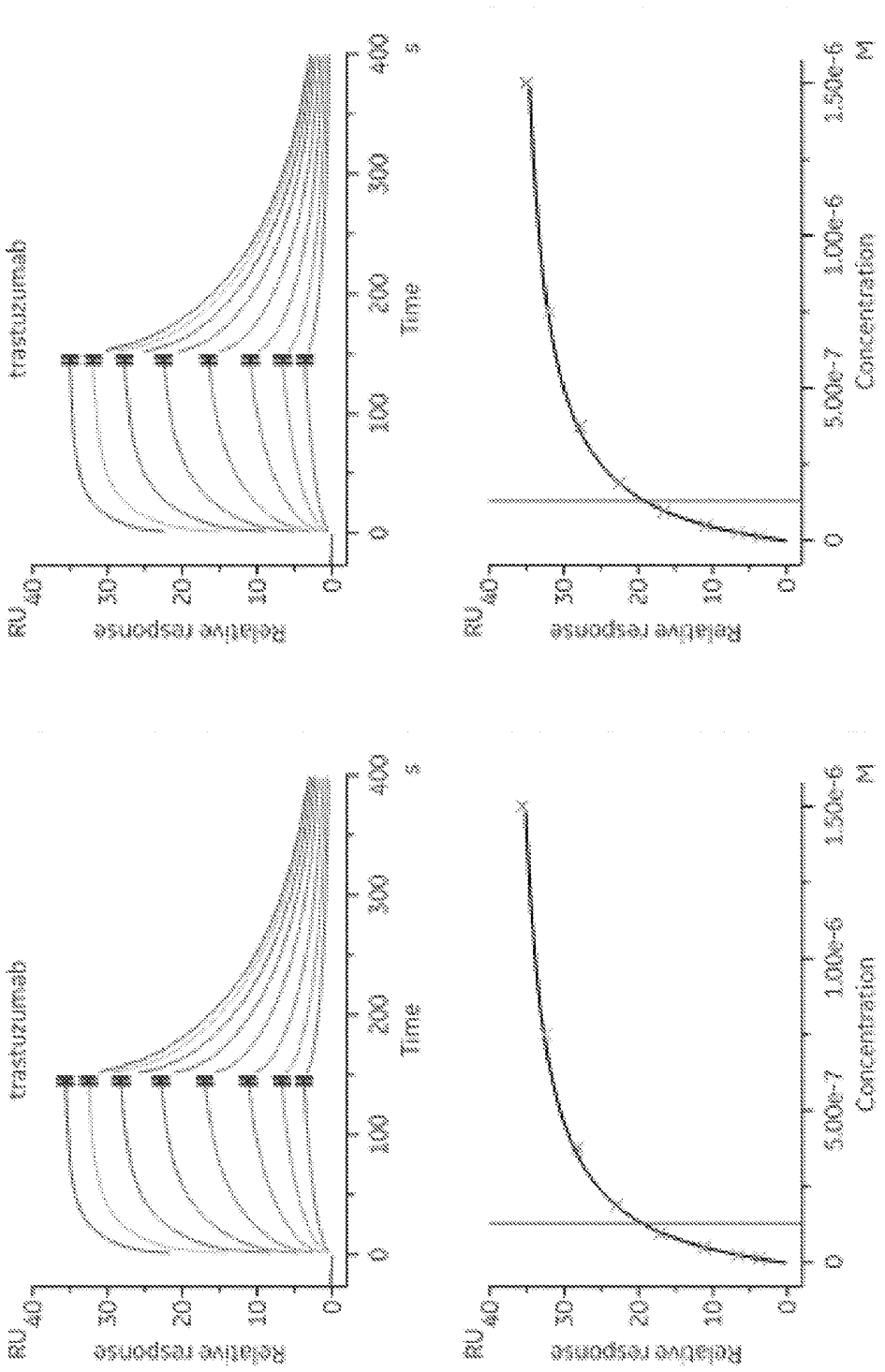


FIG. 147 Continued



trastuzumab

K_D = 128.8 ± 2.1 nM

FIG. 147 Continued

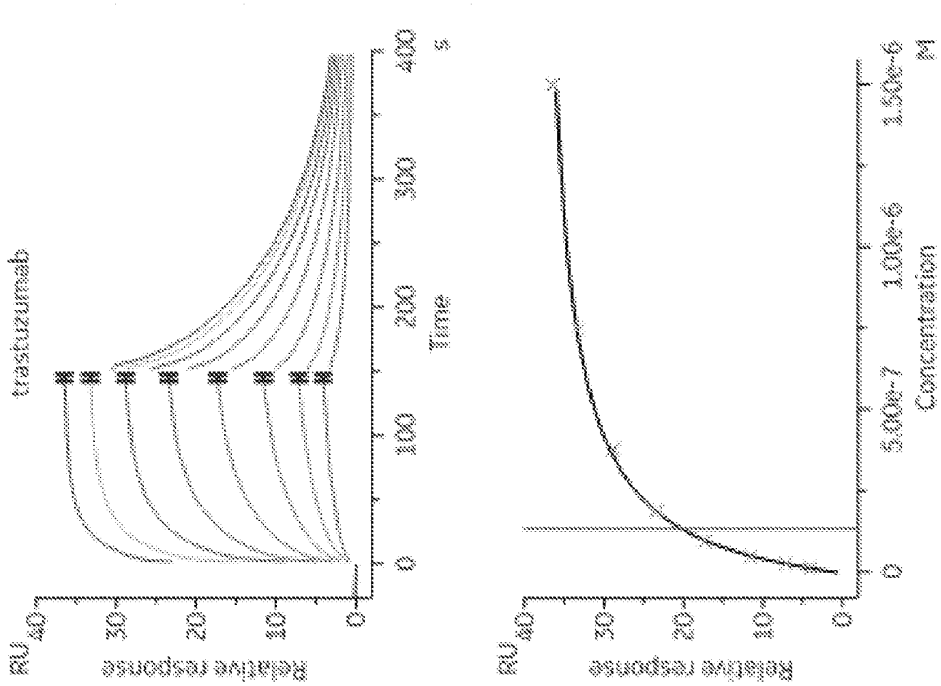
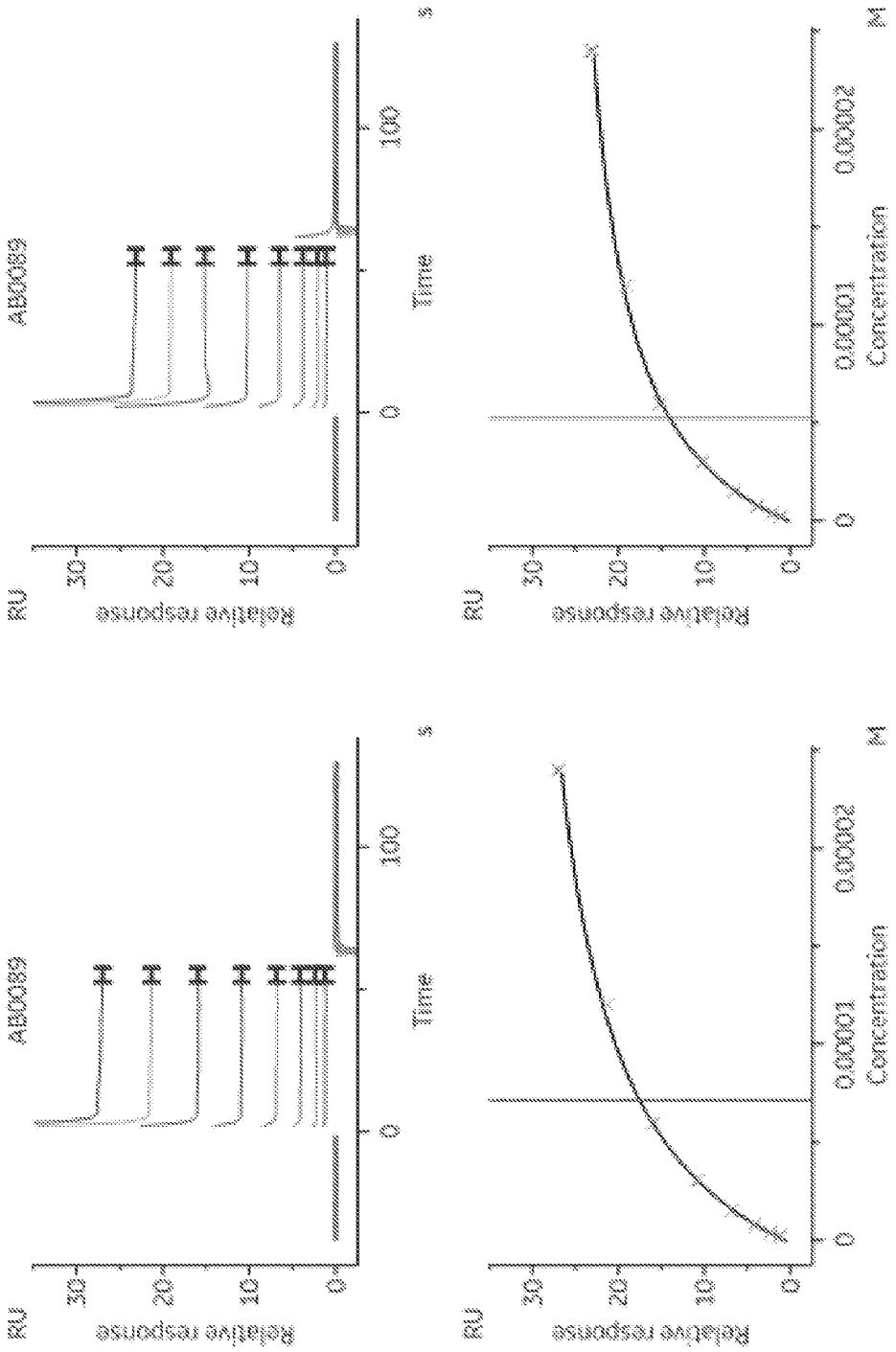


FIG. 147 Continued

trastuzumab

K_D = 128.8 ± 2.1 nM



AB0089

$K_D = 6.3 \pm 1.2\ \mu M$

FIG. 148

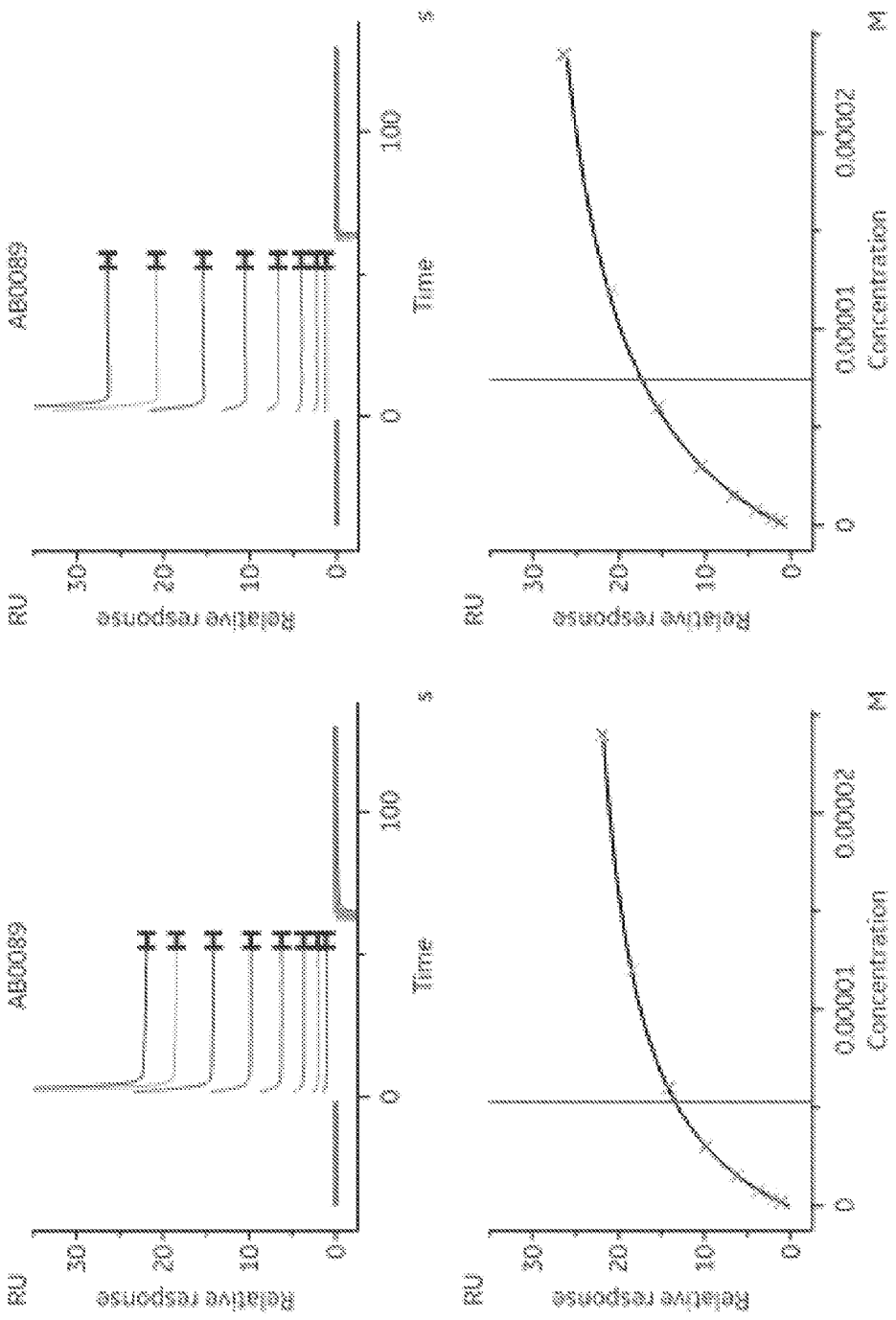
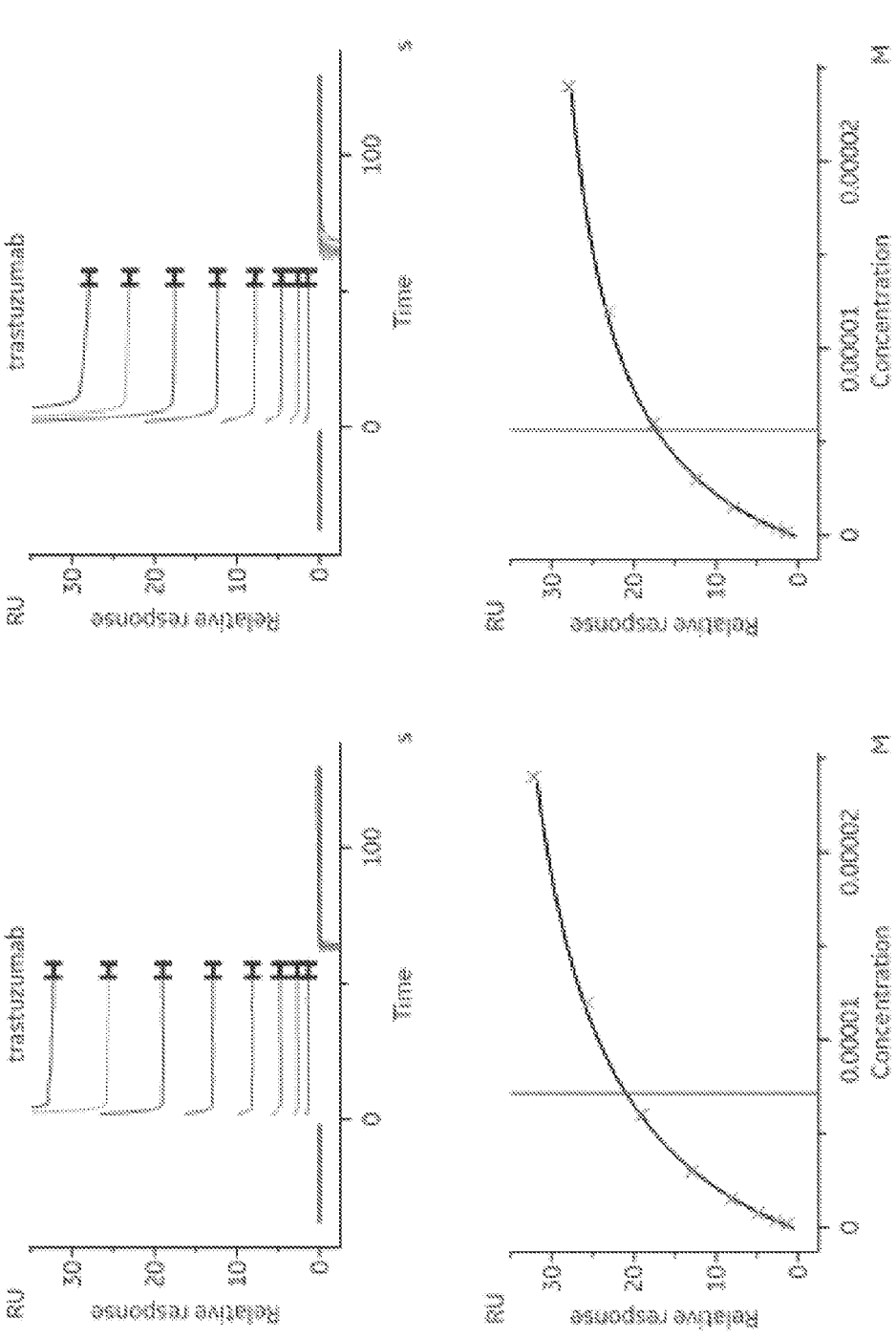


FIG. 148 Continued

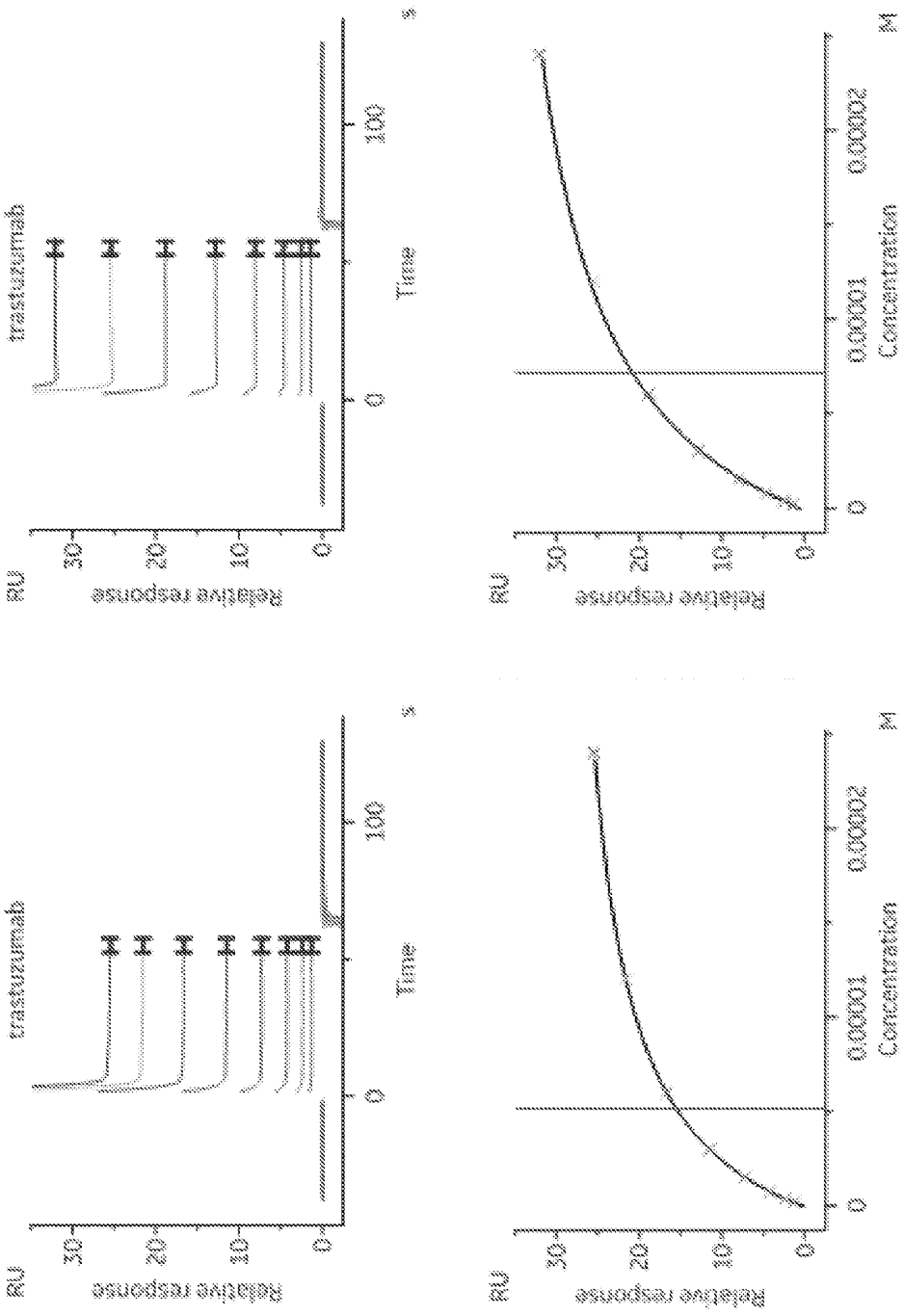
K_D = 6.3 ± 1.2 µM



trastuzumab

$K_D = 6.3 \pm 1.0\ \mu M$

FIG. 148 Continued



trastuzumab
$K_D = 6.3 \pm 1.0\ \mu M$

FIG. 148 Continued

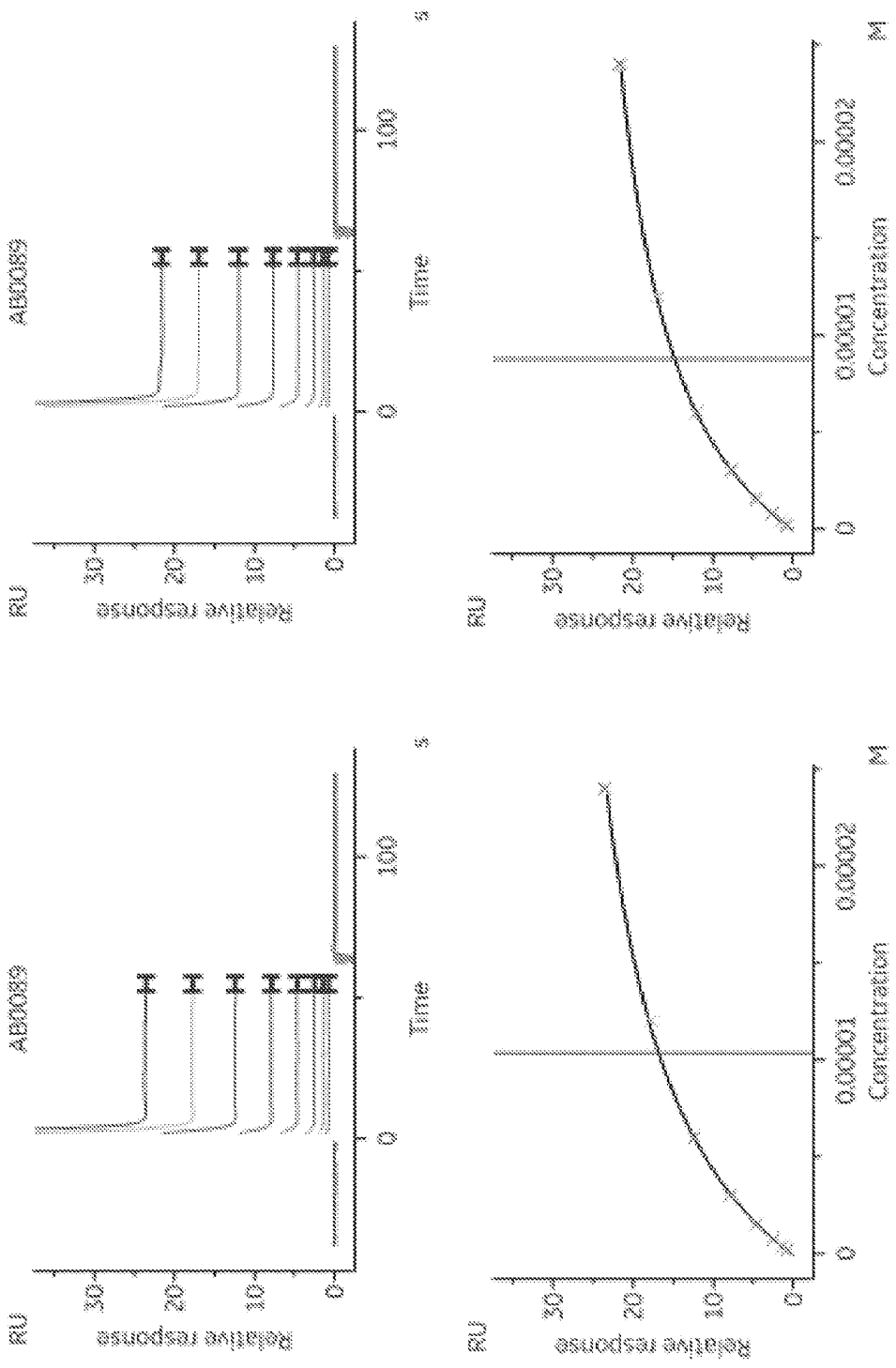
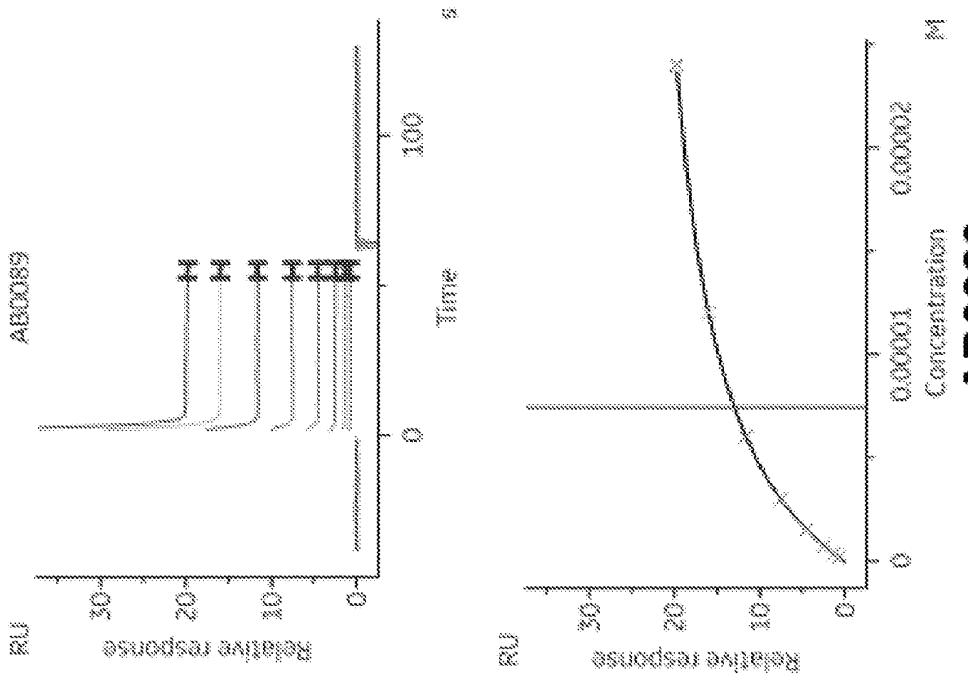
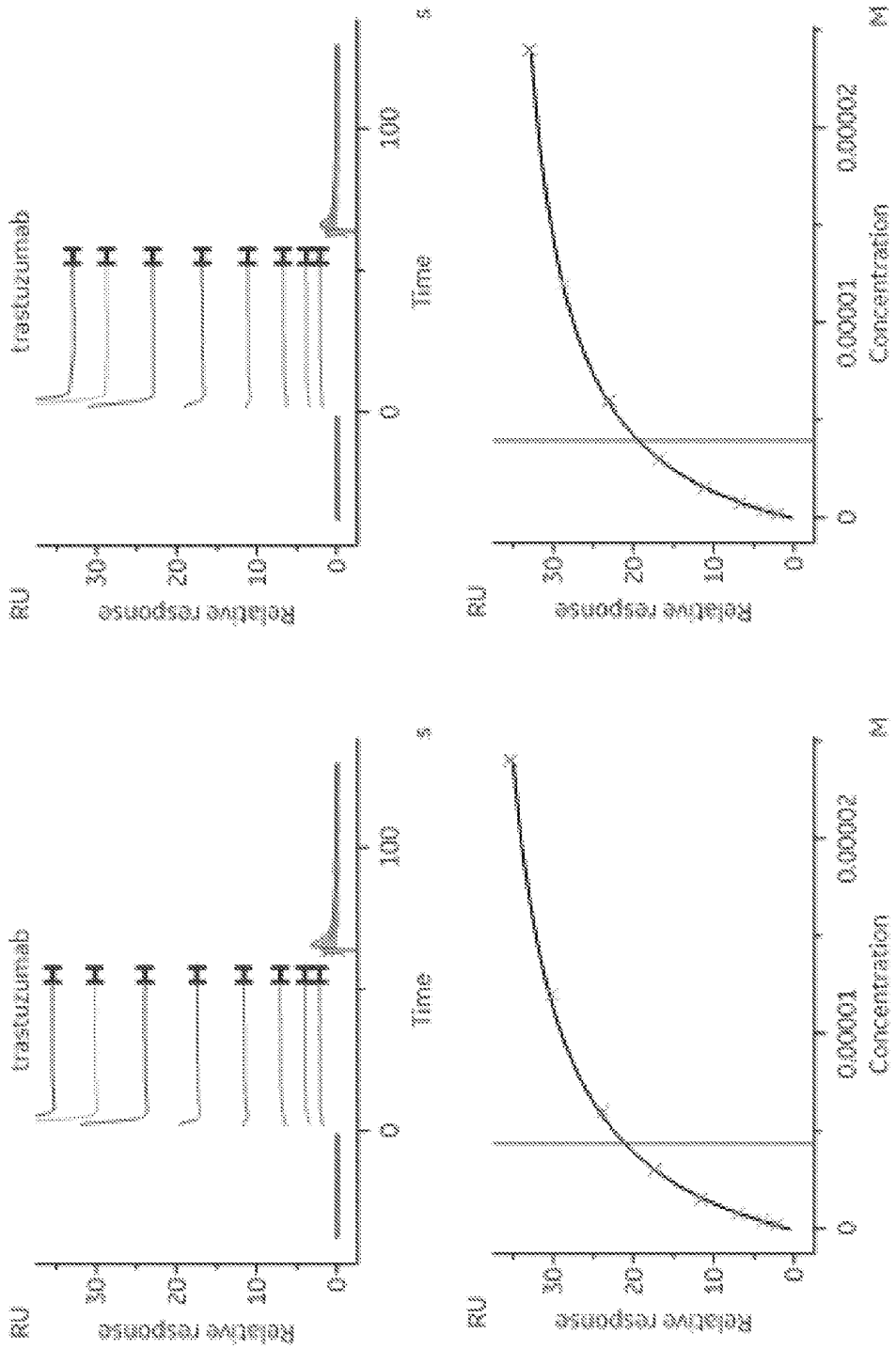

AB0089
RU
Relative response
Time
s
Concentration
M
AB0089
K_D = 8.9 ± 1.5 µM

FIG. 149



AB0089

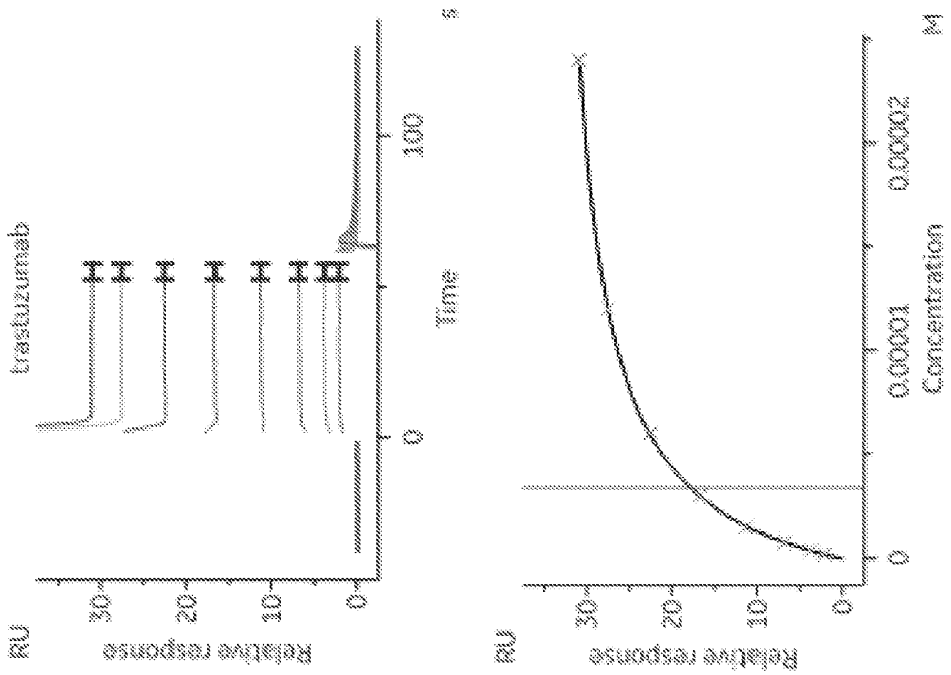
K_D = 8.9 ± 1.5 µM FIG. 149 Continued



trastuzumab

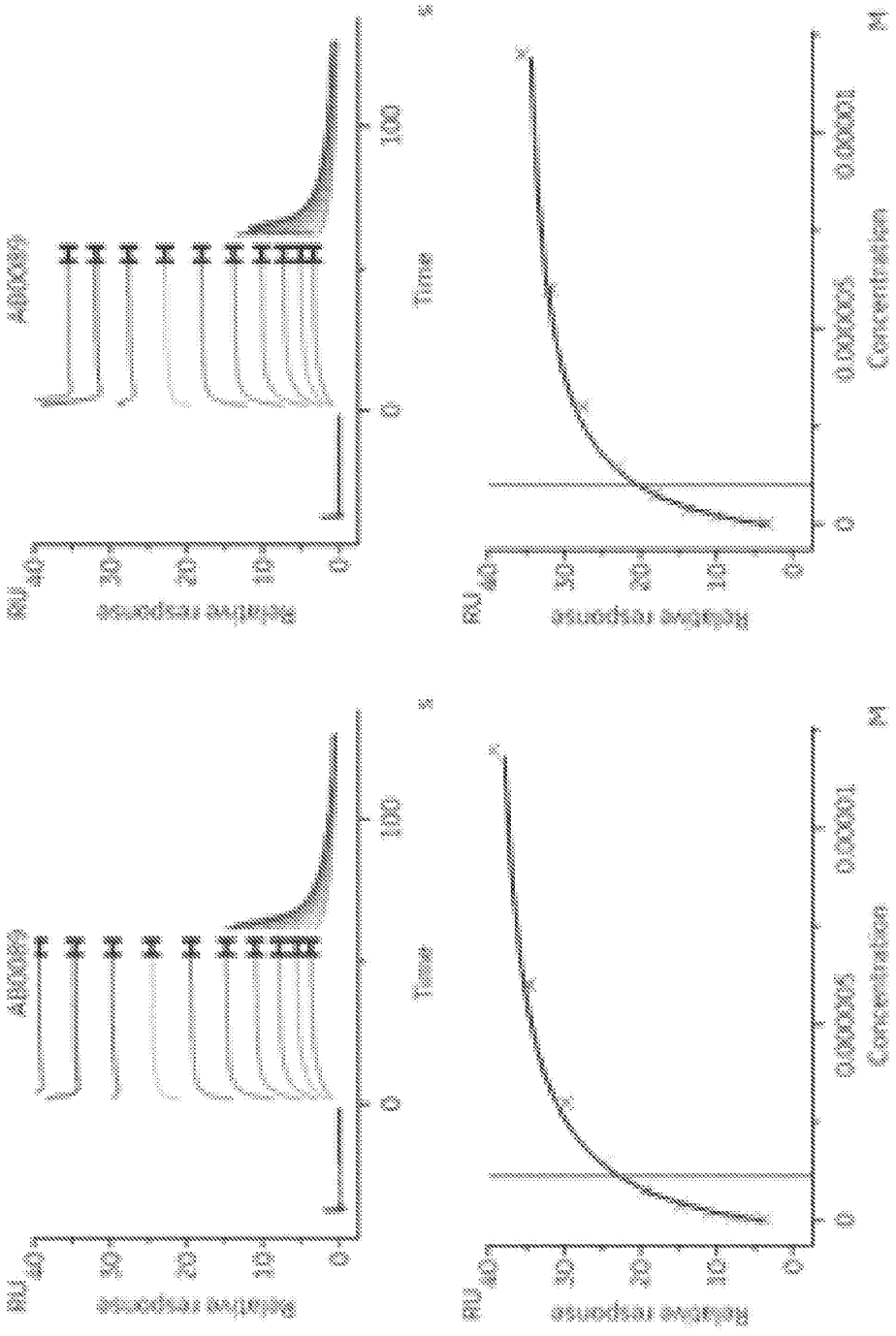
K_D = 3.9 ± 0.5 µM

FIG. 149 Continued



trastuzumab

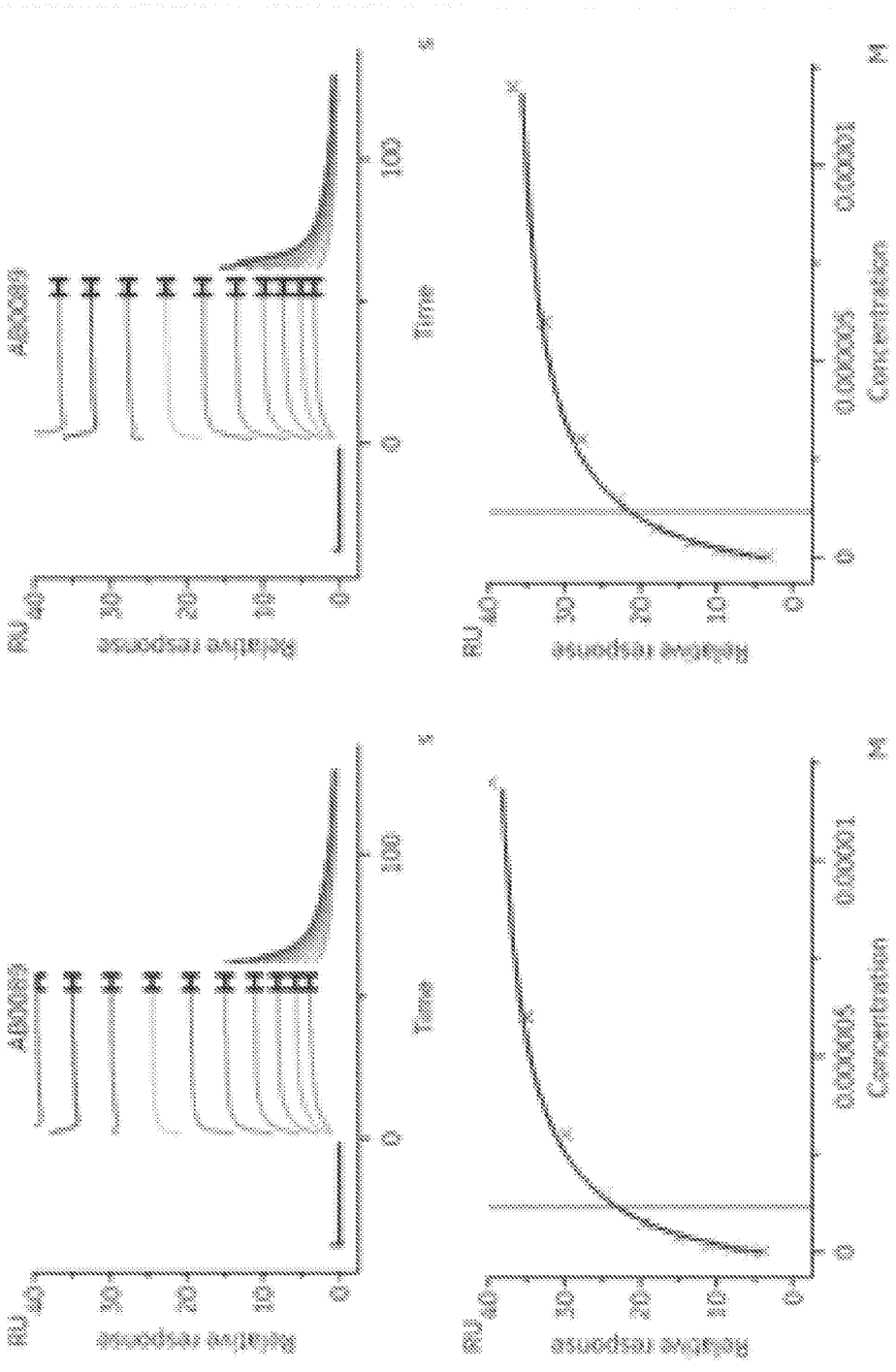
K_D = 3.9 ± 0.5 µM FIG. 149 Continued



AB0089

$K_D = 1.1 \pm 0.1\ \mu M$

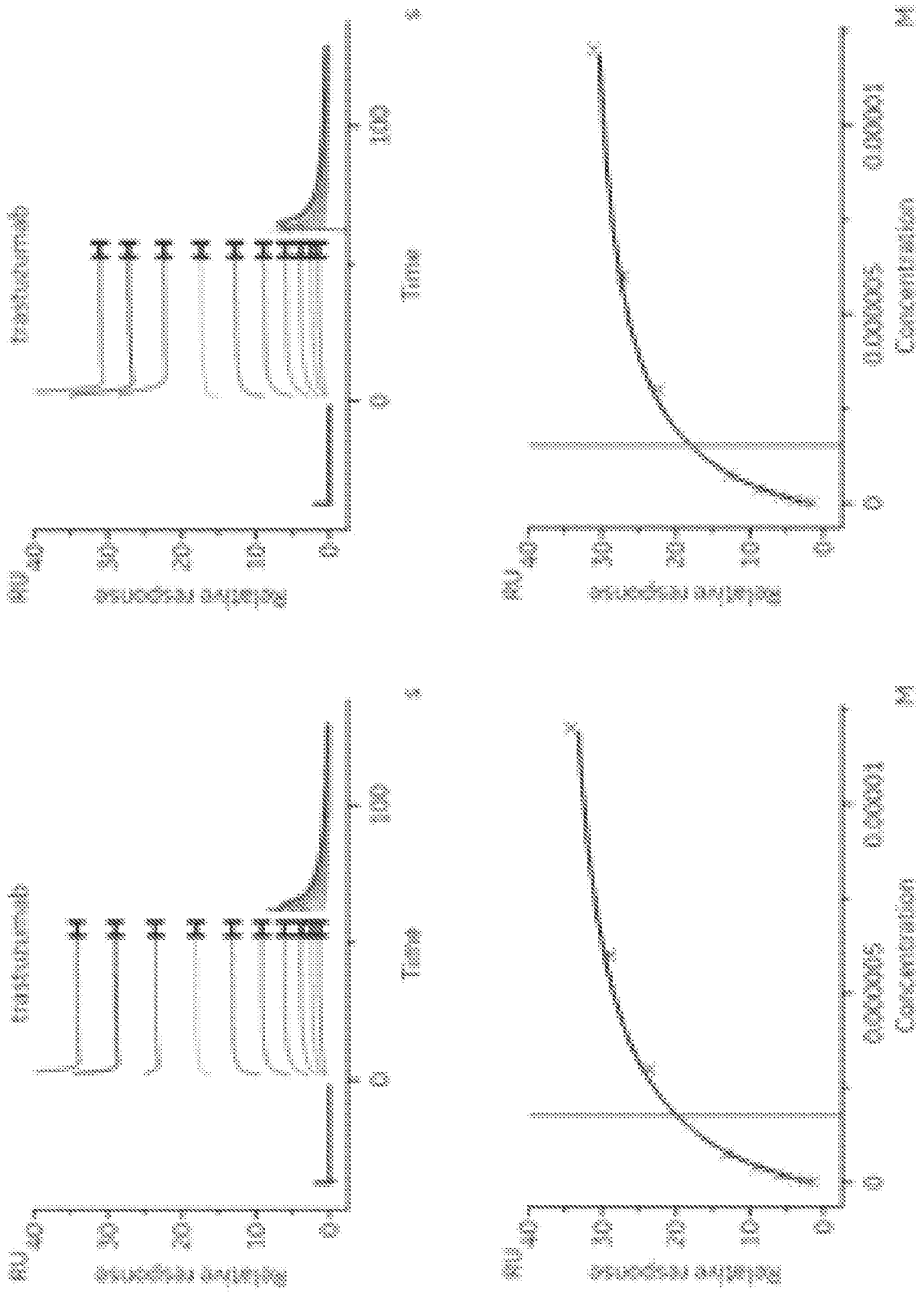
FIG. 150



AB0089

$K_D = 1.1 \pm 0.1\ \mu M$

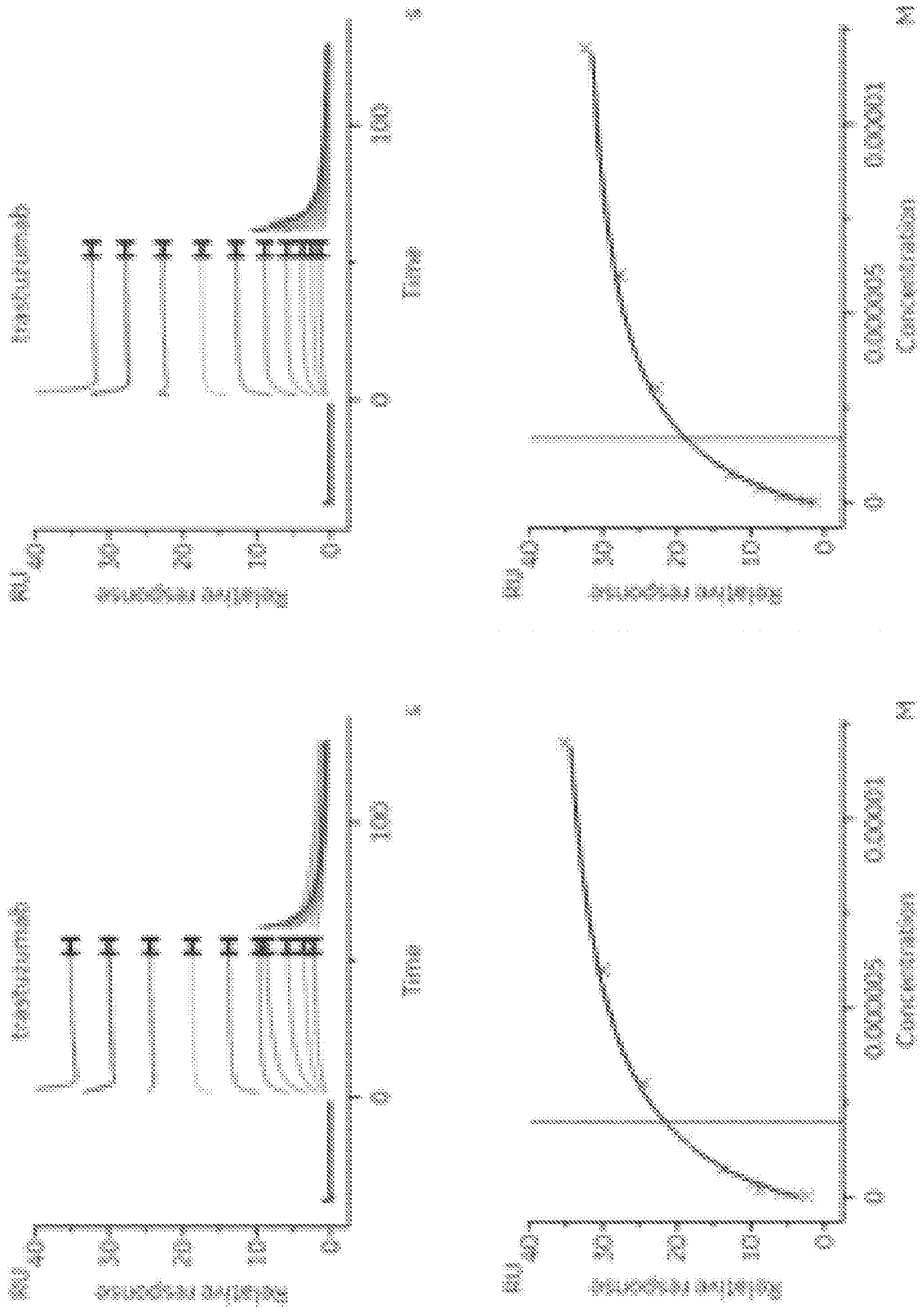
FIG. 150 Continued



trastuzumab

$K_D = 1.7 \pm 0.2\ \mu M$

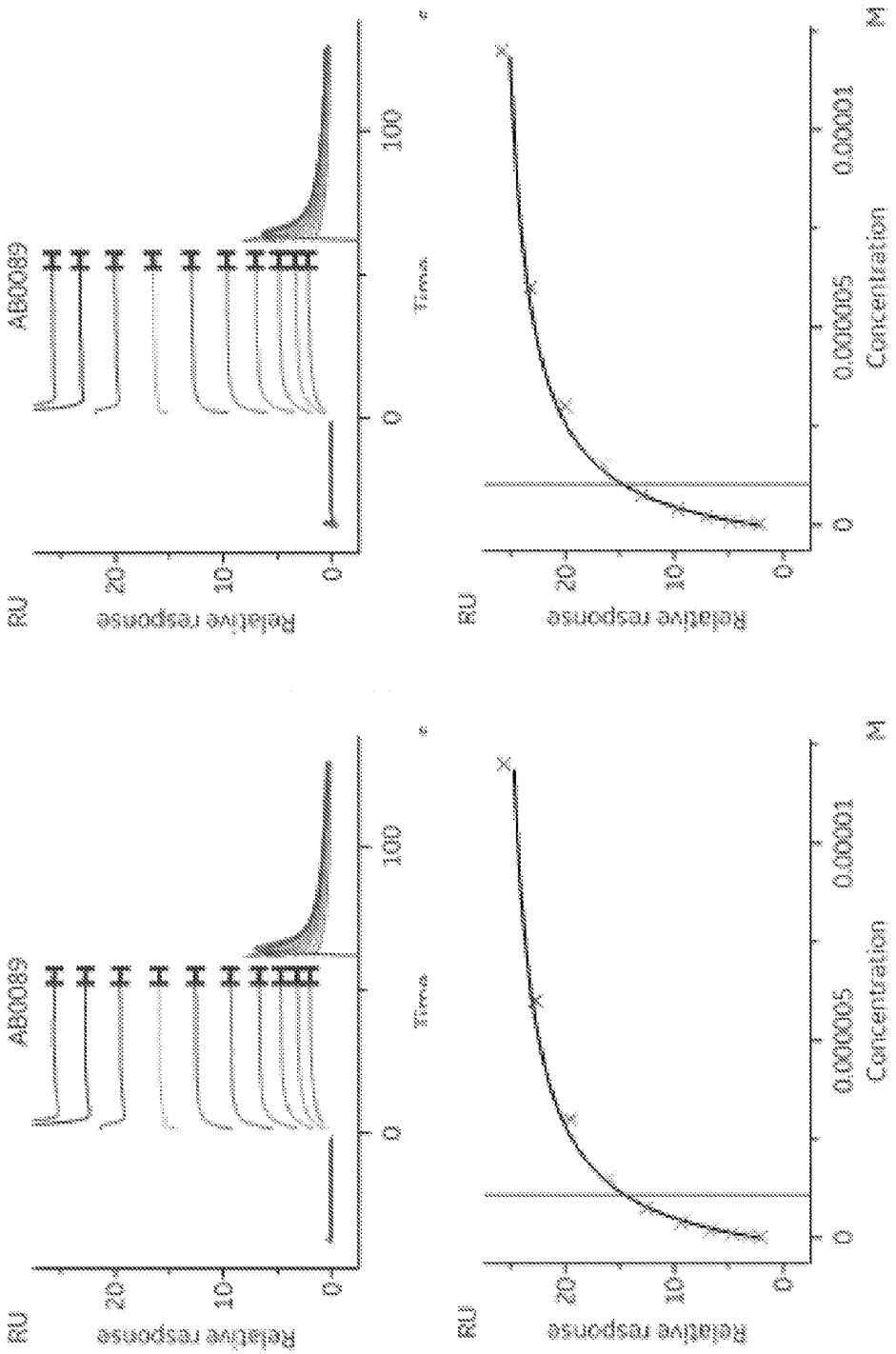
FIG. 150 Continued



trastuzumab

$K_D = 1.7 \pm 0.2\ \mu M$

FIG. 150 Continued



AB0089

$K_D = 1.0 \pm 0.1\ \mu M$

FIG. 151

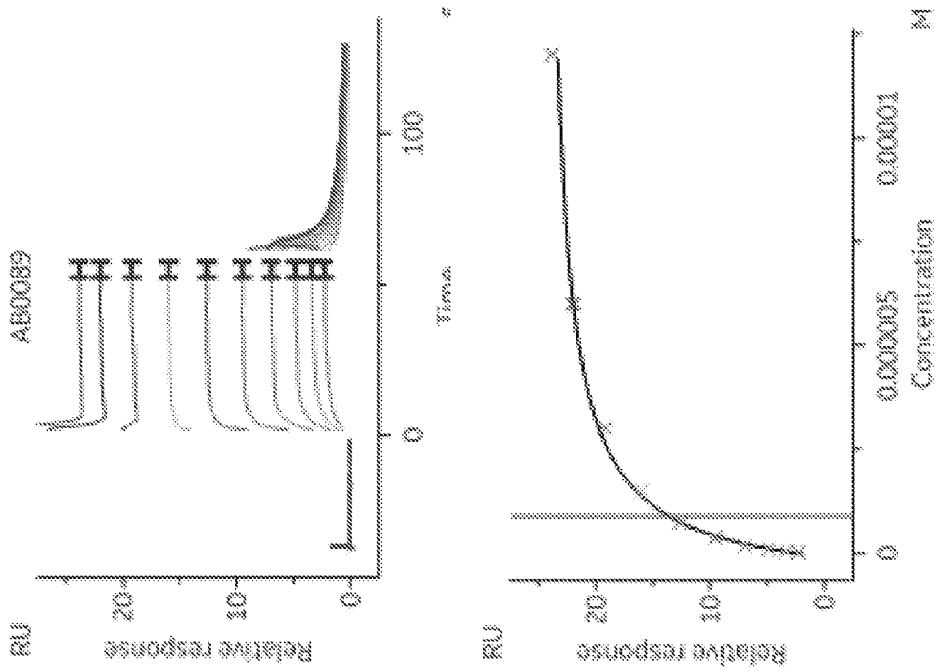
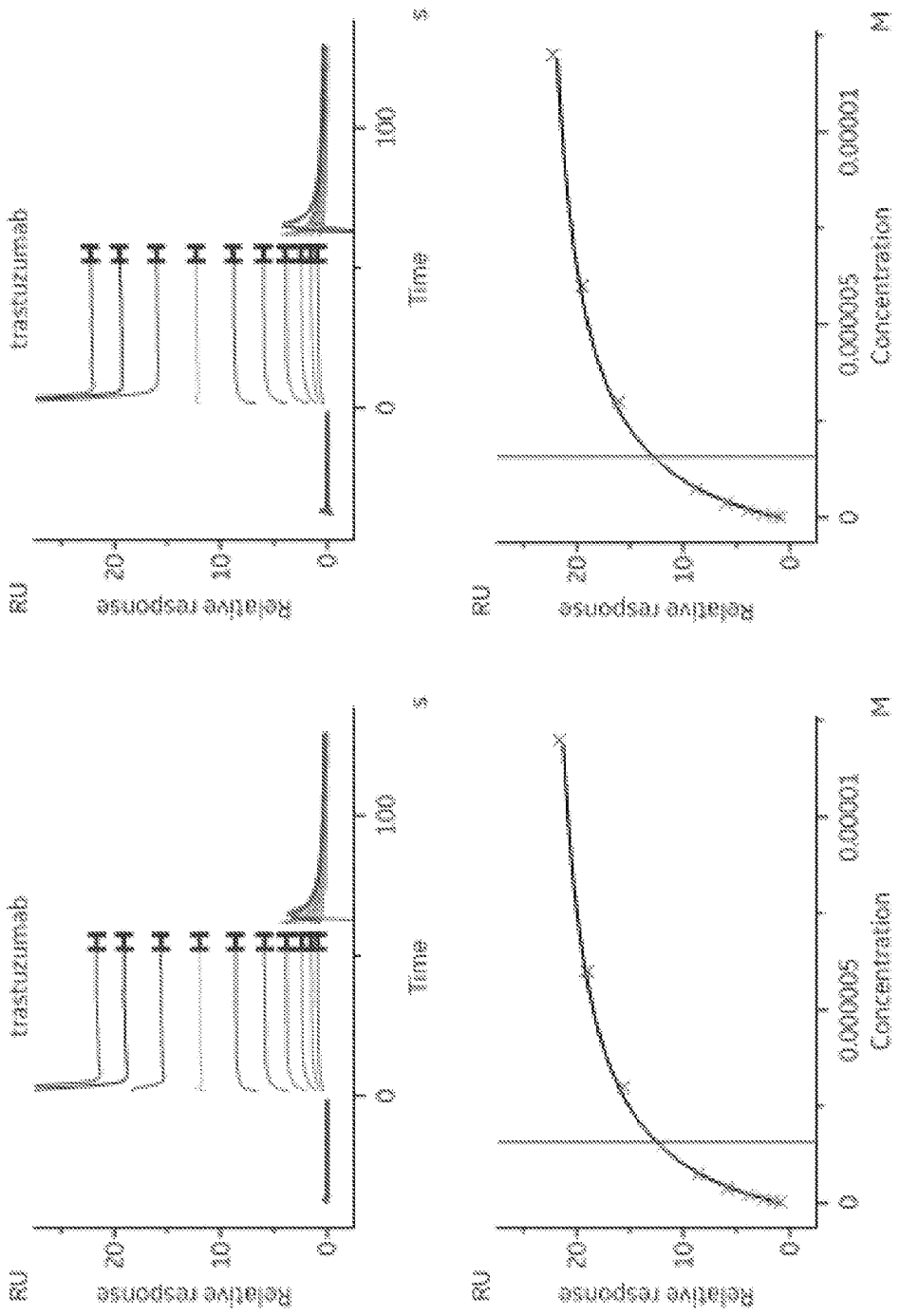


FIG. 151 Continued

AB0089

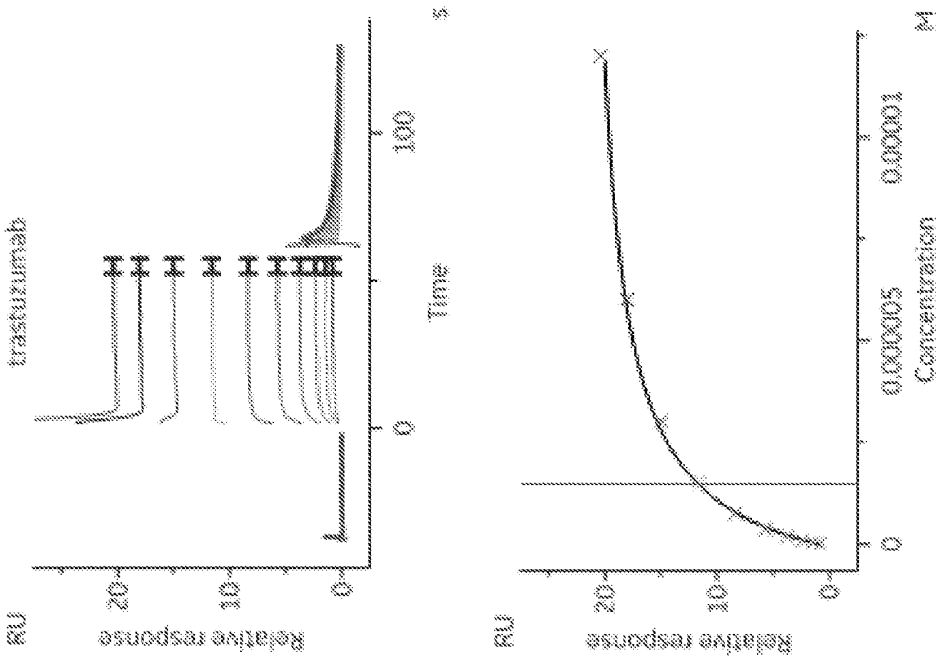
K_D = 1.0 ± 0.1 µM



trastuzumab

K_D = 1.5 ± 0.2 µM

FIG. 151 Continued



trastuzumab

$K_D = 1.5 \pm 0.2\ \mu M$

FIG. 151 Continued

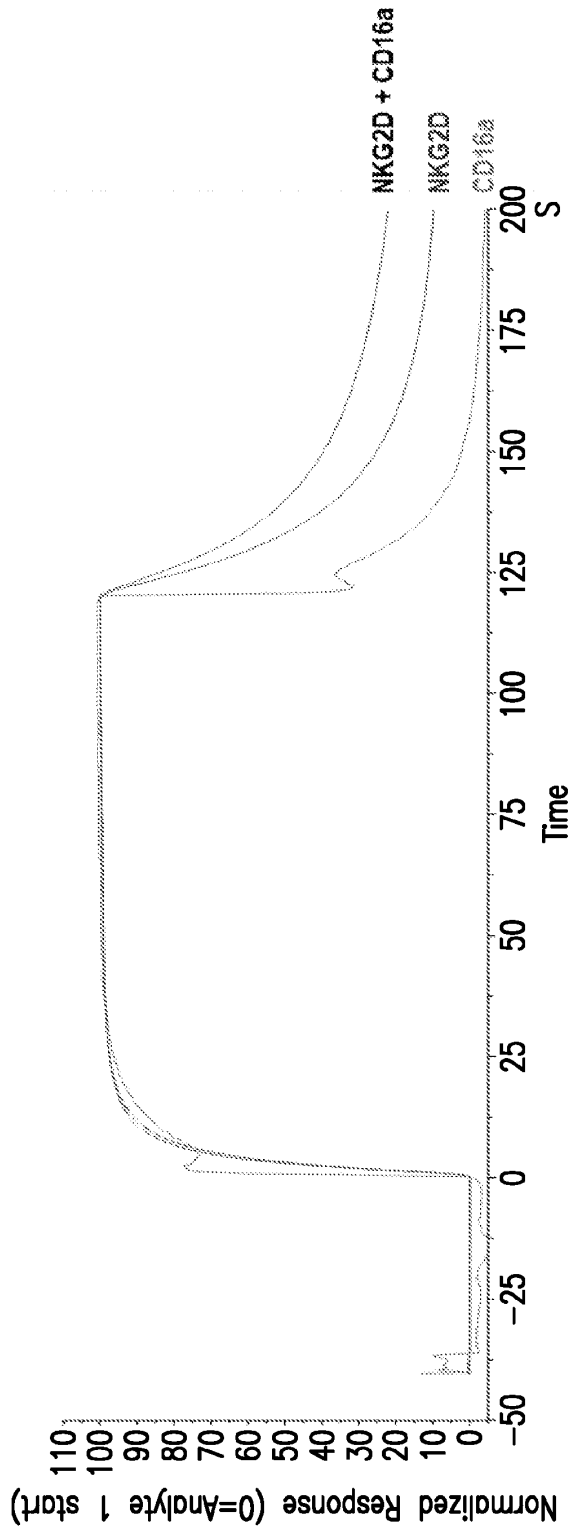

NKG2D + CD16a
NKG2D
CD16a
Normalized Response (0=Analyte 1 start)
110
100
90
80
70
60
50
40
30
20
10
0
−50
−25
0
25
50
75
100
125
150
175
200
Time
S

FIG. 152

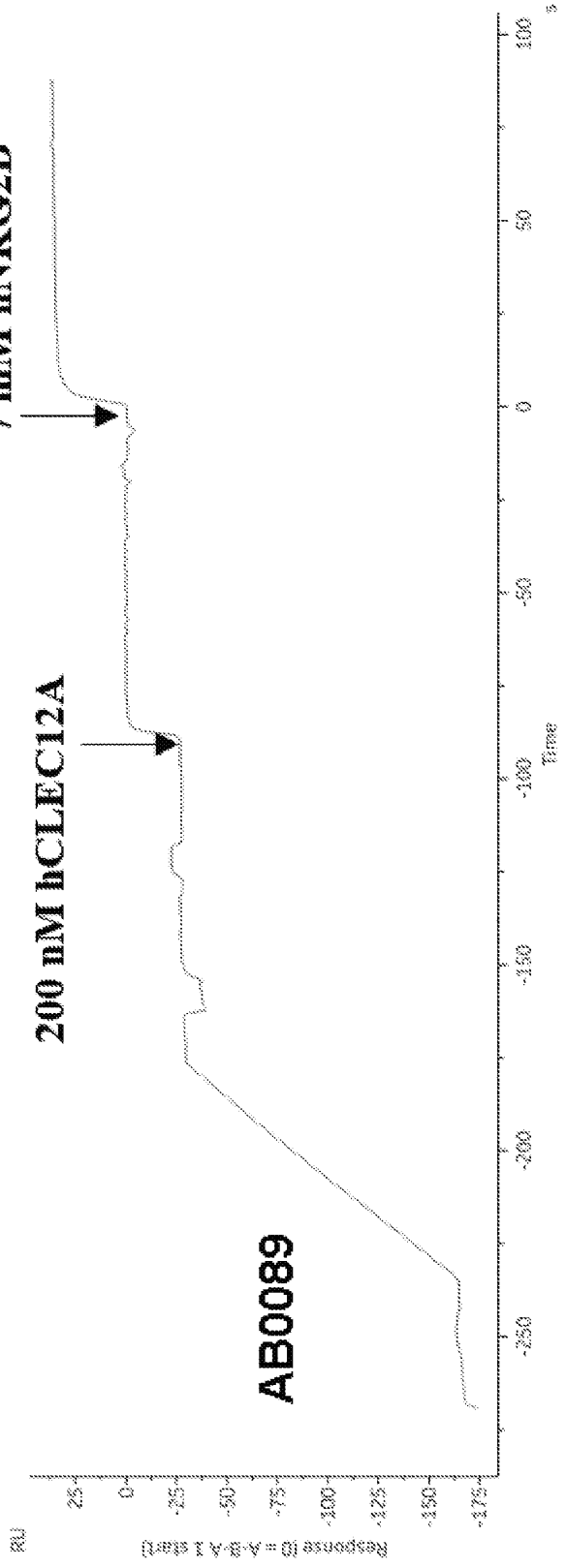

7 nM hNKG2D
200 nM hCLEC12A
AB0089
Response (D = A-B-A 1 start)
RU
Time
s
25
0
-25
-50
-75
-100
-125
-150
-175
100
50
0
-50
-100
-150
-200
-250

FIG. 153

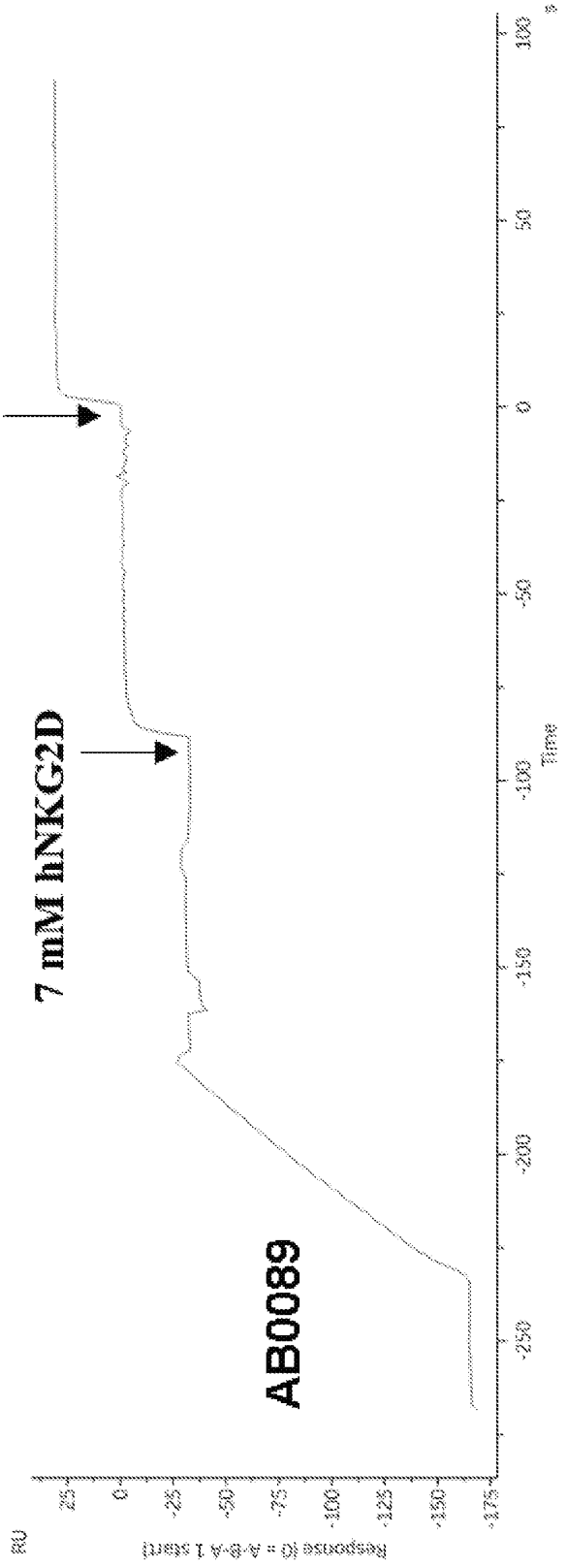


FIG. 153 Continued

FIG. 154

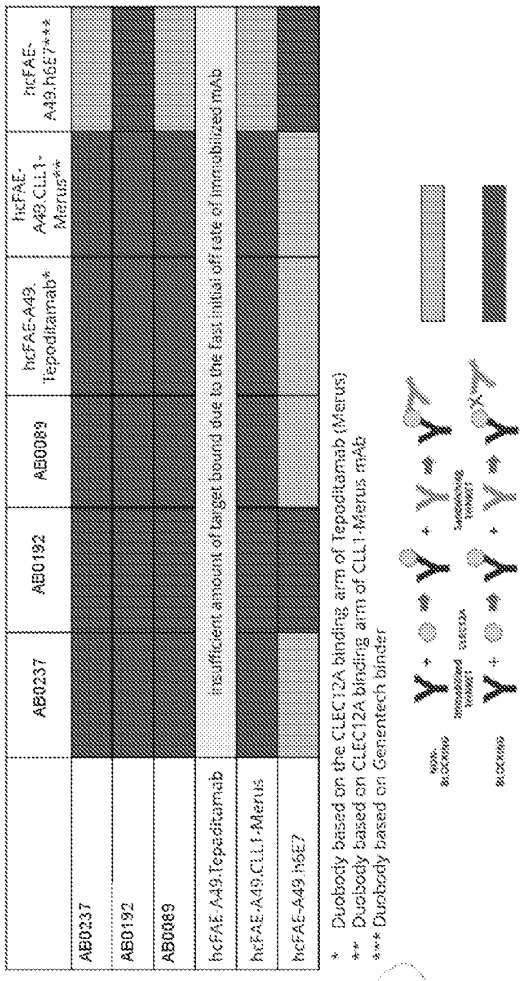

AB0237
AB0192
AB0089
hFAE-A49.Tepoditamab
hcFAE-A49.CLL1-Merus
hcFAE-A49.h6E7
* Duobody based on the CLEC12A binding arm of Tepoditamab (Merus)
** Duobody based on CLEC12A binding arm of CLL1-Merus mAb
*** Duobody based on Genentech binder

FIG. 155

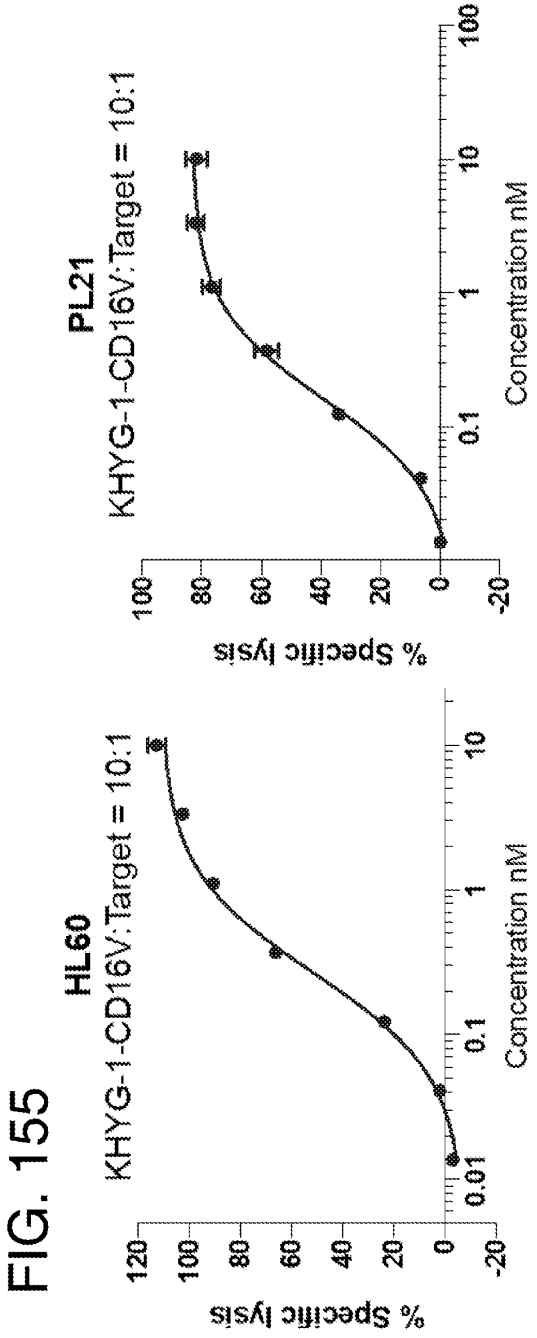

HL60
KHYG-1-CD16V:Target = 10:1
% Specific lysis
120
100
80
60
40
20
0
-20
0.01
0.1
1
10
Concentration nM
PL21
KHYG-1-CD16V:Target = 10:1
% Specific lysis
100
80
60
40
20
0
-20
0.1
1
10
100
Concentration nM

FIG. 156

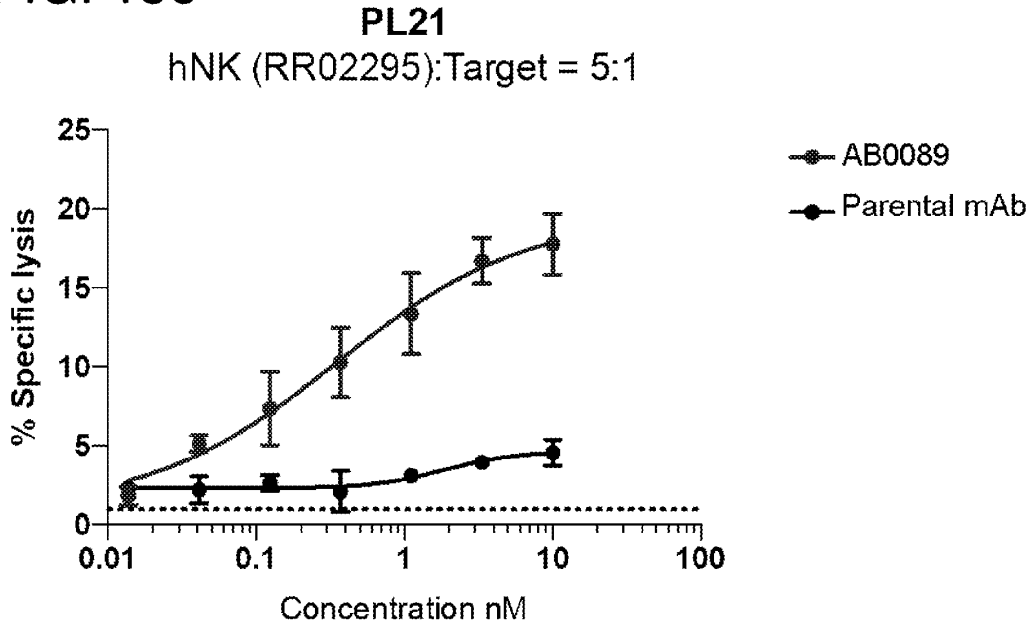
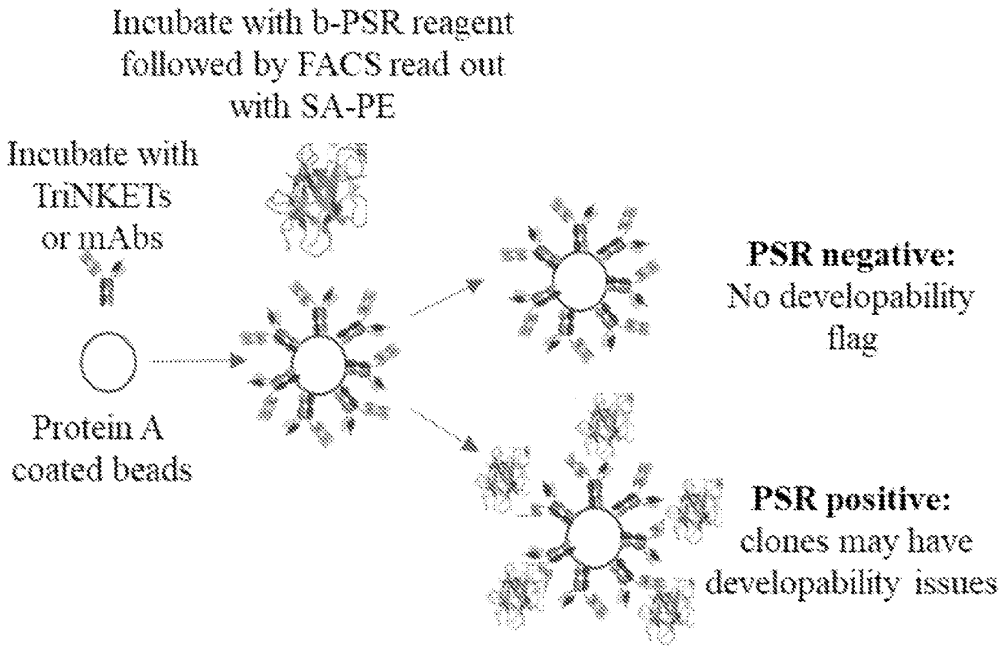

PL21
hNK (RR02295):Target = 5:1
% Specific lysis
25
20
15
10
5
0
0.01
0.1
1
10
100
Concentration nM
AB0089
Parental mAb

FIG. 157A


Incubate with b-PSR reagent
followed by FACS read out
with SA-PE
Incubate with
TriNKETs
or mAbs
Protein A
coated beads
PSR negative:
No developability
flag
PSR positive:
clones may have
developability issues

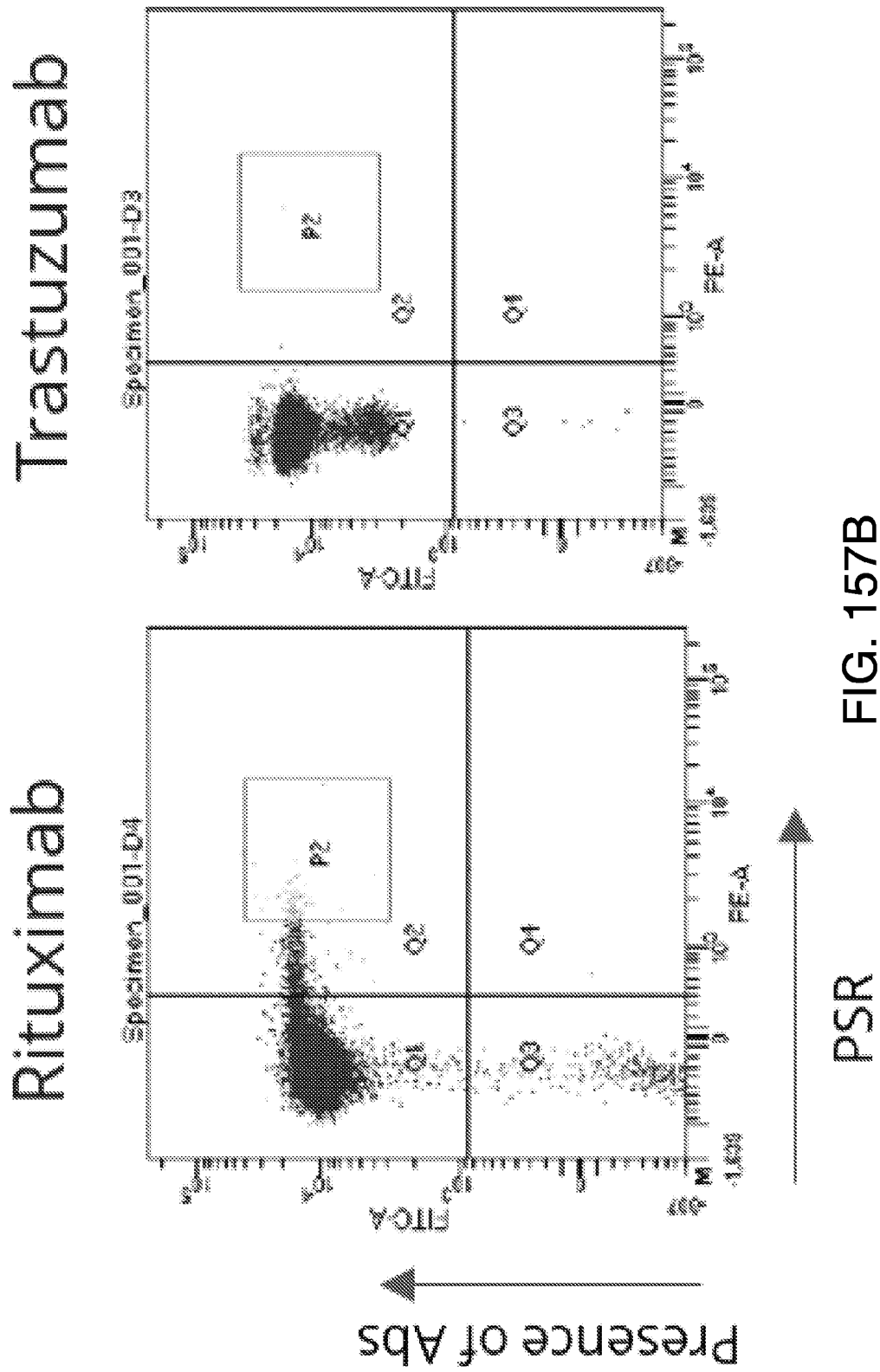

Trastuzumab
Rituximab
Specimen_001-03
Specimen_001-04
P2
Q2
Q1
Q3
Q4
FITC-A
PE-A
Presence of Abs
PSR

FIG. 157B

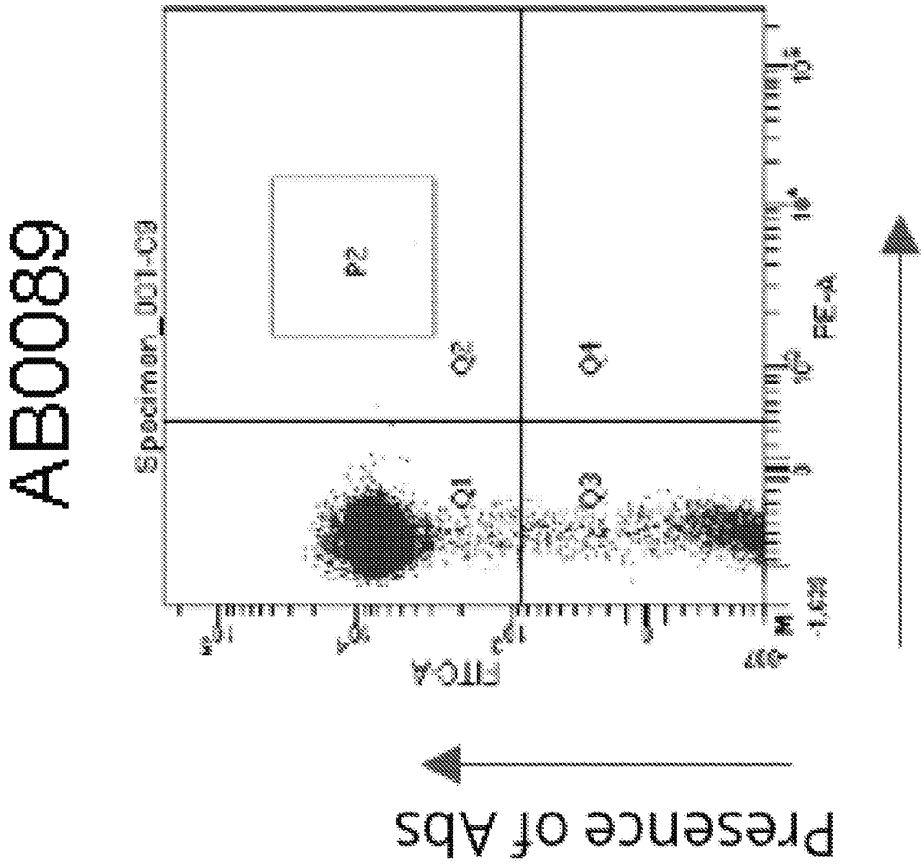
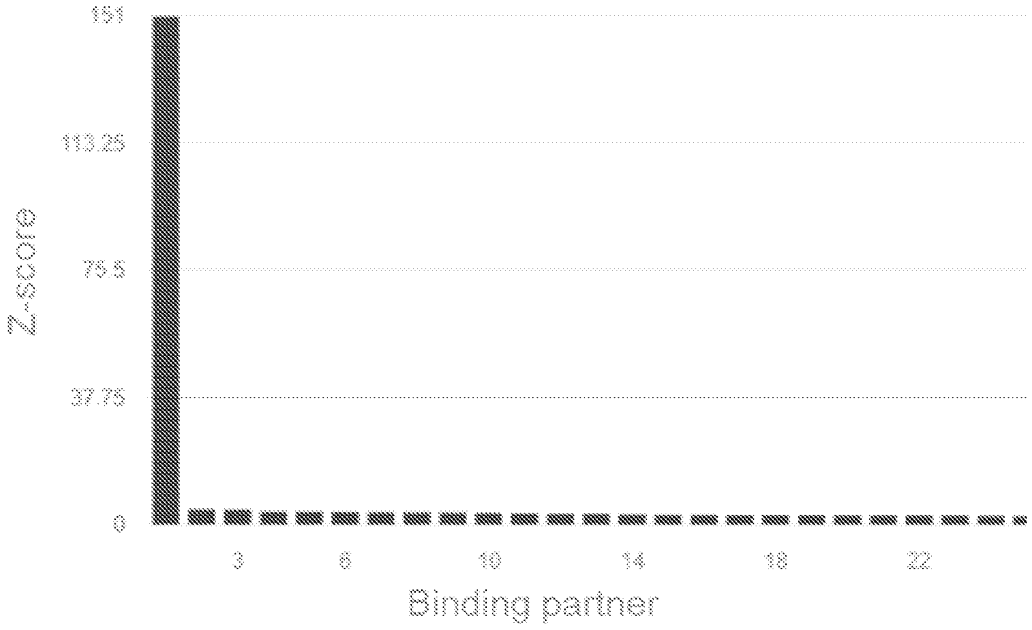


FIG. 157B Continued

FIG. 158



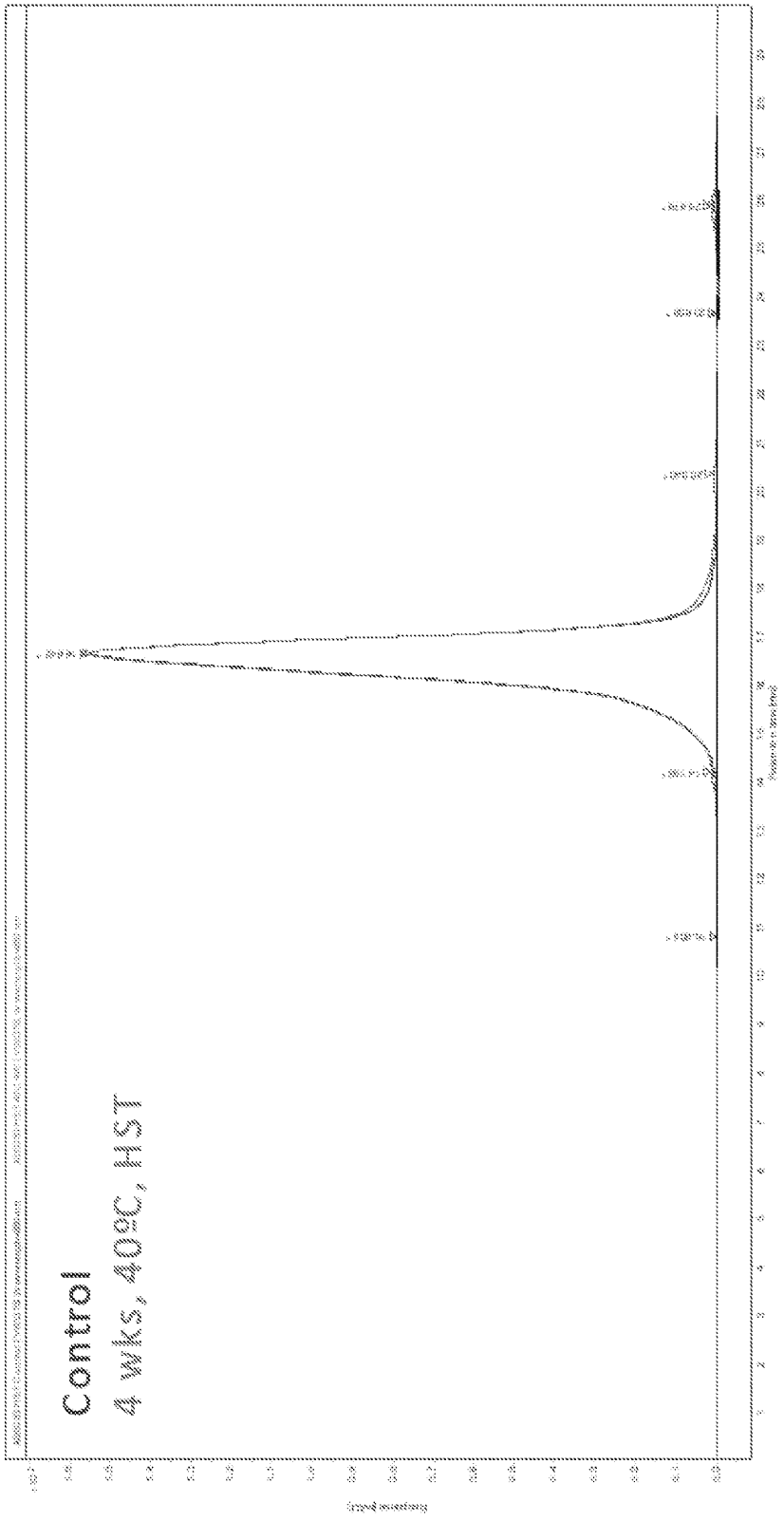


FIG. 159

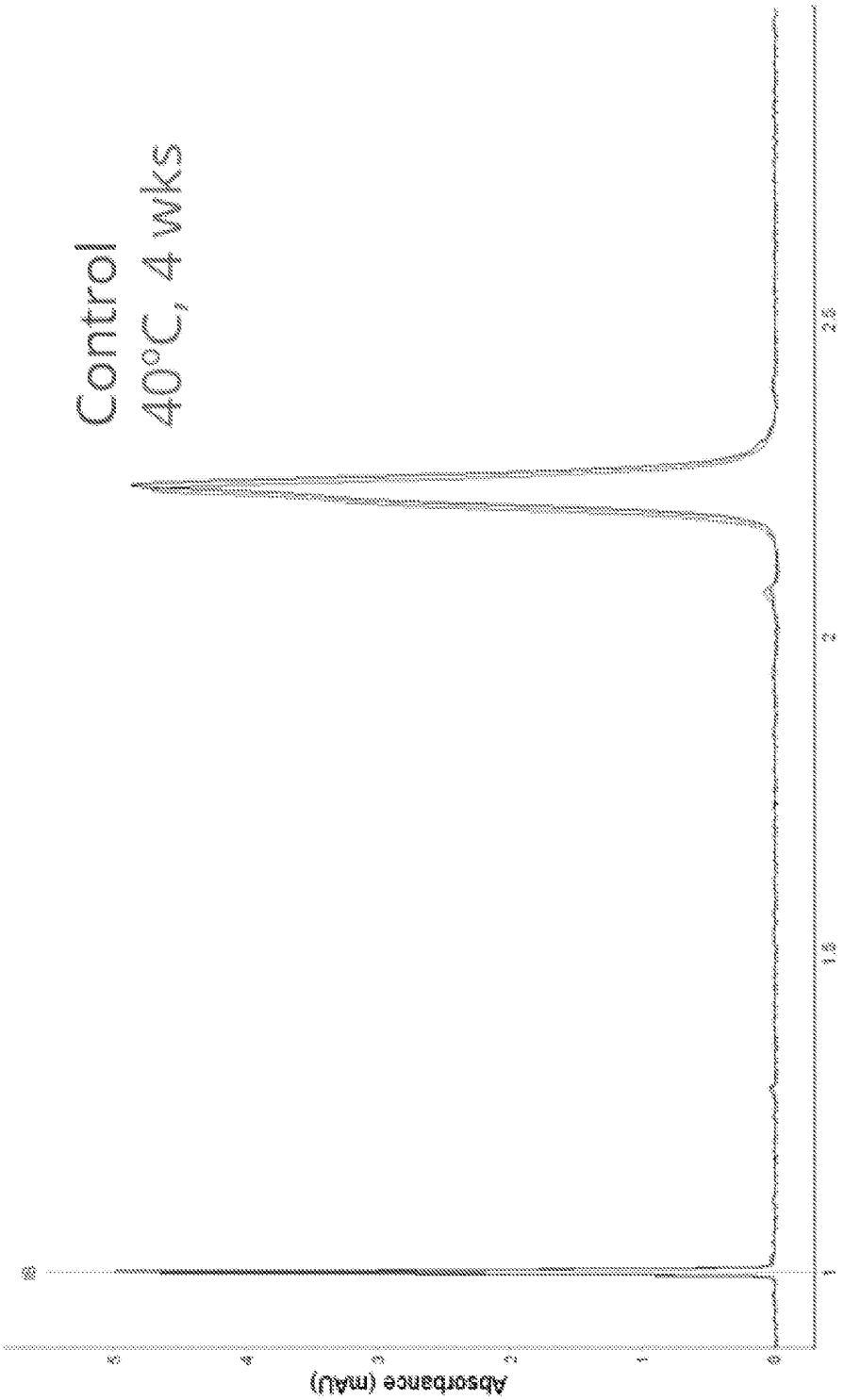


FIG. 160

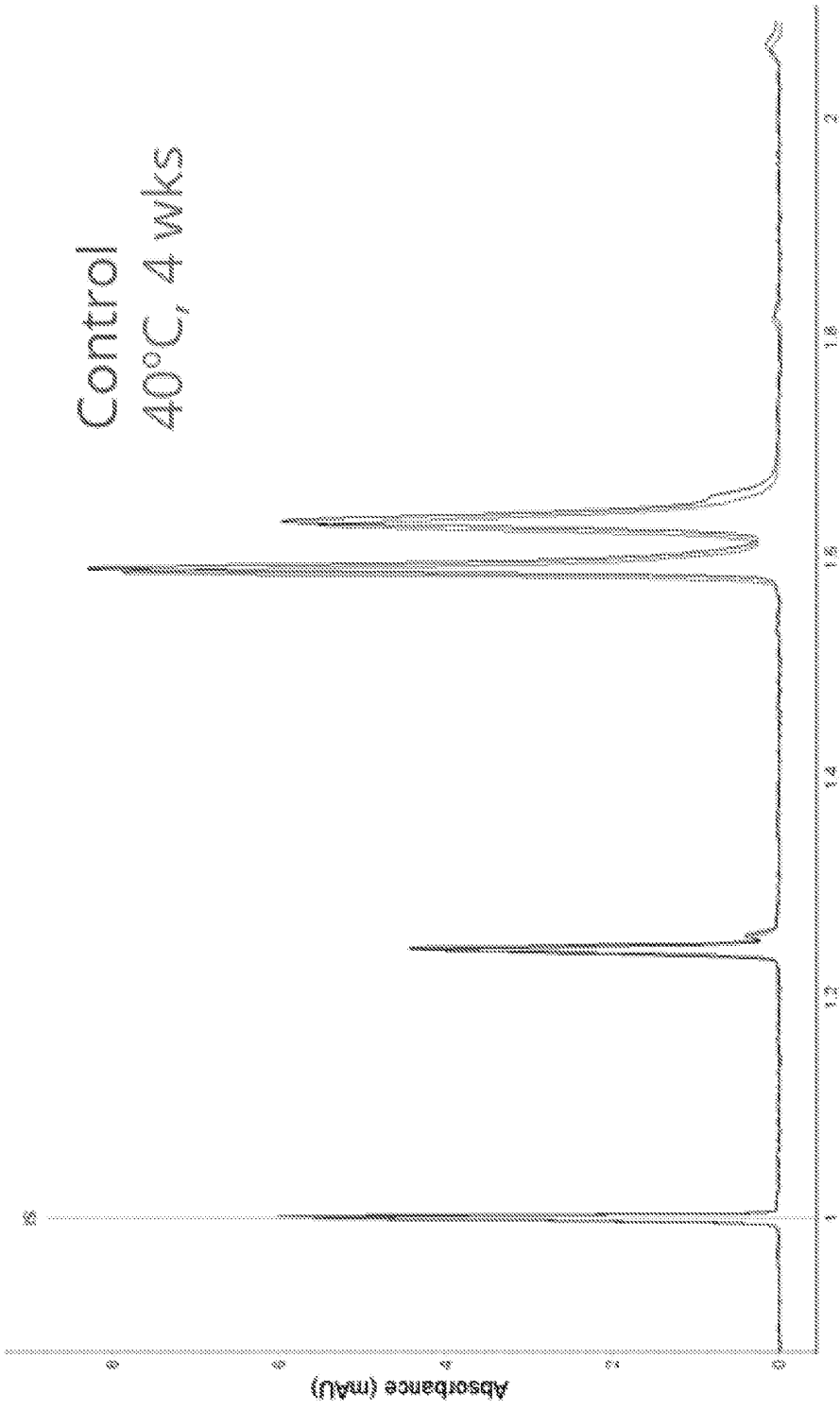
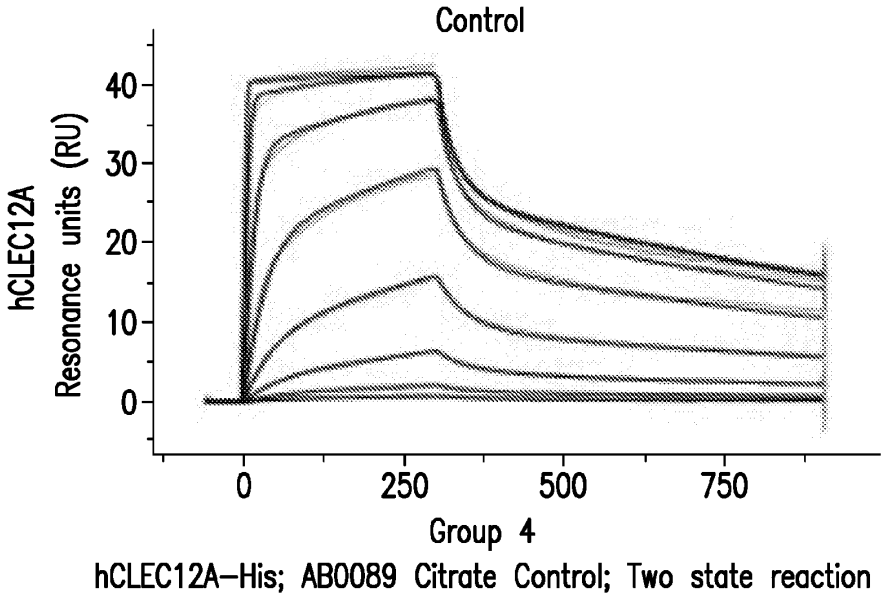


FIG. 160 Continued


Control
hCLEC12A
Resonance units (RU)
40
30
20
10
0
0
250
500
750
Group 4
hCLEC12A–His; AB0089 Citrate Control; Two state reaction

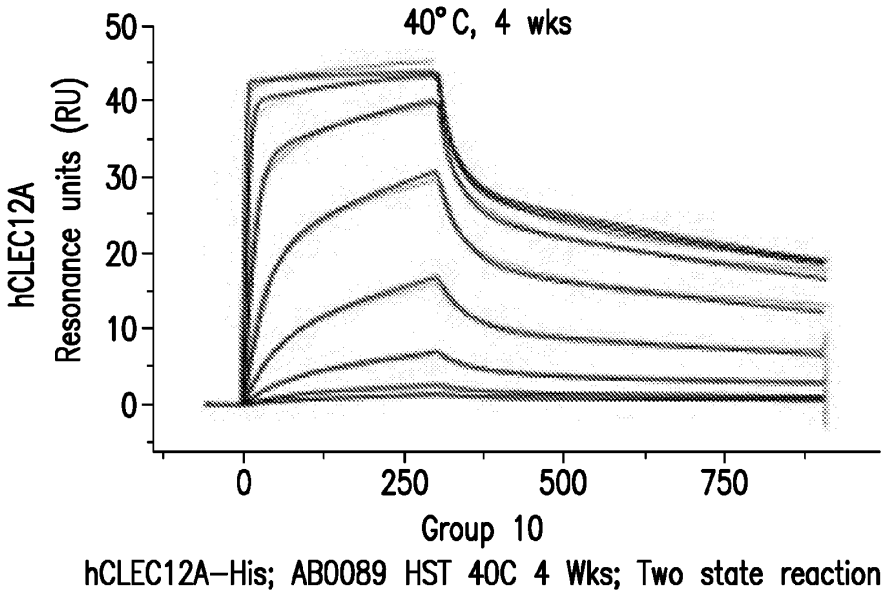
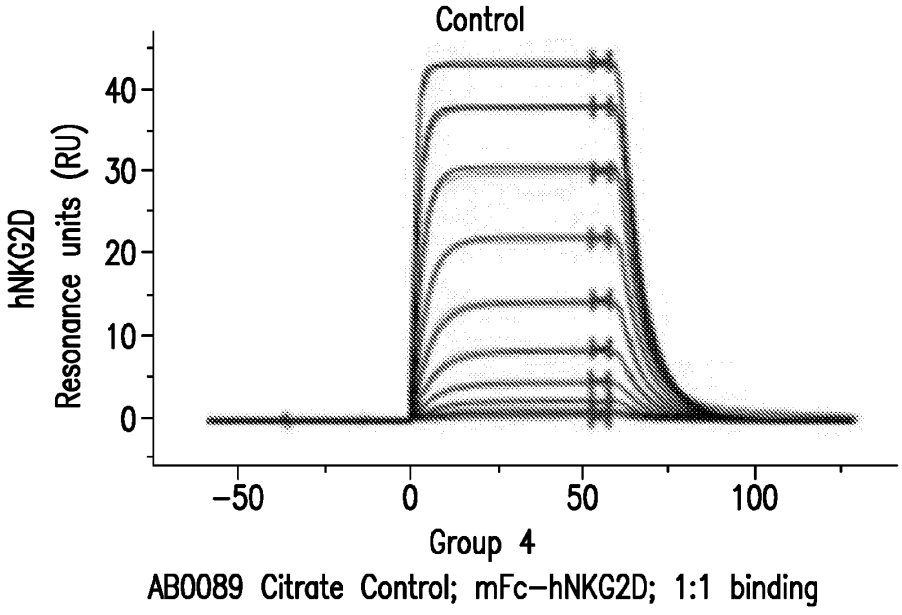

40° C, 4 wks
hCLEC12A
Resonance units (RU)
50
40
30
20
10
0
0
250
500
750
Group 10
hCLEC12A–His; AB0089 HST 40C 4 Wks; Two state reaction

FIG. 161A


Control
hNKG2D
Resonance units (RU)
40
30
20
10
0
-50
0
50
100
Group 4
AB0089 Citrate Control; mFc-hNKG2D; 1:1 binding

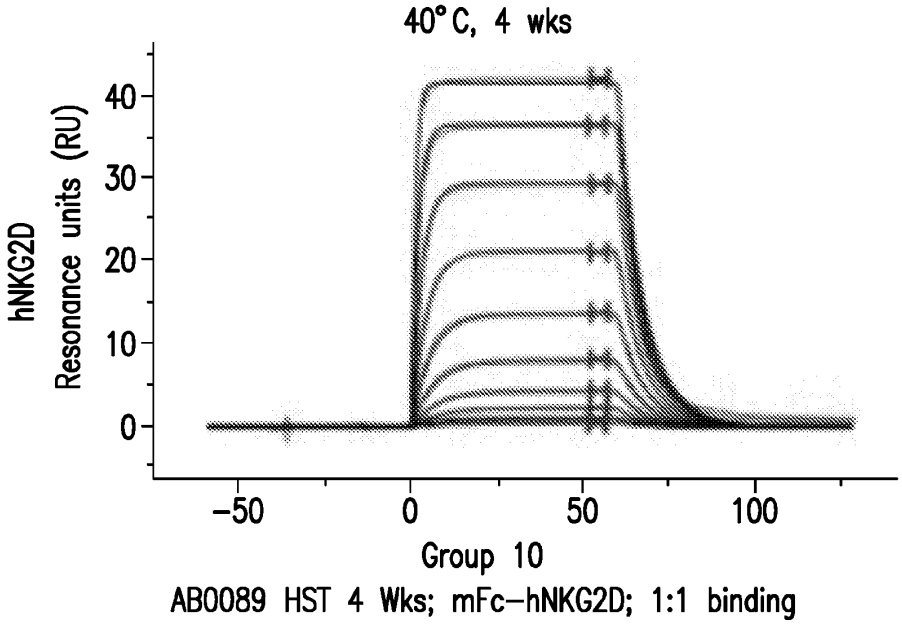

40° C, 4 wks
hNKG2D
Resonance units (RU)
40
30
20
10
0
-50
0
50
100
Group 10
AB0089 HST 4 Wks; mFc-hNKG2D; 1:1 binding

FIG. 161B

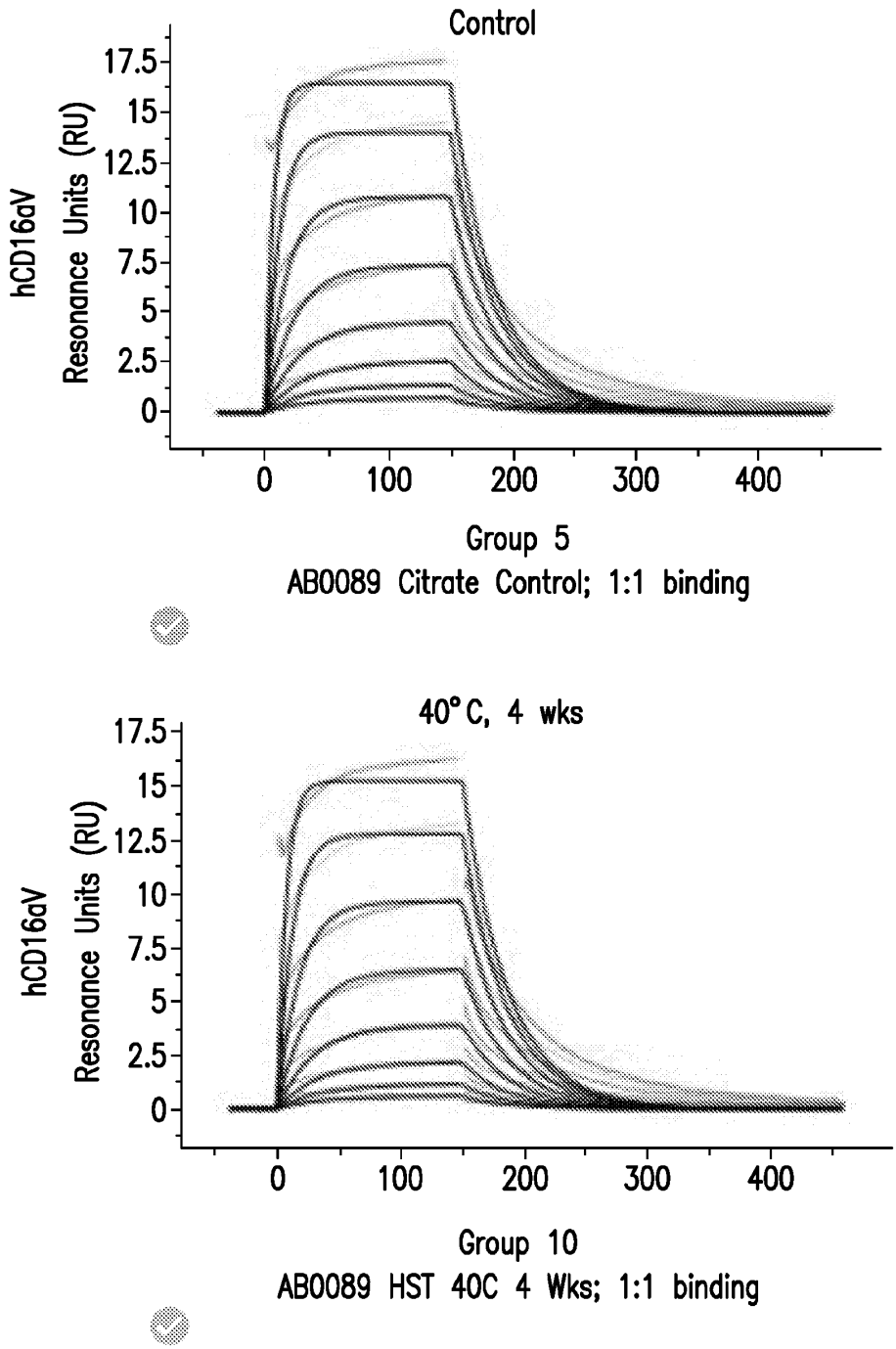
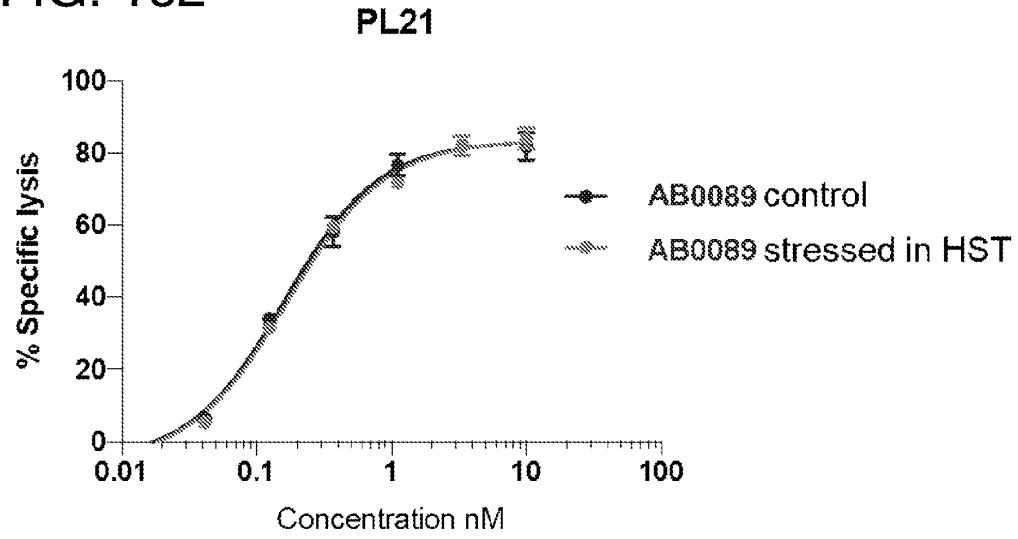

Control
hCD16aV
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
0
100
200
300
400
Group 5
AB0089 Citrate Control; 1:1 binding
40° C, 4 wks
hCD16aV
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
0
100
200
300
400
Group 10
AB0089 HST 40C 4 Wks; 1:1 binding

FIG. 161C

FIG. 162


PL21
% Specific lysis
100
80
60
40
20
0
0.01
0.1
1
10
100
Concentration nM
AB0089 control
AB0089 stressed in HST

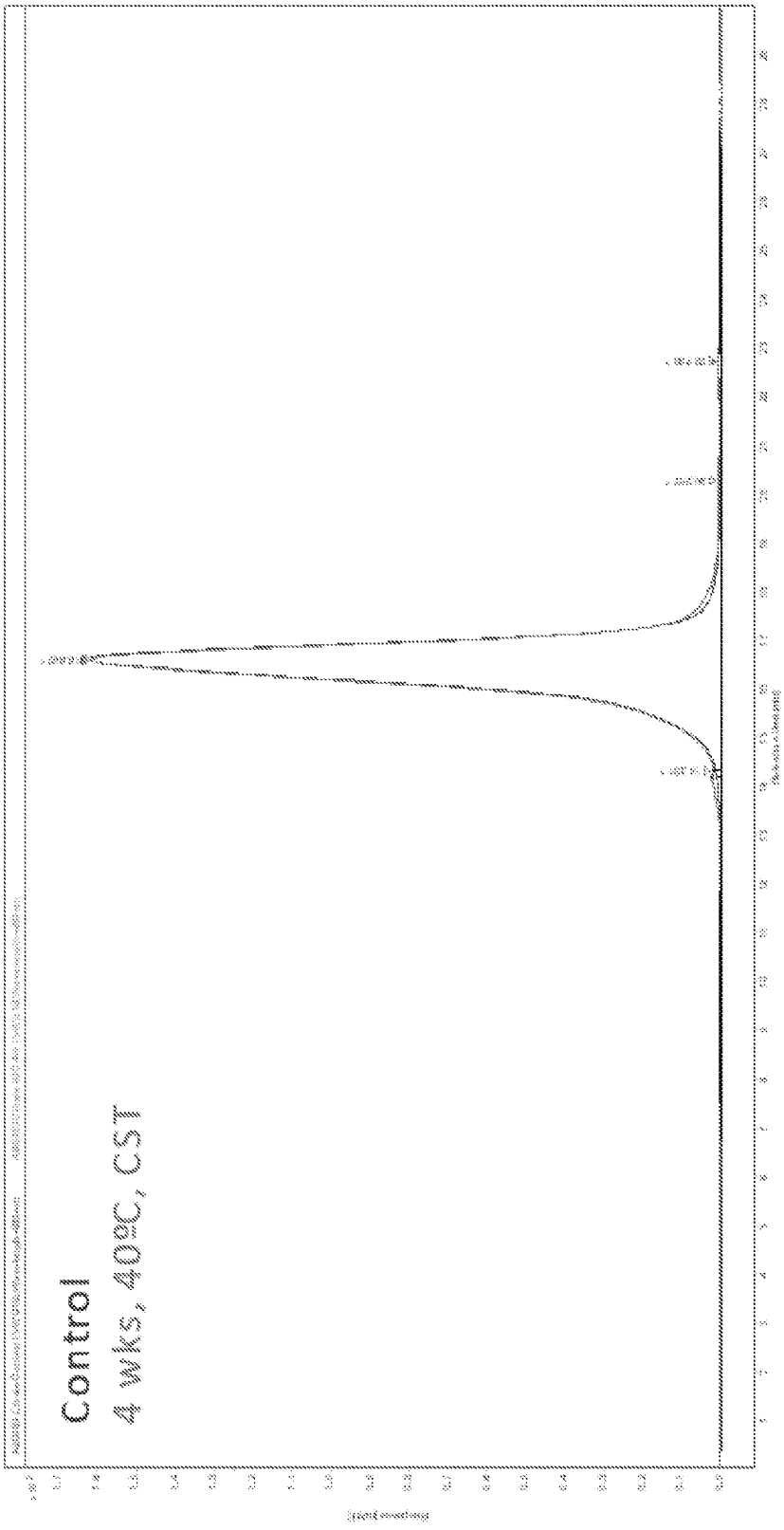


FIG. 163

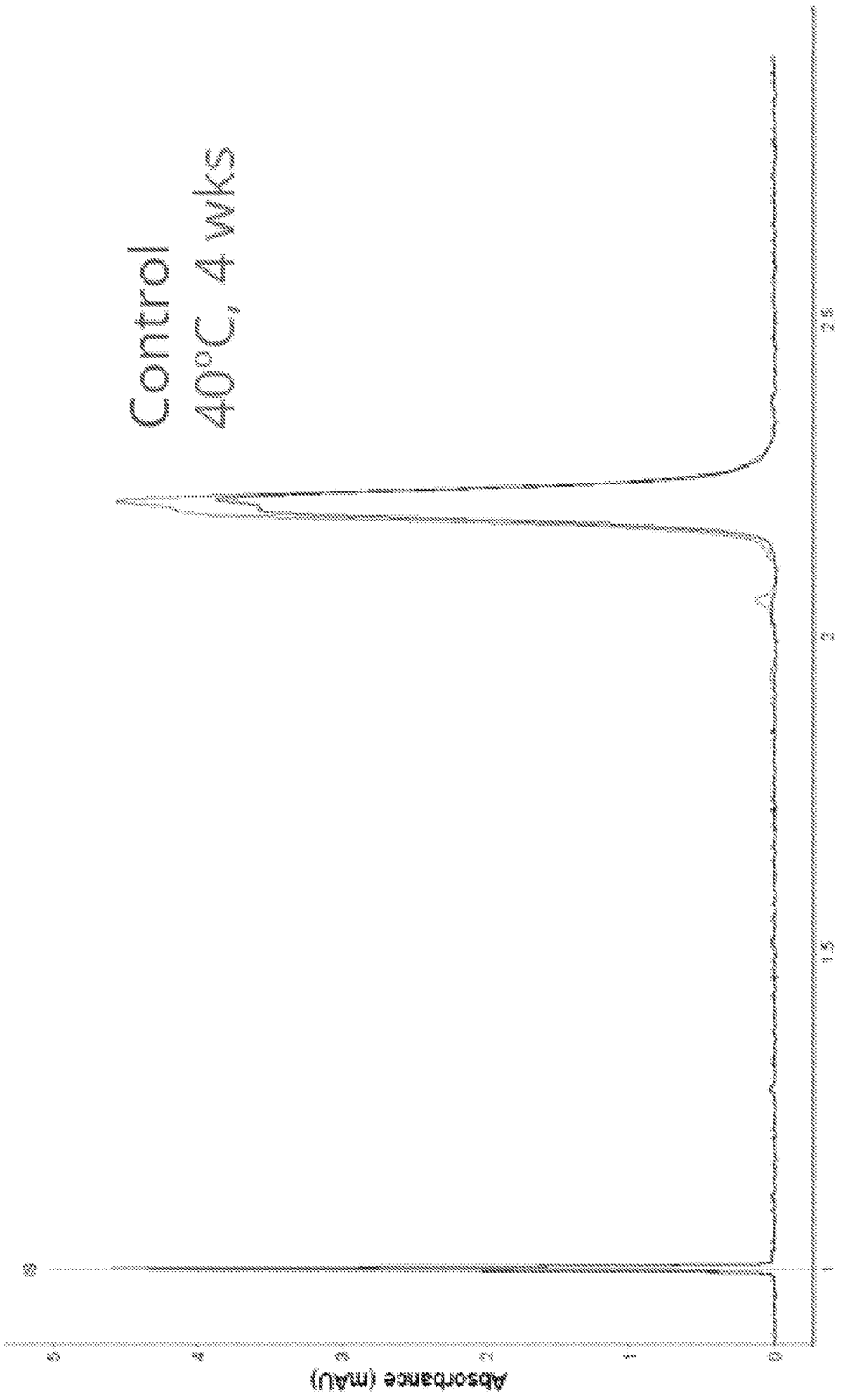


FIG. 164

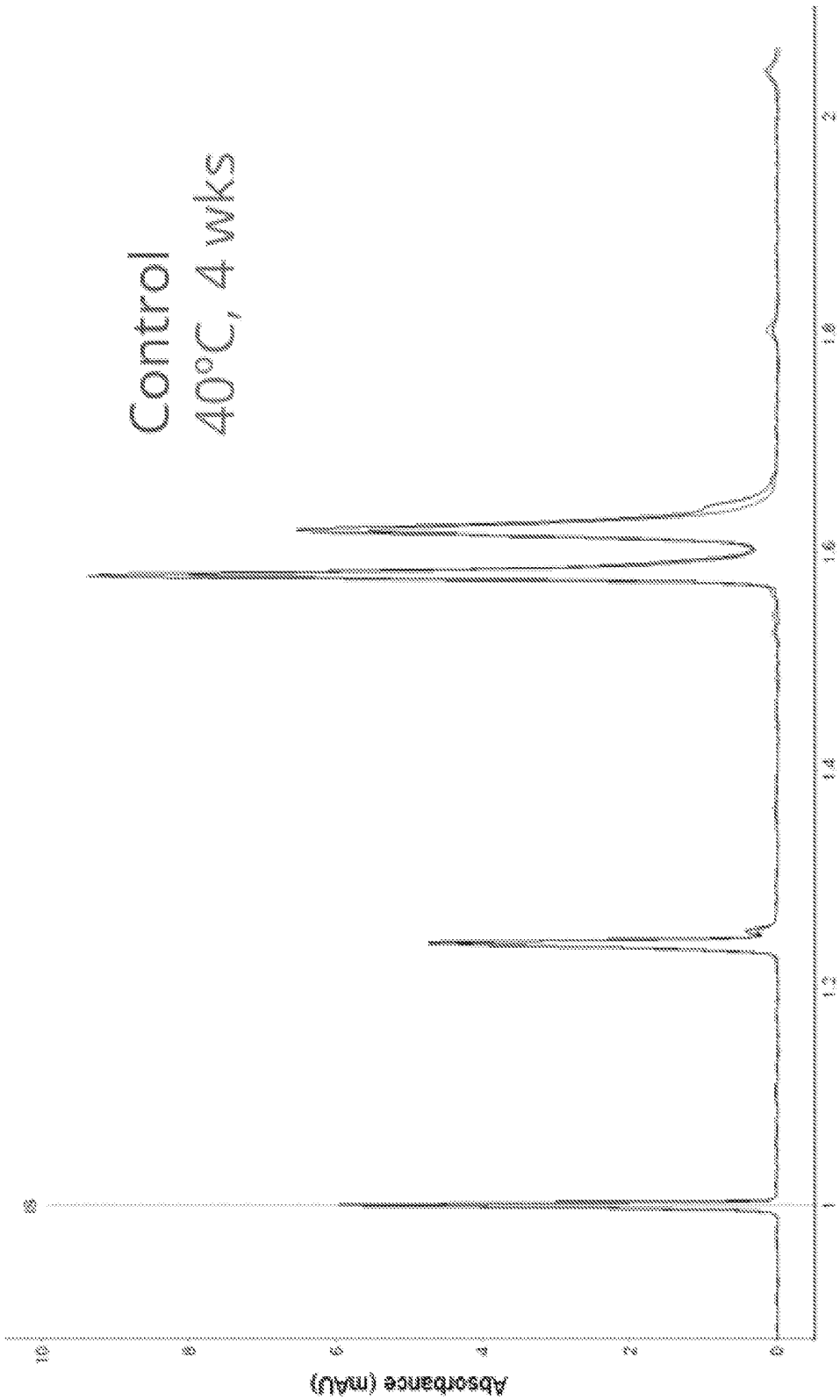
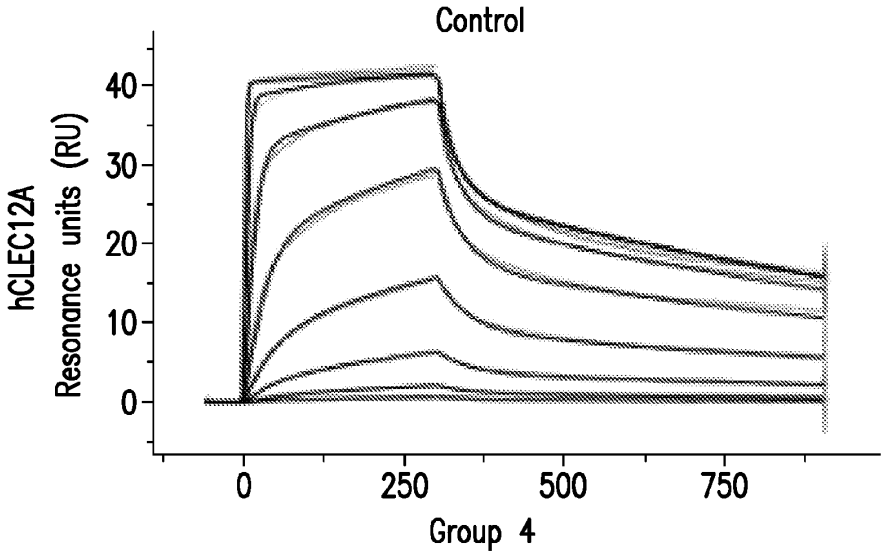
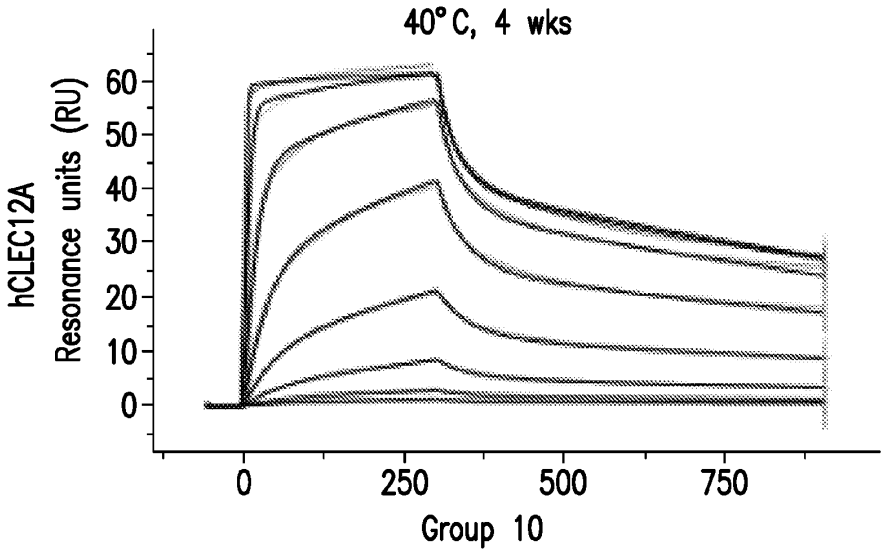


FIG. 164 Continued

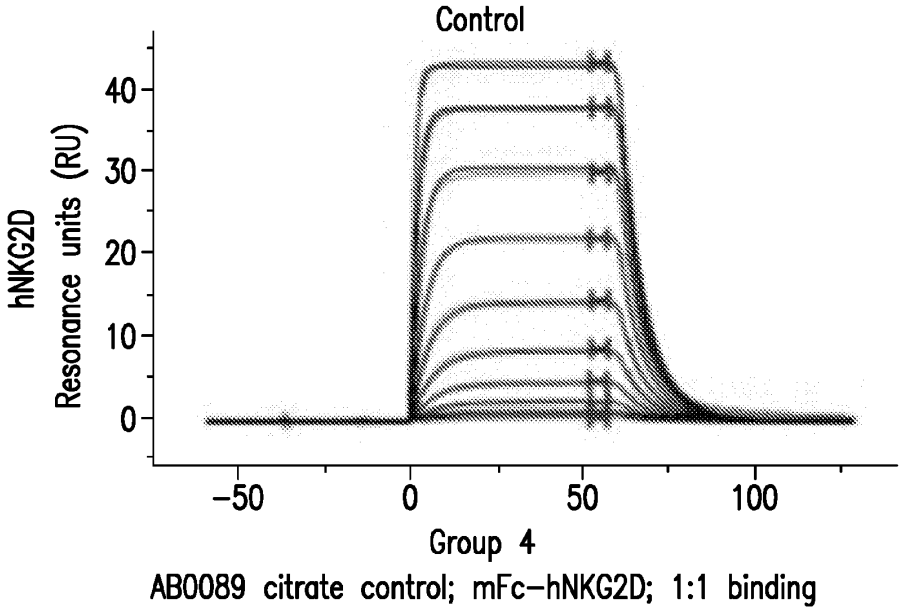


hCLEC12A–His; AB0089 Citrate Control; Two state reaction



hCLEC12A–His; AB0089 Citrate 40C 4 Wks; Two state reaction

FIG. 165A


Control
hNKG2D
Resonance units (RU)
40
30
20
10
0
−50
0
50
100
Group 4
AB0089 citrate control; mFc−hNKG2D; 1:1 binding

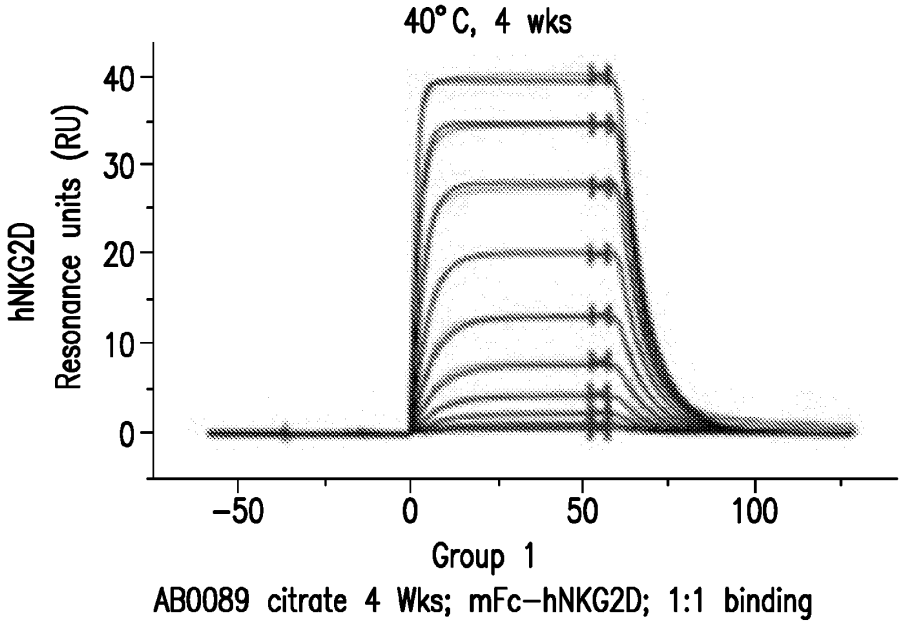
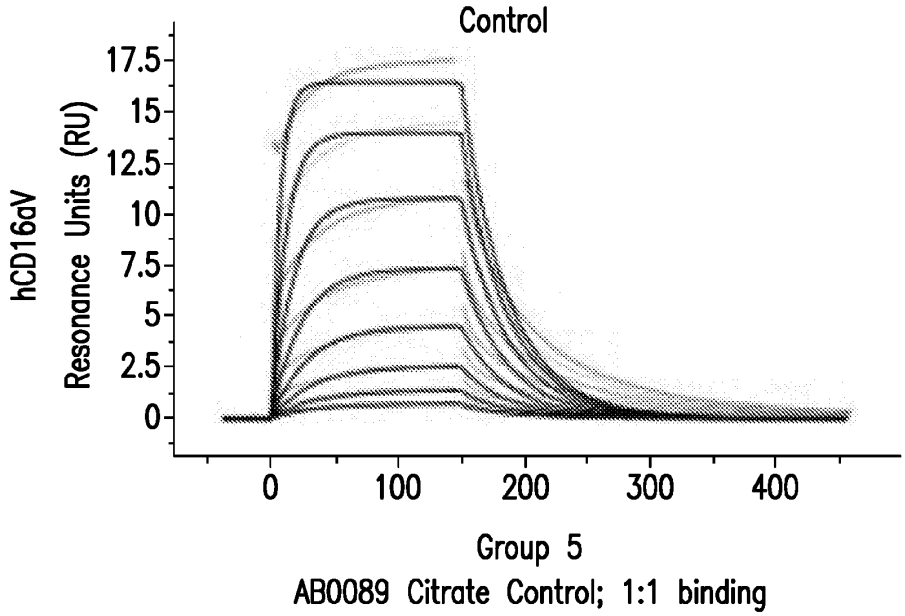

40° C, 4 wks
hNKG2D
Resonance units (RU)
40
30
20
10
0
−50
0
50
100
Group 1
AB0089 citrate 4 Wks; mFc−hNKG2D; 1:1 binding

FIG. 165B


Control
hCD16aV
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
0
100
200
300
400
Group 5
AB0089 Citrate Control; 1:1 binding

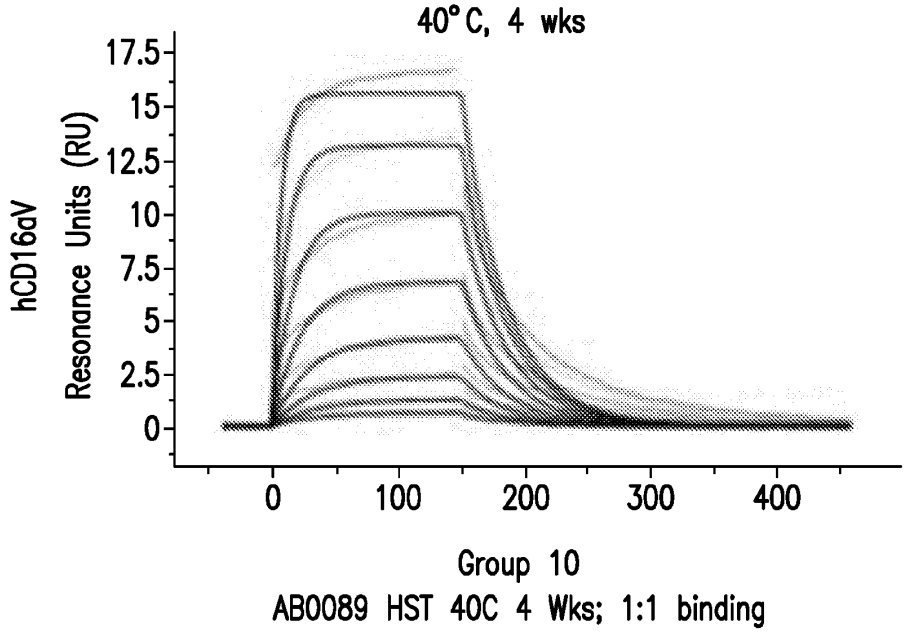
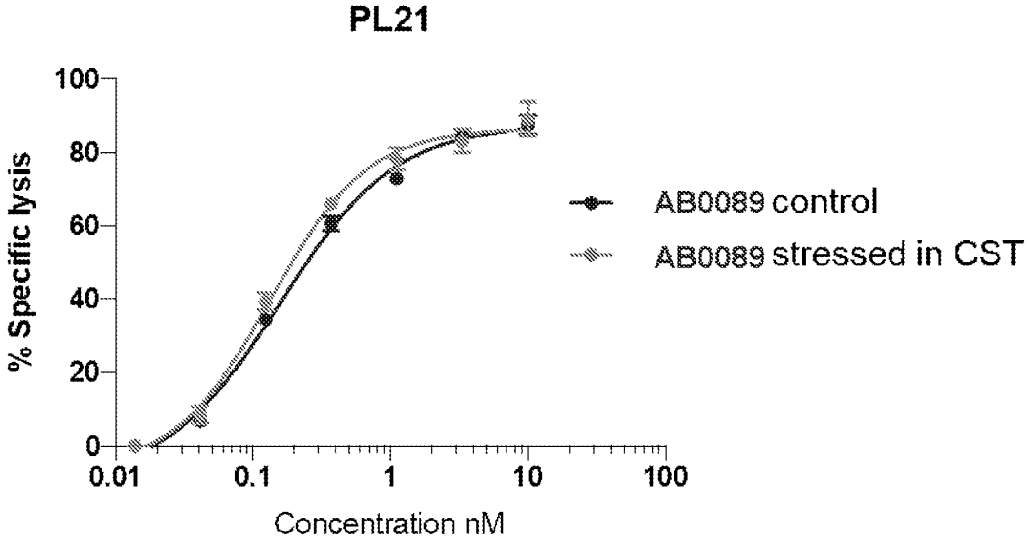

40° C, 4 wks
hCD16aV
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
0
100
200
300
400
Group 10
AB0089 HST 40C 4 Wks; 1:1 binding

FIG. 165C

FIG. 166


PL21
% Specific lysis
100
80
60
40
20
0
0.01
0.1
1
10
100
Concentration nM
AB0089 control
AB0089 stressed in CST

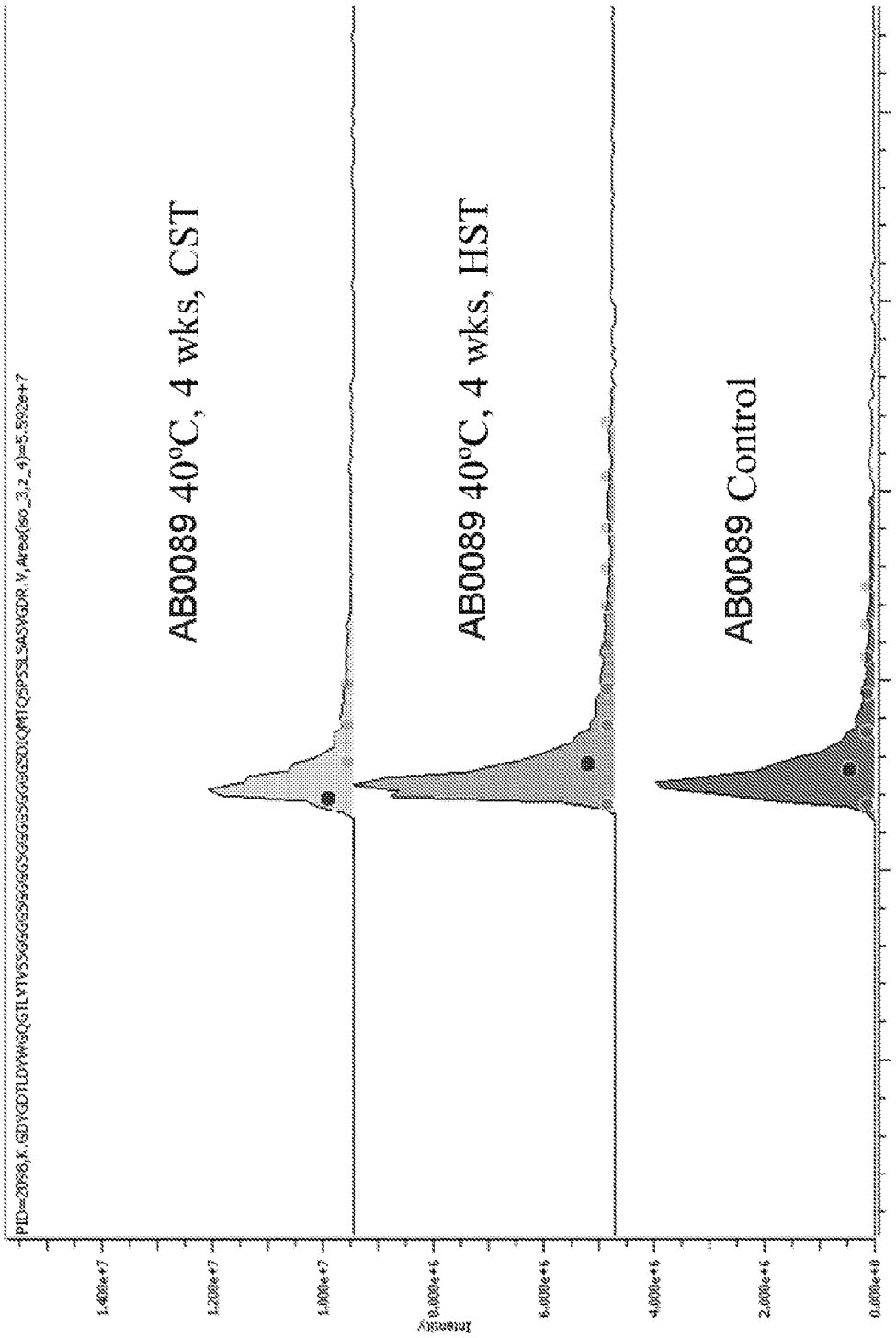

AB0089 40°C, 4 wks, CST
AB0089 40°C, 4 wks, HST
AB0089 Control
Intensity
1.400e+7
1.200e+7
1.000e+7
8.000e+6
6.000e+6
4.000e+6
2.000e+6
0.000e+0

FIG. 167

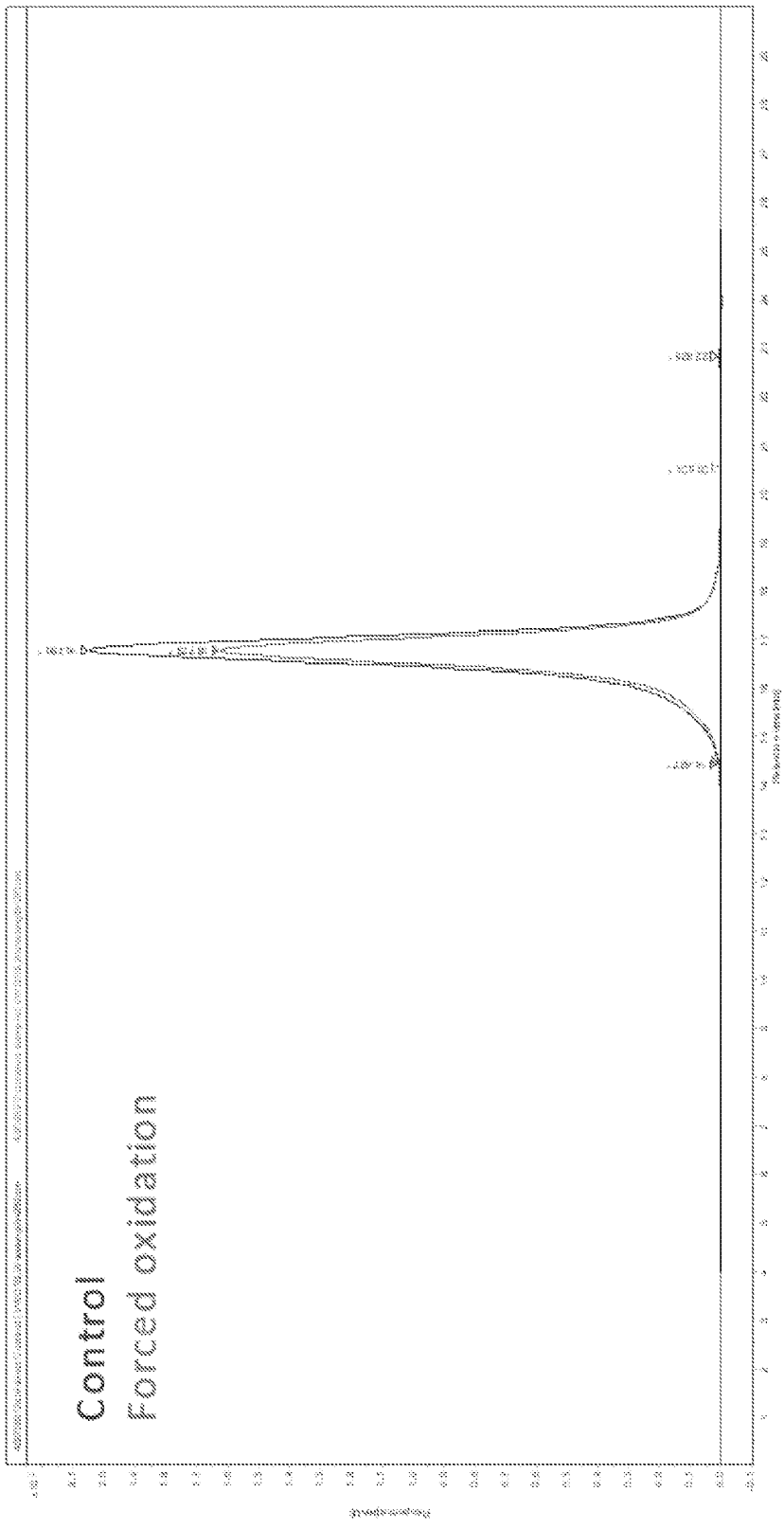


FIG. 168

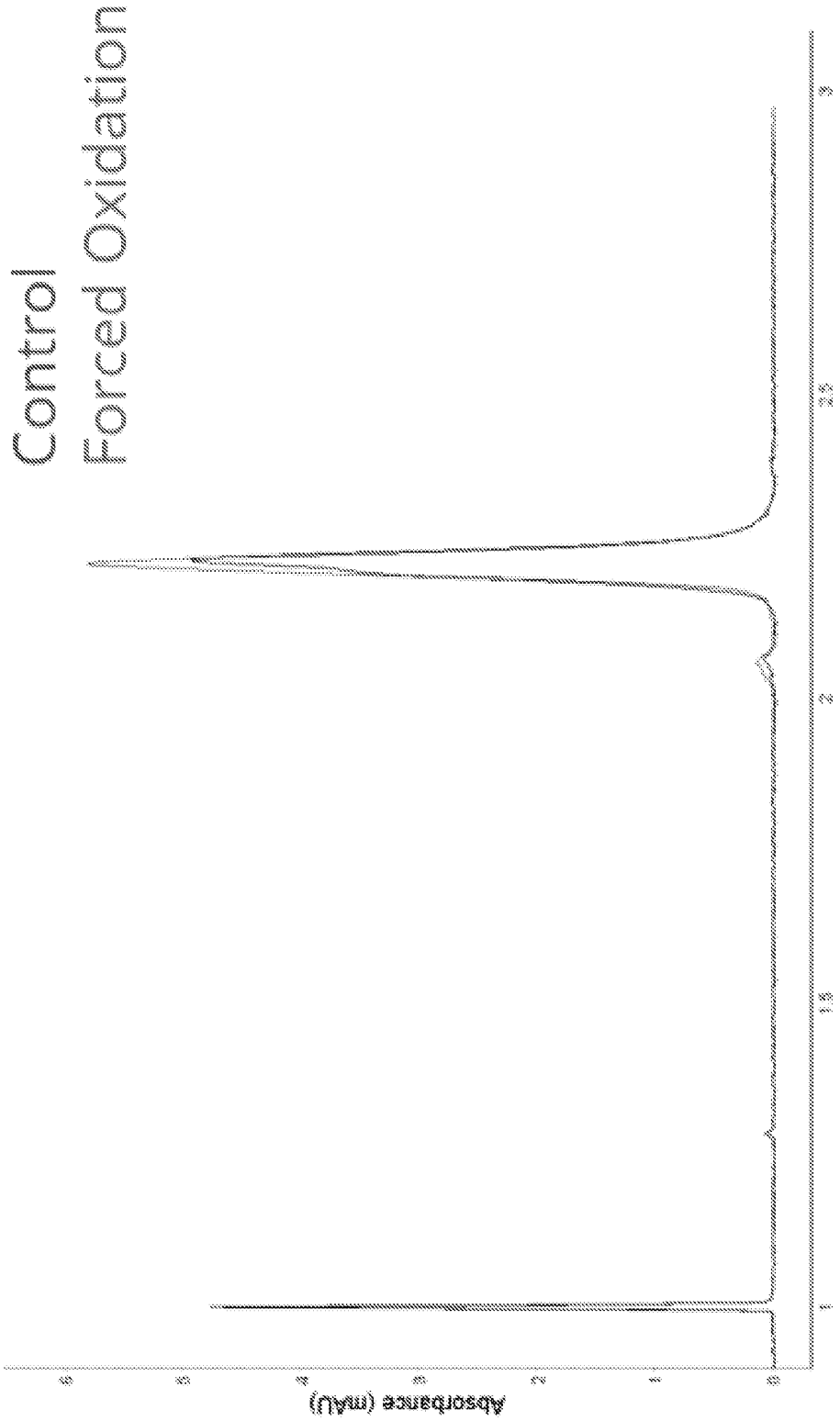

Control
Forced Oxidation
Absorbance (mAU)
Relative Migration Time

FIG. 169

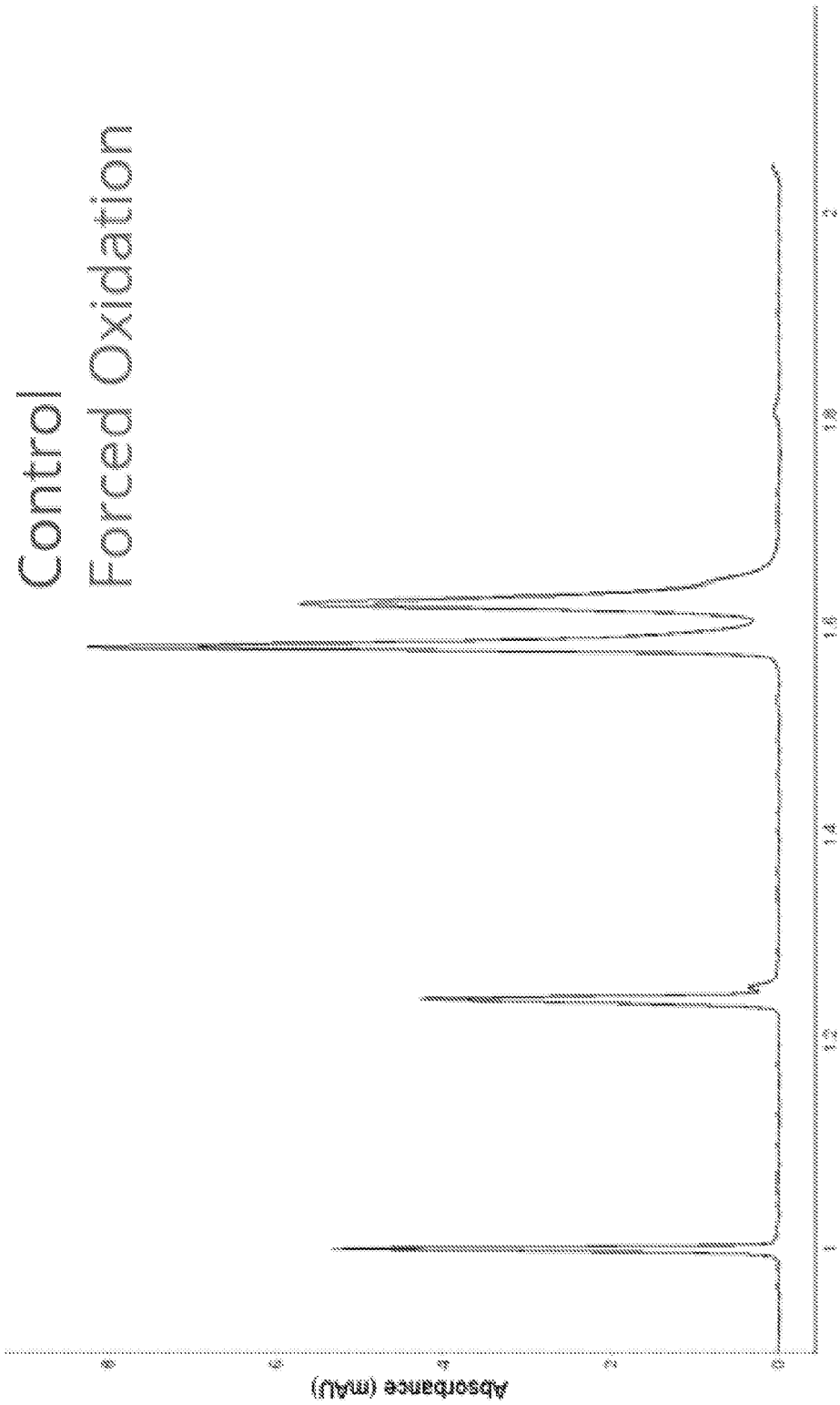
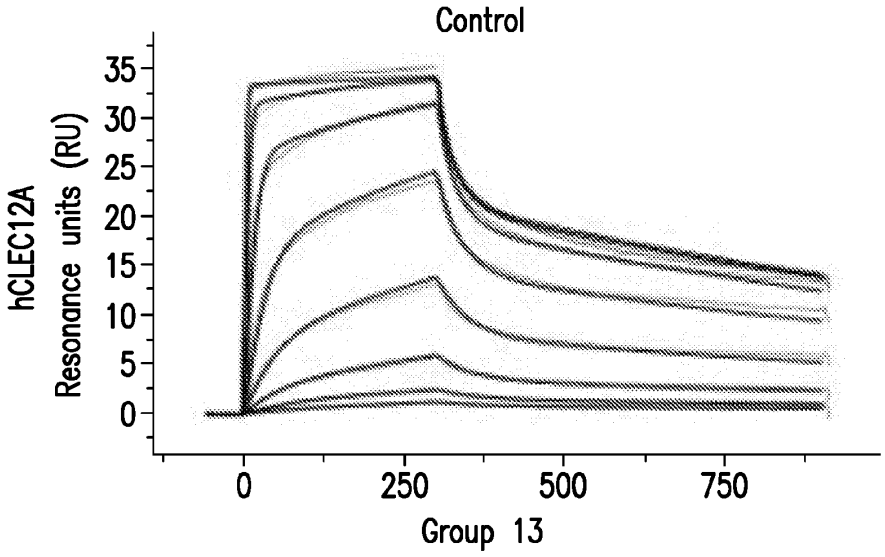
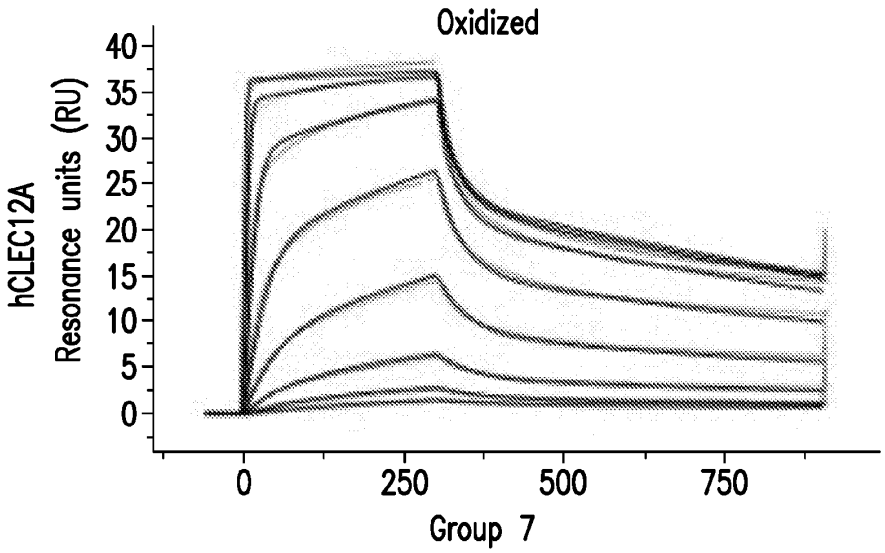


FIG. 169 Continued



hCLEC12A–His; AB0089 Oxidation Control; Two state reaction



hCLEC12A–His; AB0089 Forced Oxidation; Two state reaction

FIG. 170A

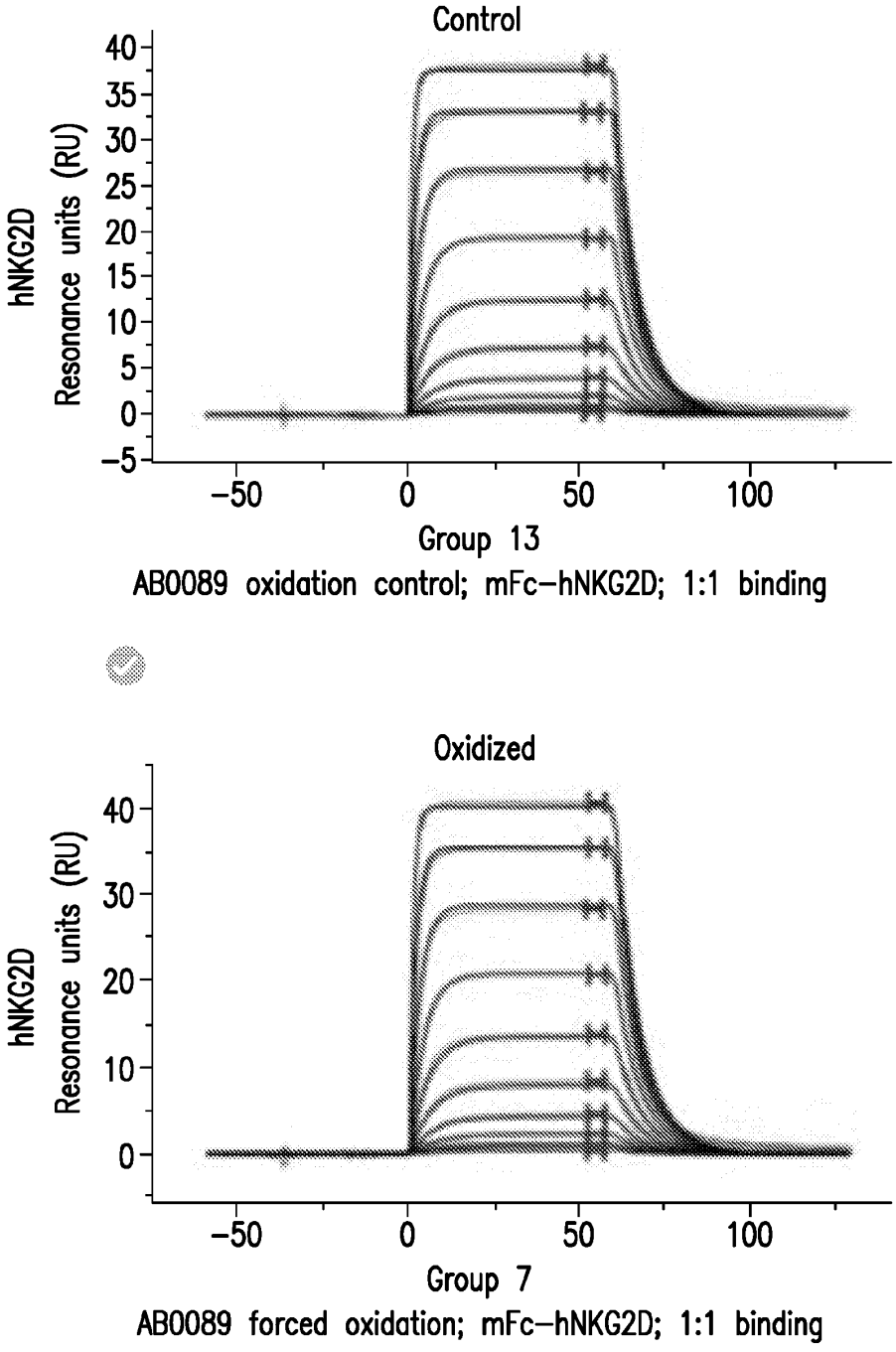
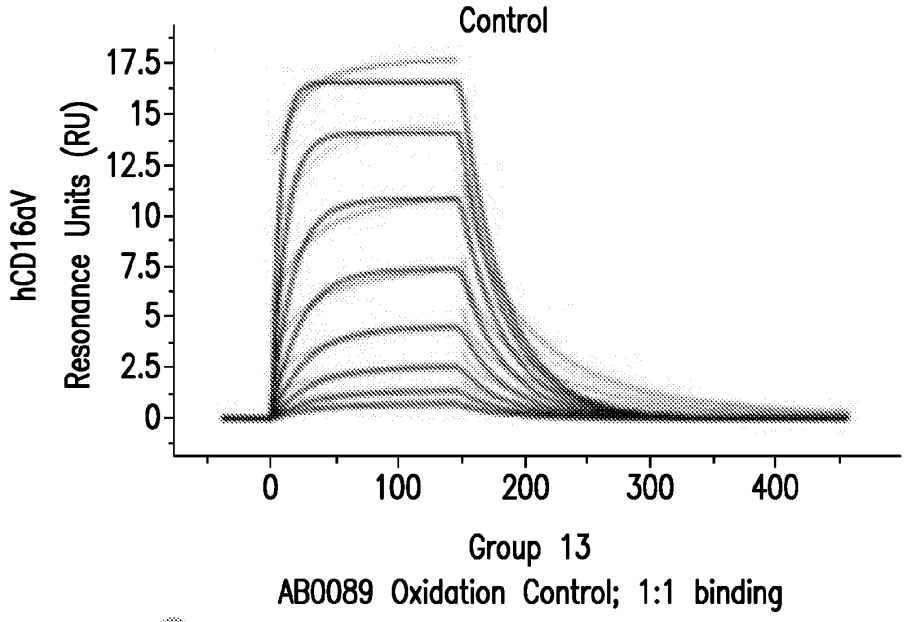

Control
hNKG2D
Resonance units (RU)
40
35
30
25
20
15
10
5
0
-5
-50
0
50
100
Group 13
AB0089 oxidation control; mFc-hNKG2D; 1:1 binding
Oxidized
hNKG2D
Resonance units (RU)
40
30
20
10
0
-50
0
50
100
Group 7
AB0089 forced oxidation; mFc-hNKG2D; 1:1 binding

FIG. 170B


Control
hCD16aV
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
0
100
200
300
400
Group 13
AB0089 Oxidation Control; 1:1 binding

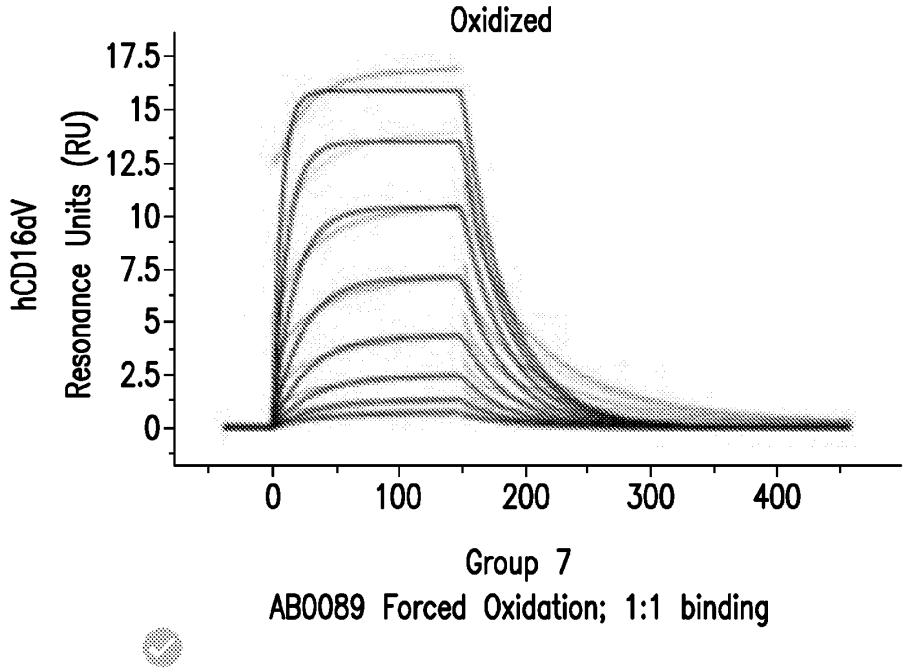
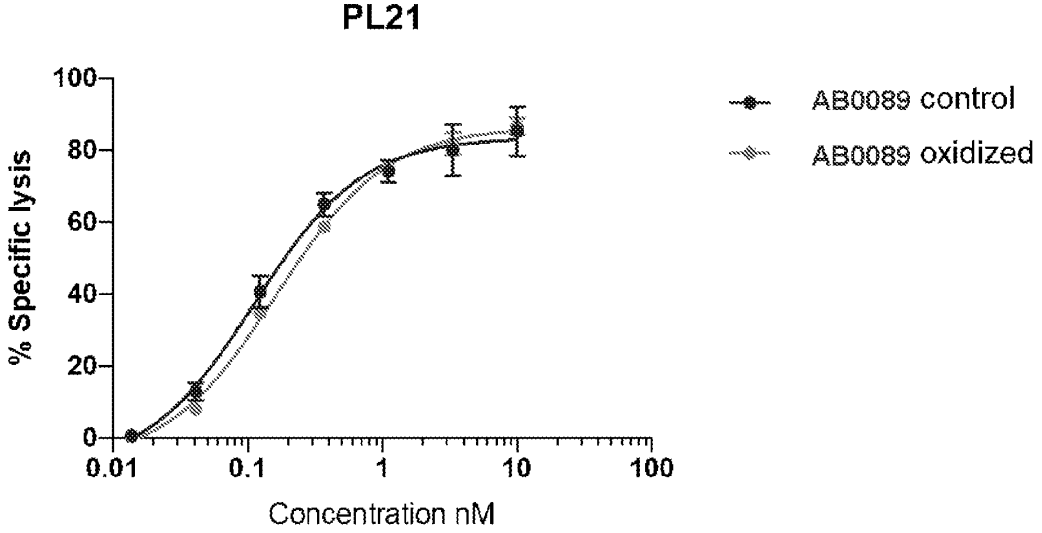

Oxidized
hCD16aV
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
0
100
200
300
400
Group 7
AB0089 Forced Oxidation; 1:1 binding

FIG. 170C

FIG. 171


PL21
% Specific lysis
100
80
60
40
20
0
0.01
0.1
1
10
100
Concentration nM
AB0089 control
AB0089 oxidized

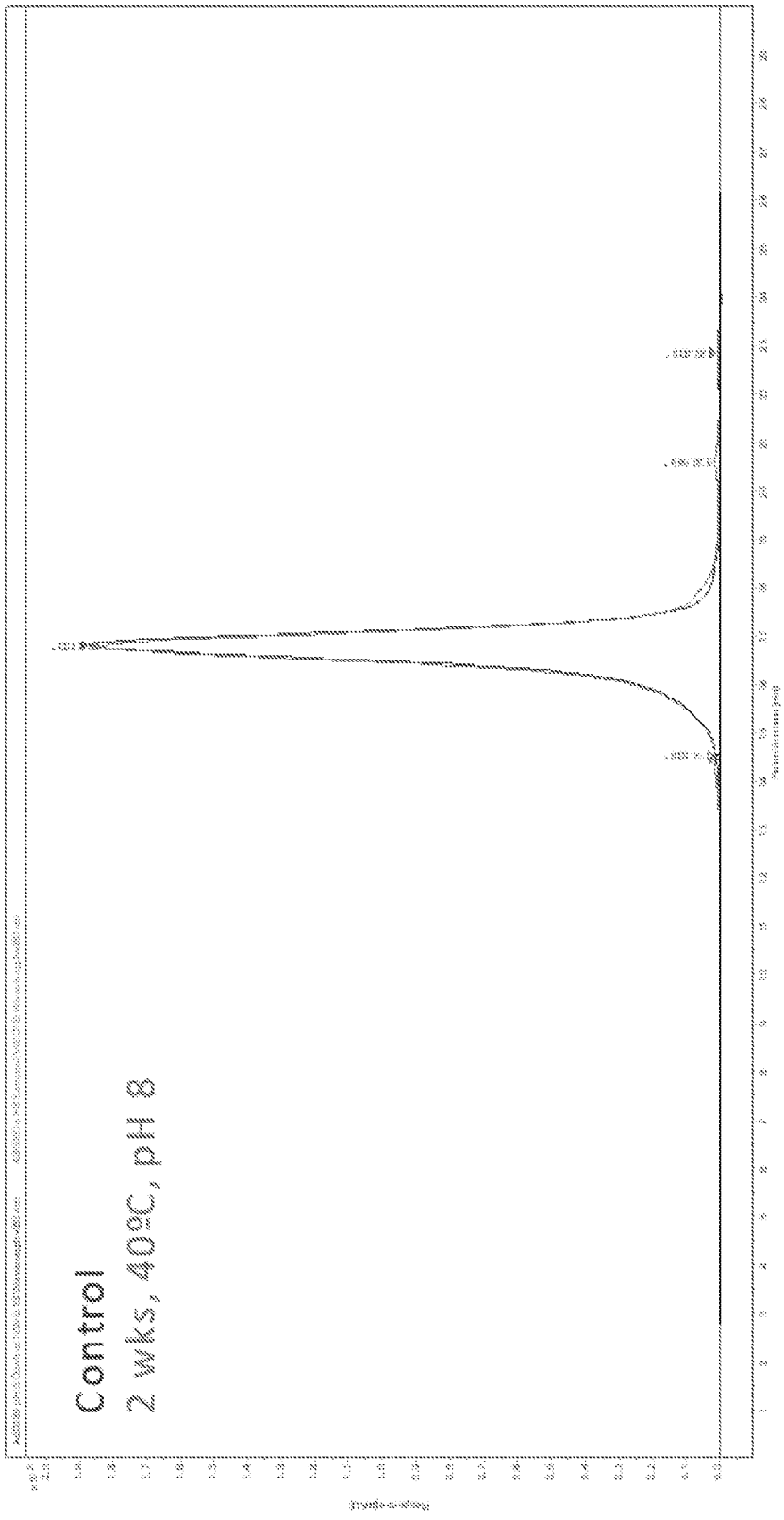


FIG. 172

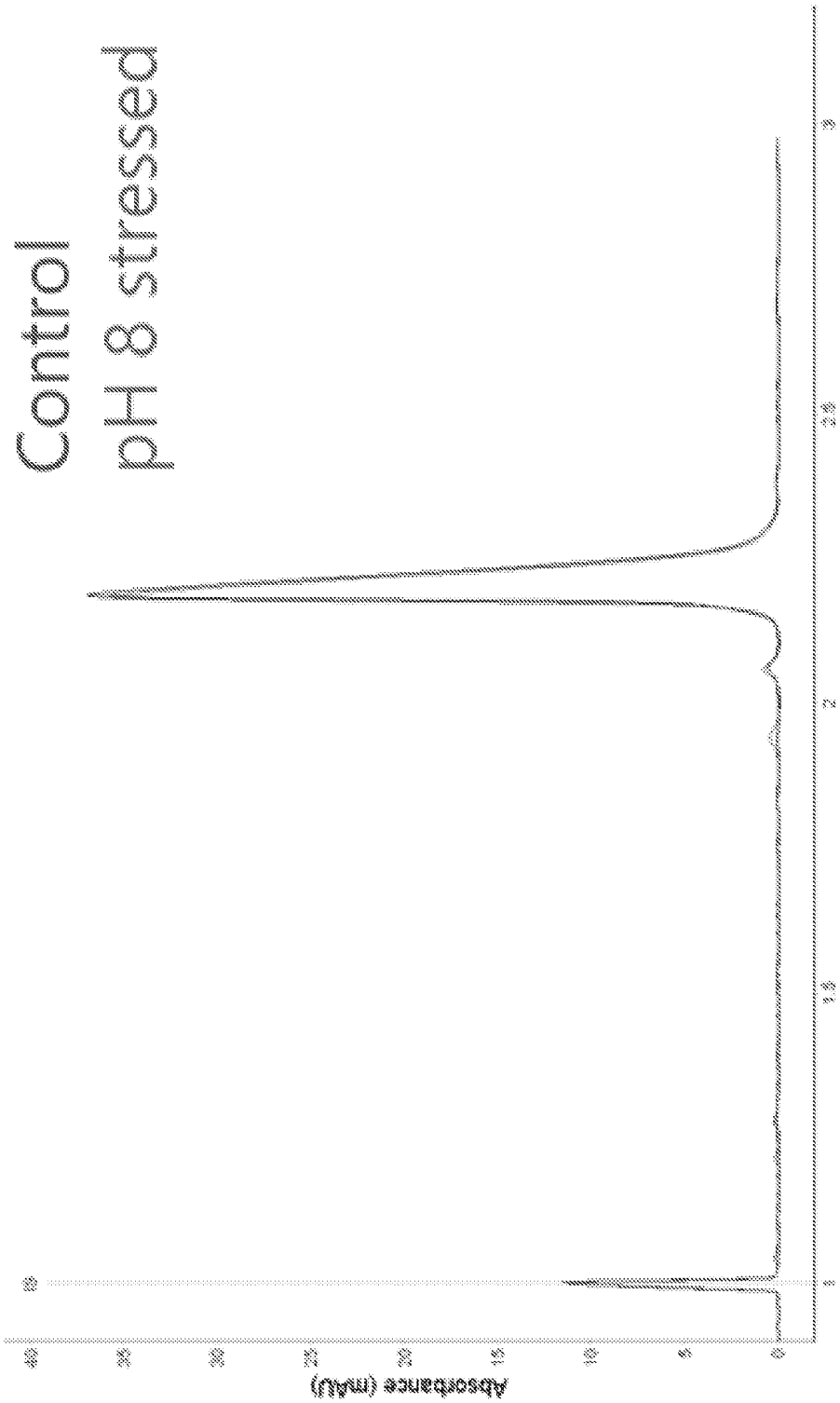

Control
pH stressed
Absorbance (mAU)
Relative Migration Time

FIG. 173

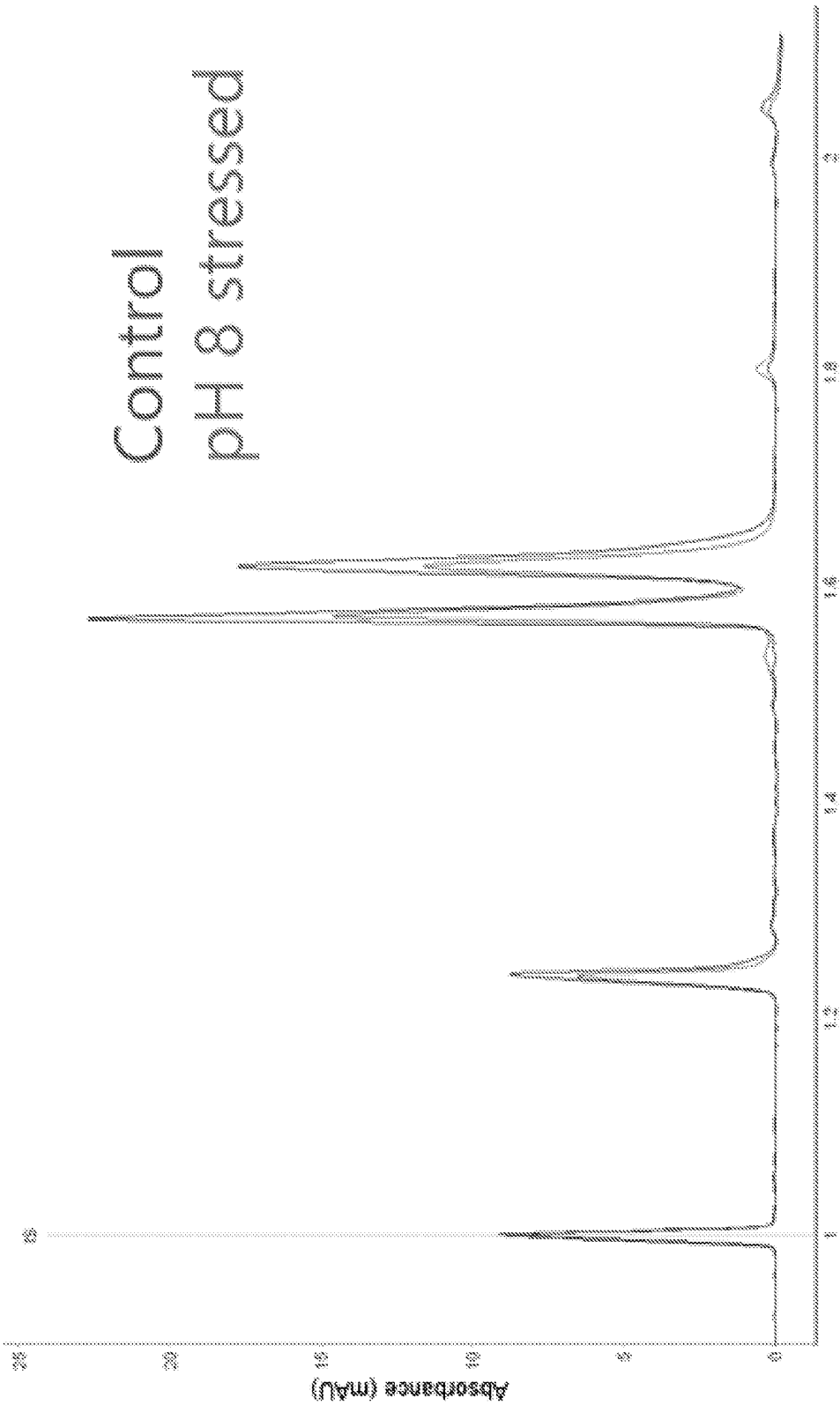


FIG. 173 Continued

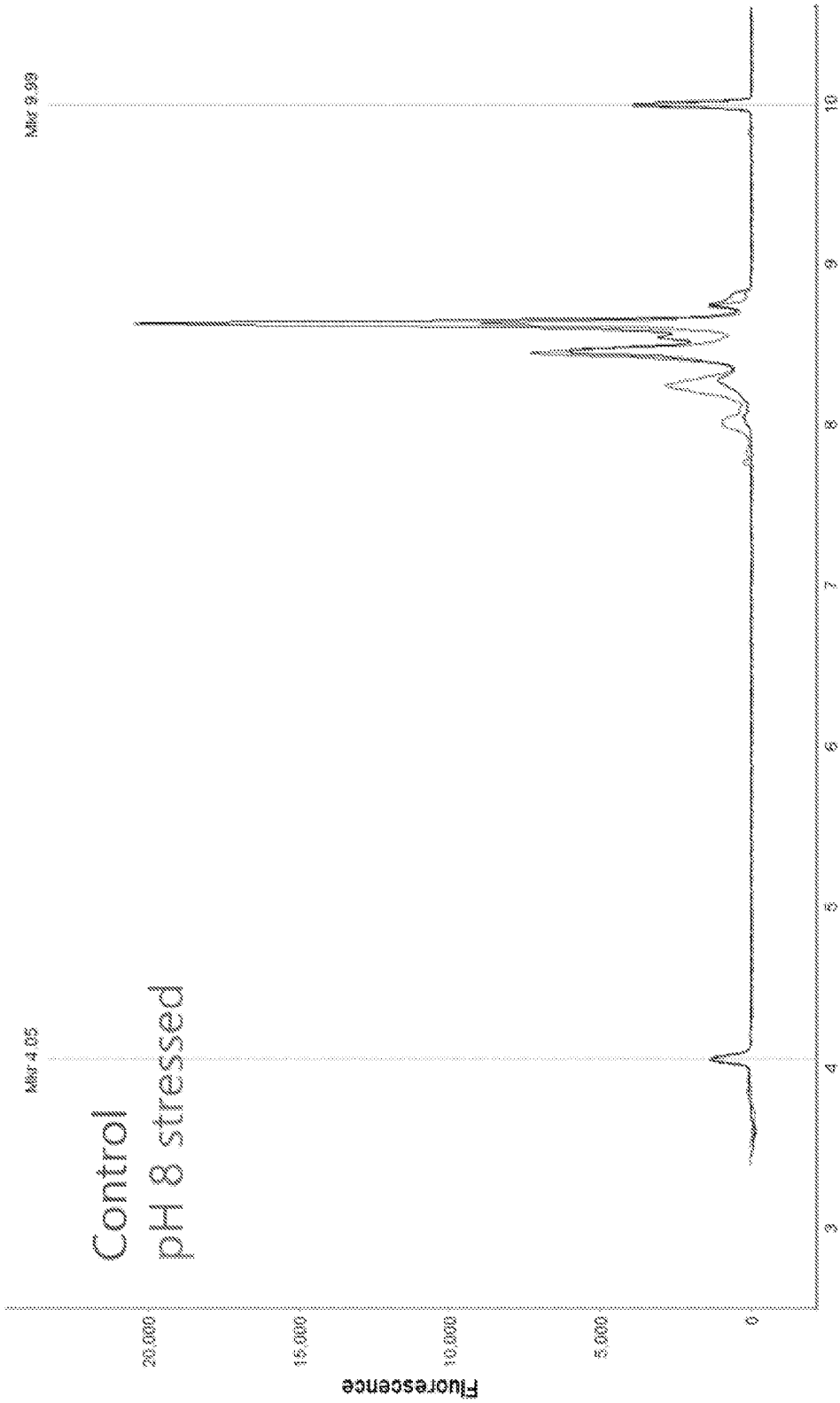
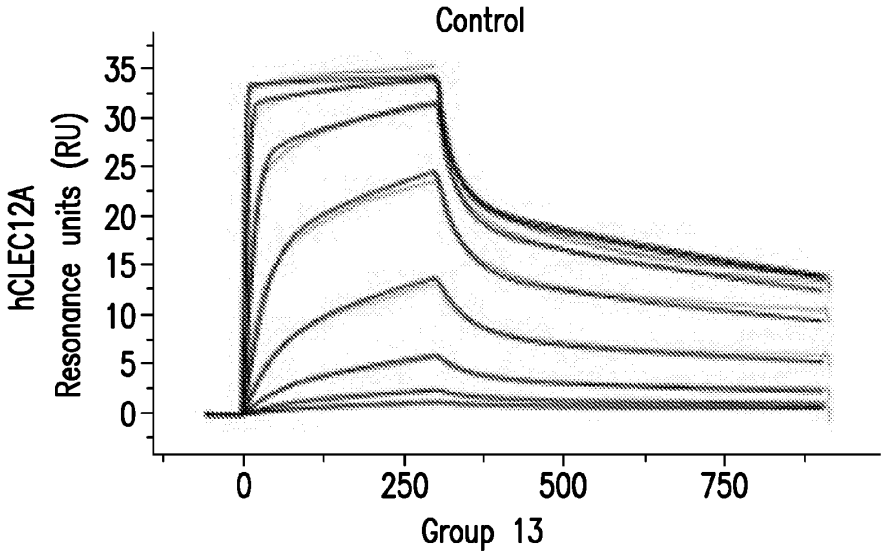
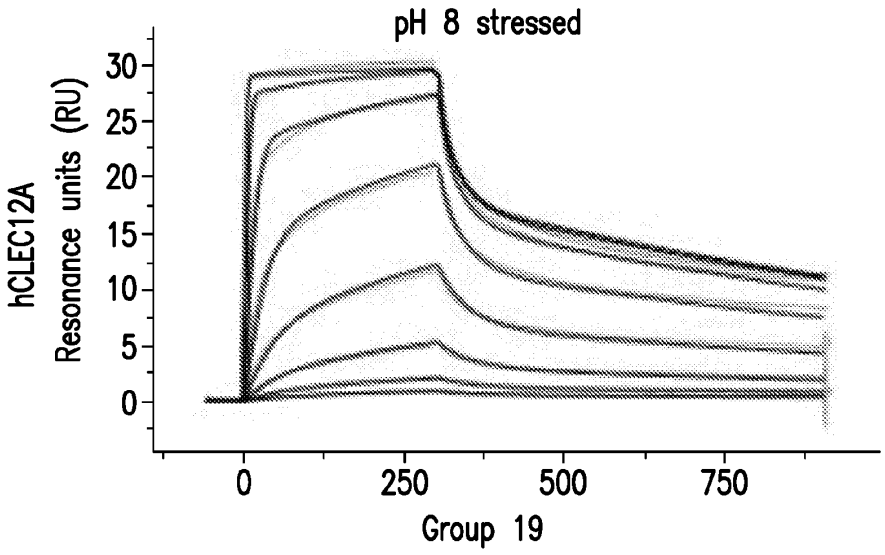

Mkr 9.99
Mkr 4.05
Control
pH 8 stressed
Fluorescence
20,000
15,000
10,000
5,000
0
3
4
5
6
7
8
9
10
pI

FIG. 174

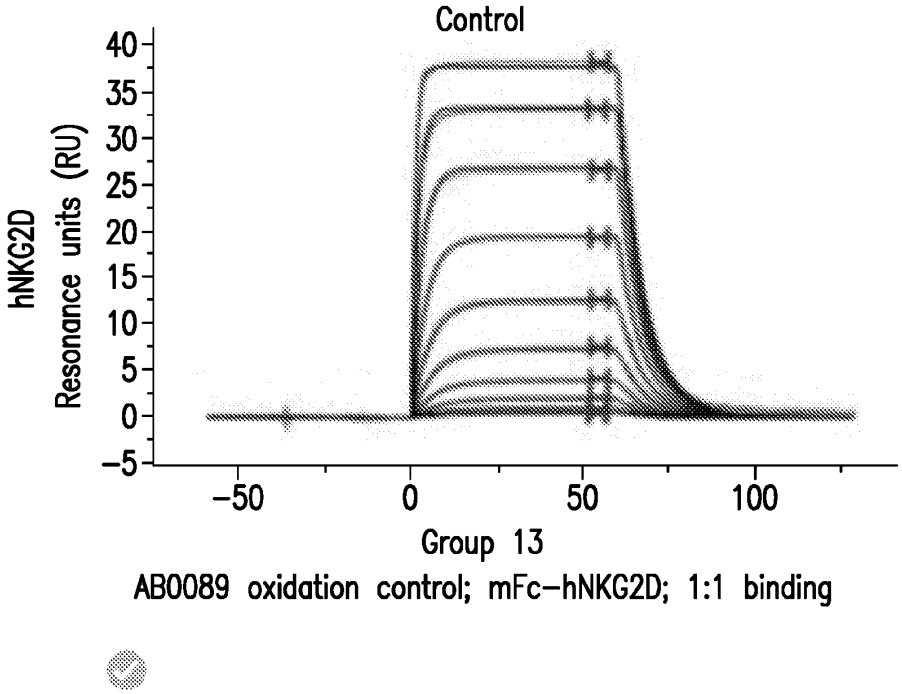


hCLEC12A–His; AB0089 Oxidation Control; Two state reaction



hCLEC12A–His; AB0089 pH 8 40C 2 Wks; Two state reaction

FIG. 175A


Control
hNKG2D
Resonance units (RU)
40
35
30
25
20
15
10
5
0
−5
−50
0
50
100
Group 13
AB0089 oxidation control; mFc−hNKG2D; 1:1 binding

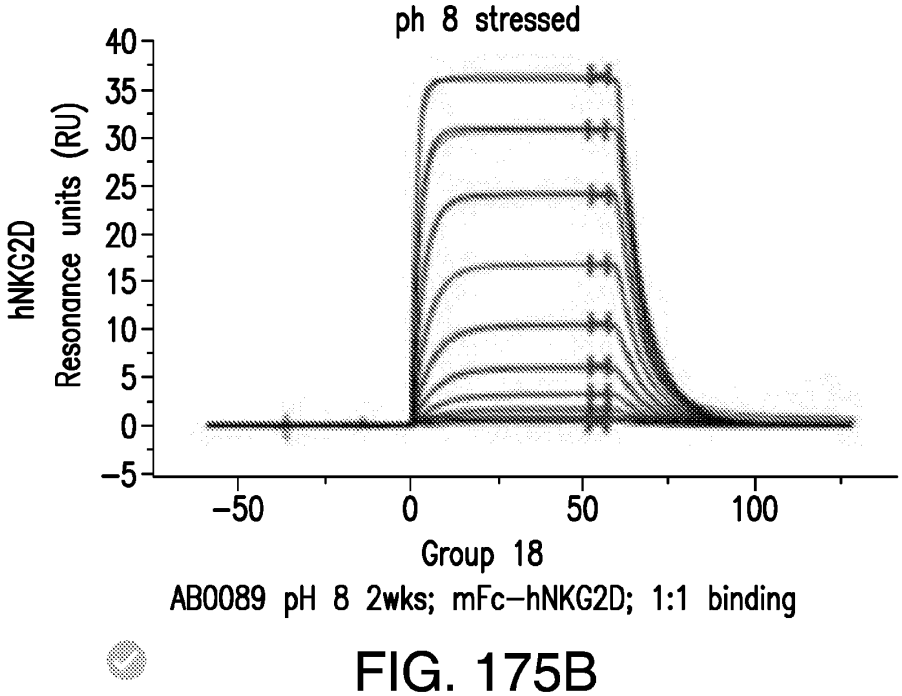
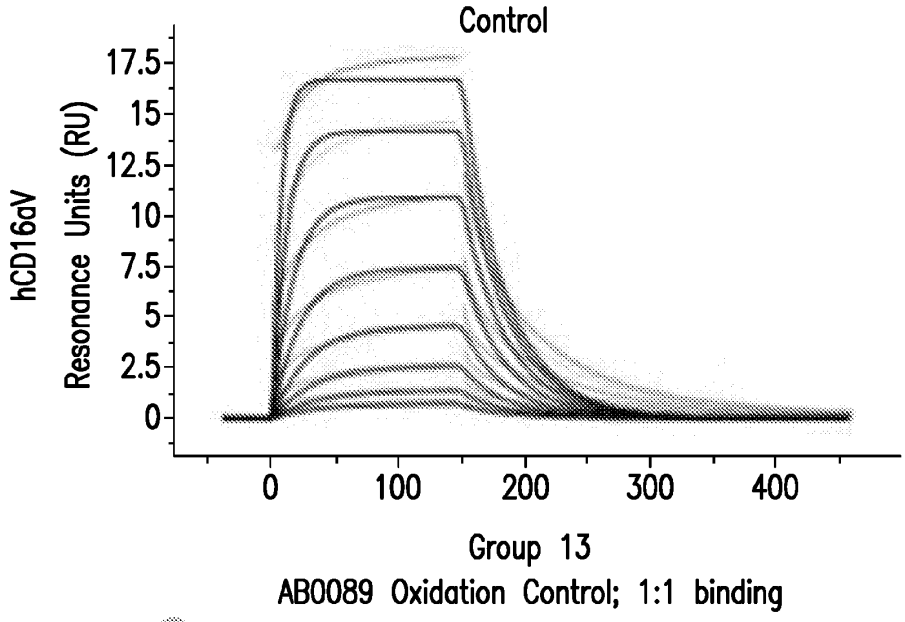

ph 8 stressed
hNKG2D
Resonance units (RU)
40
35
30
25
20
15
10
5
0
−5
−50
0
50
100
Group 18
AB0089 pH 8 2wks; mFc−hNKG2D; 1:1 binding

FIG. 175B


Control
hCD16aV
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
0
100
200
300
400
Group 13
AB0089 Oxidation Control; 1:1 binding

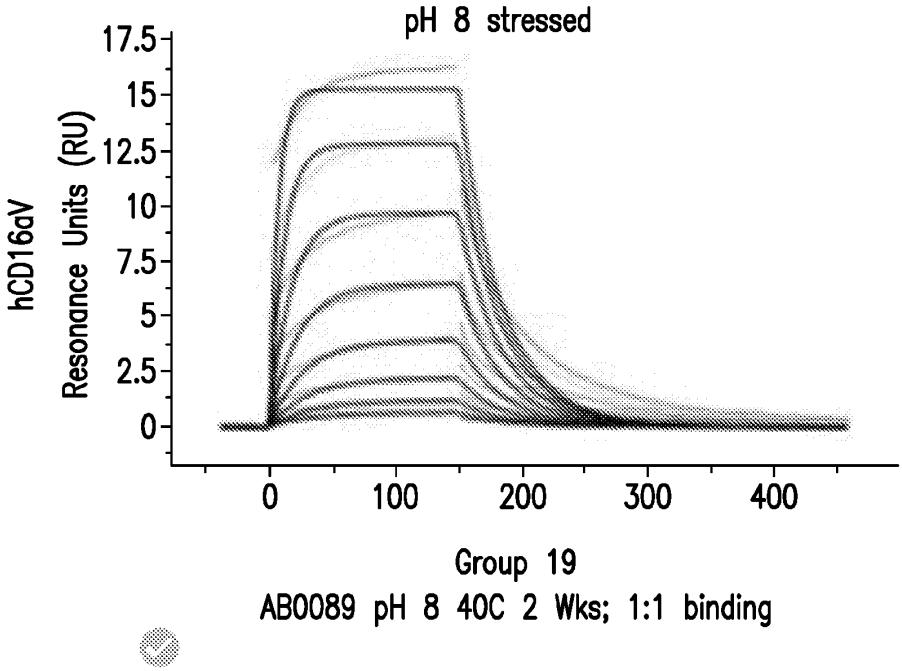
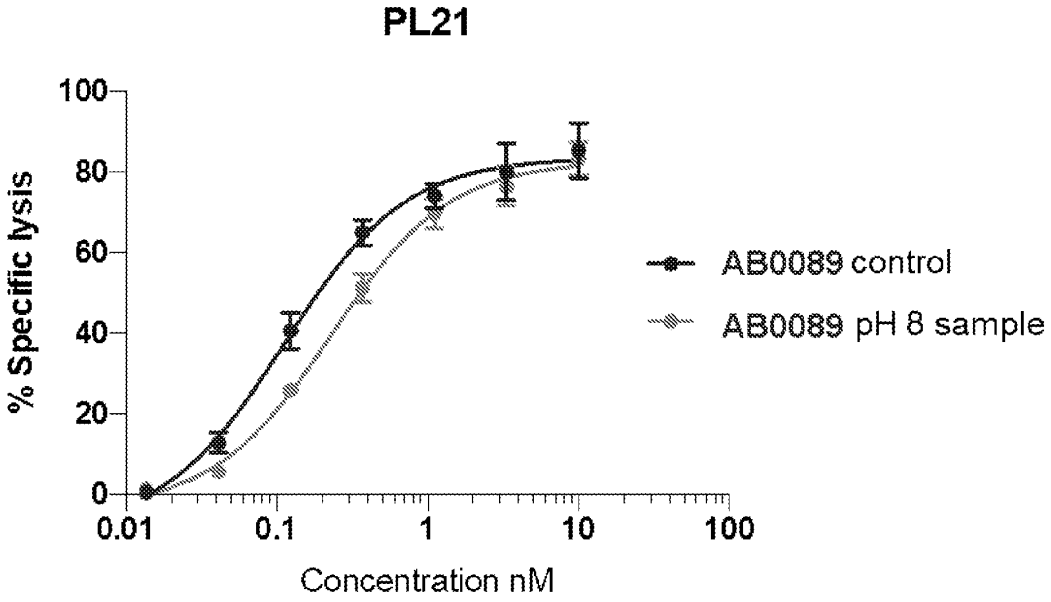

pH 8 stressed
hCD16aV
Resonance Units (RU)
17.5
15
12.5
10
7.5
5
2.5
0
0
100
200
300
400
Group 19
AB0089 pH 8 40C 2 Wks; 1:1 binding

FIG. 175C

FIG. 176


PL21
% Specific lysis
100
80
60
40
20
0
0.01
0.1
1
10
100
Concentration nM
AB0089 control
AB0089 pH 8 sample

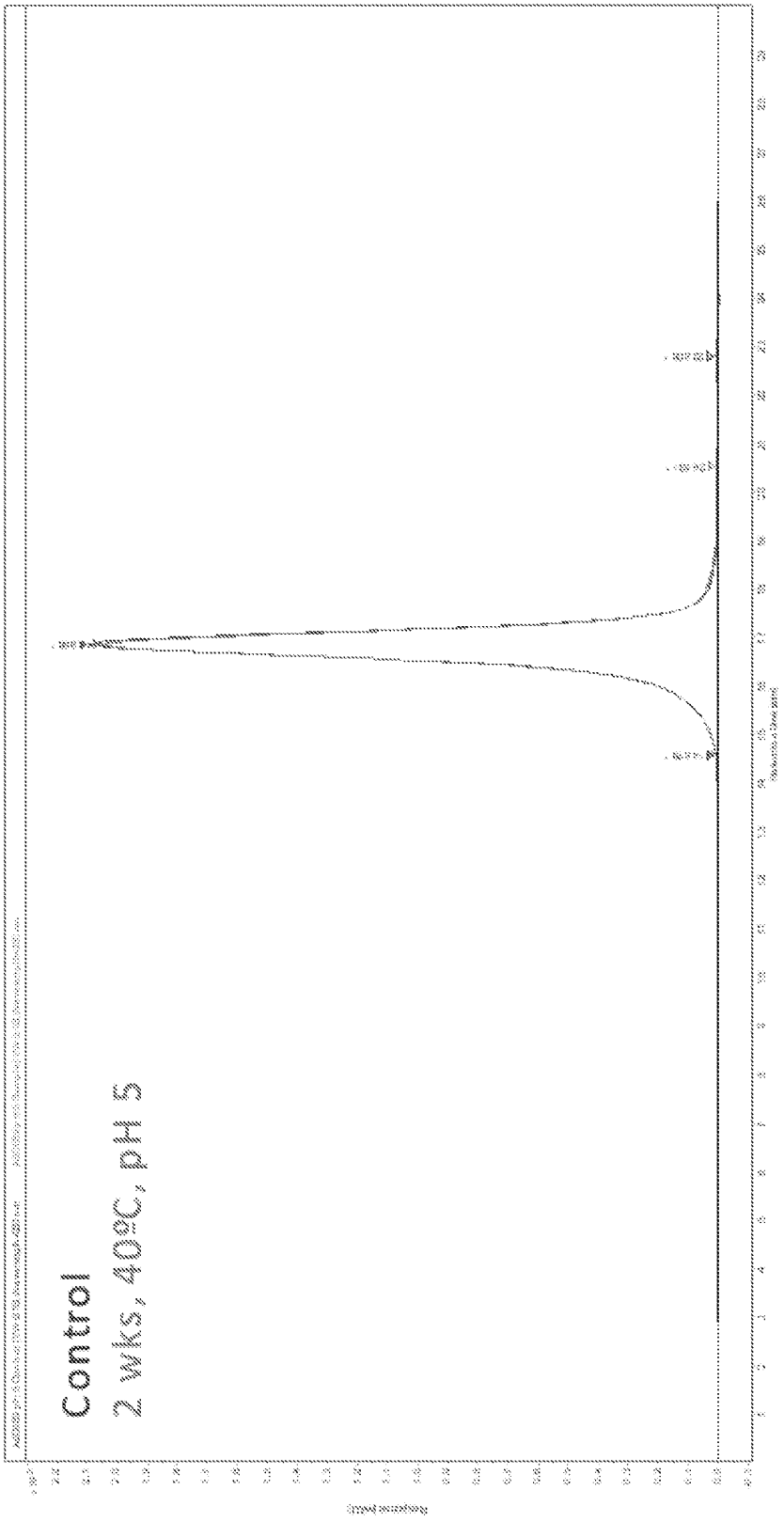


FIG. 177

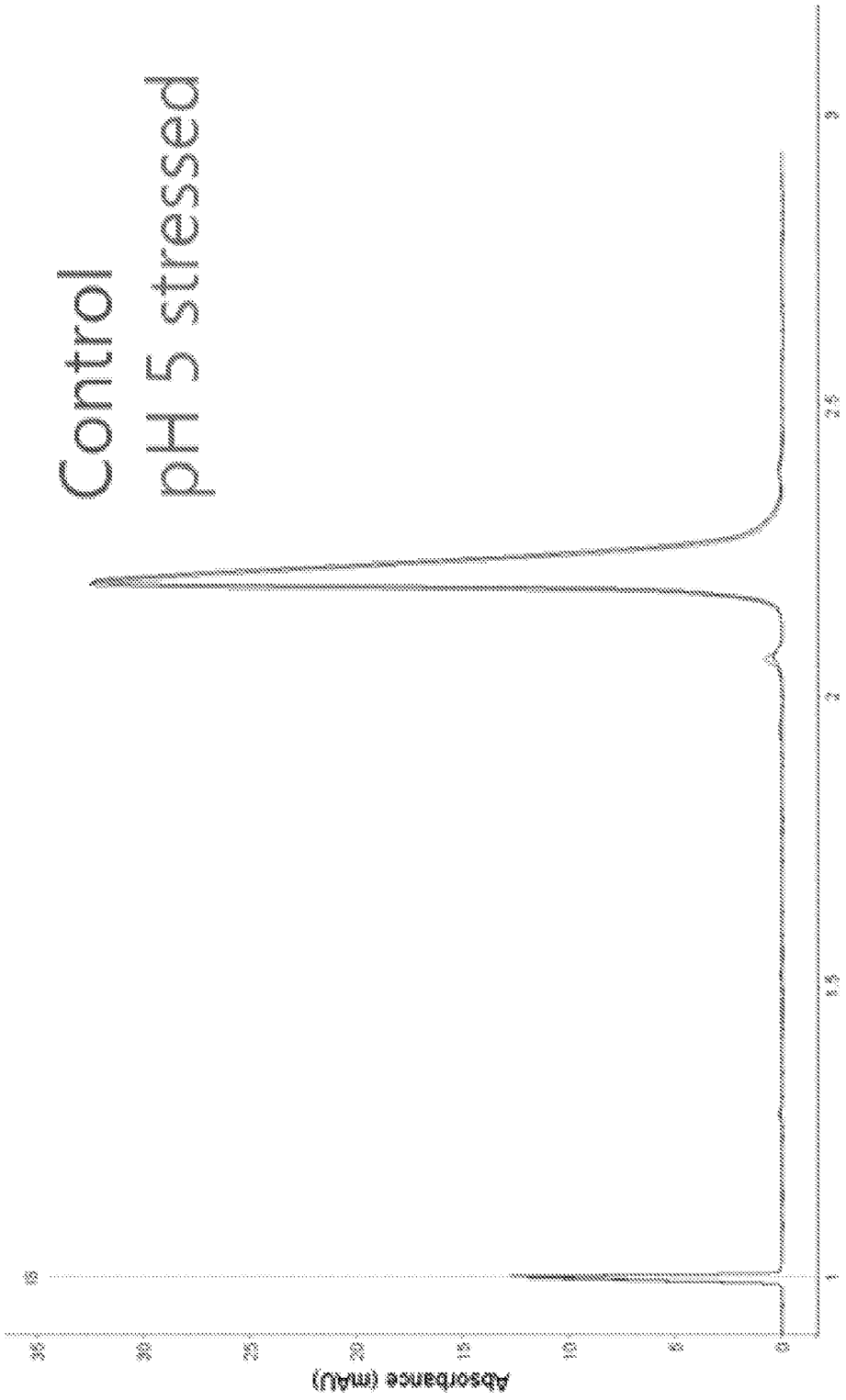

Control
pH 5 stressed
Absorbance (mAU)
Relative Migration Time

FIG. 178

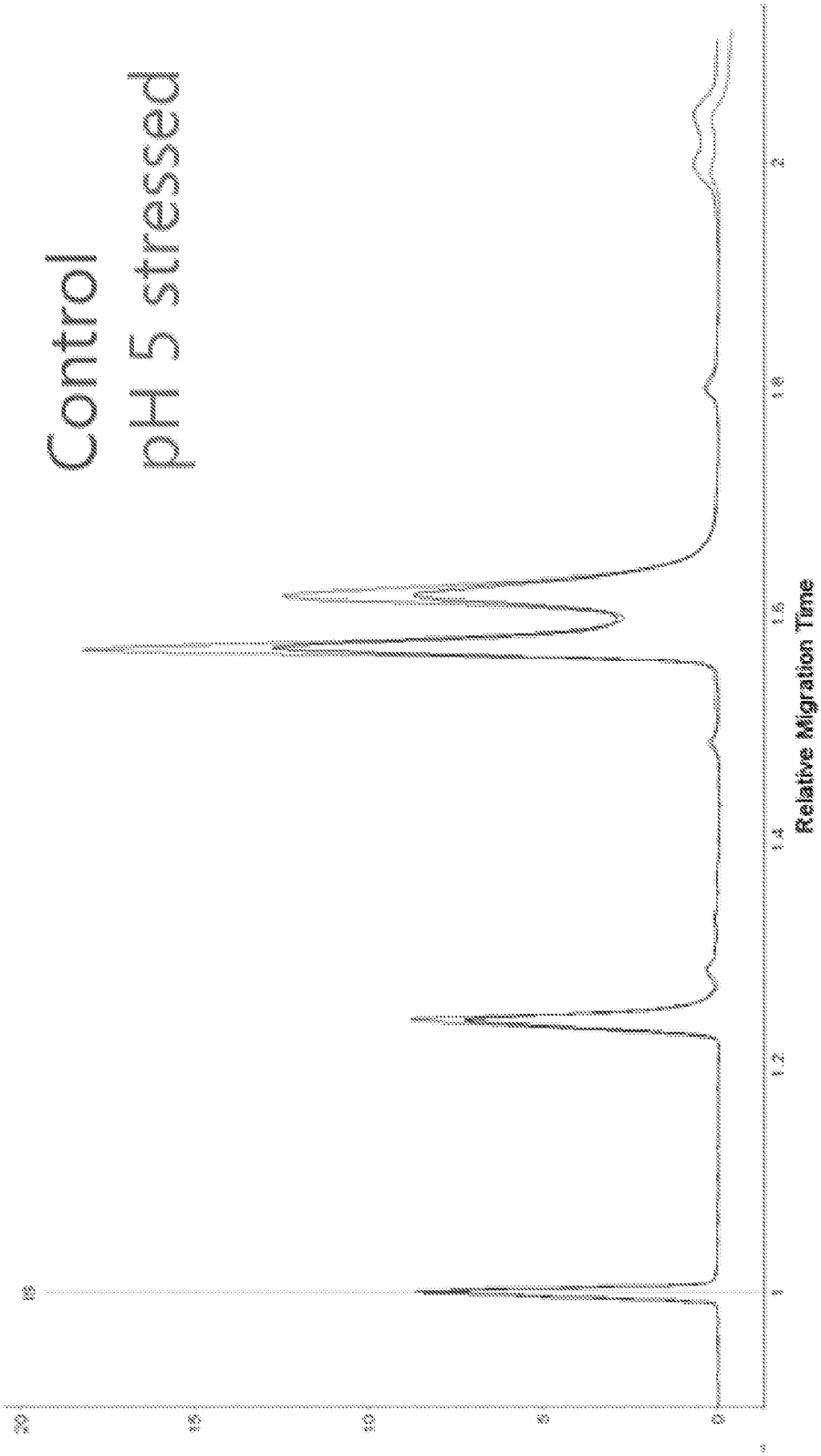


FIG. 178 Continued

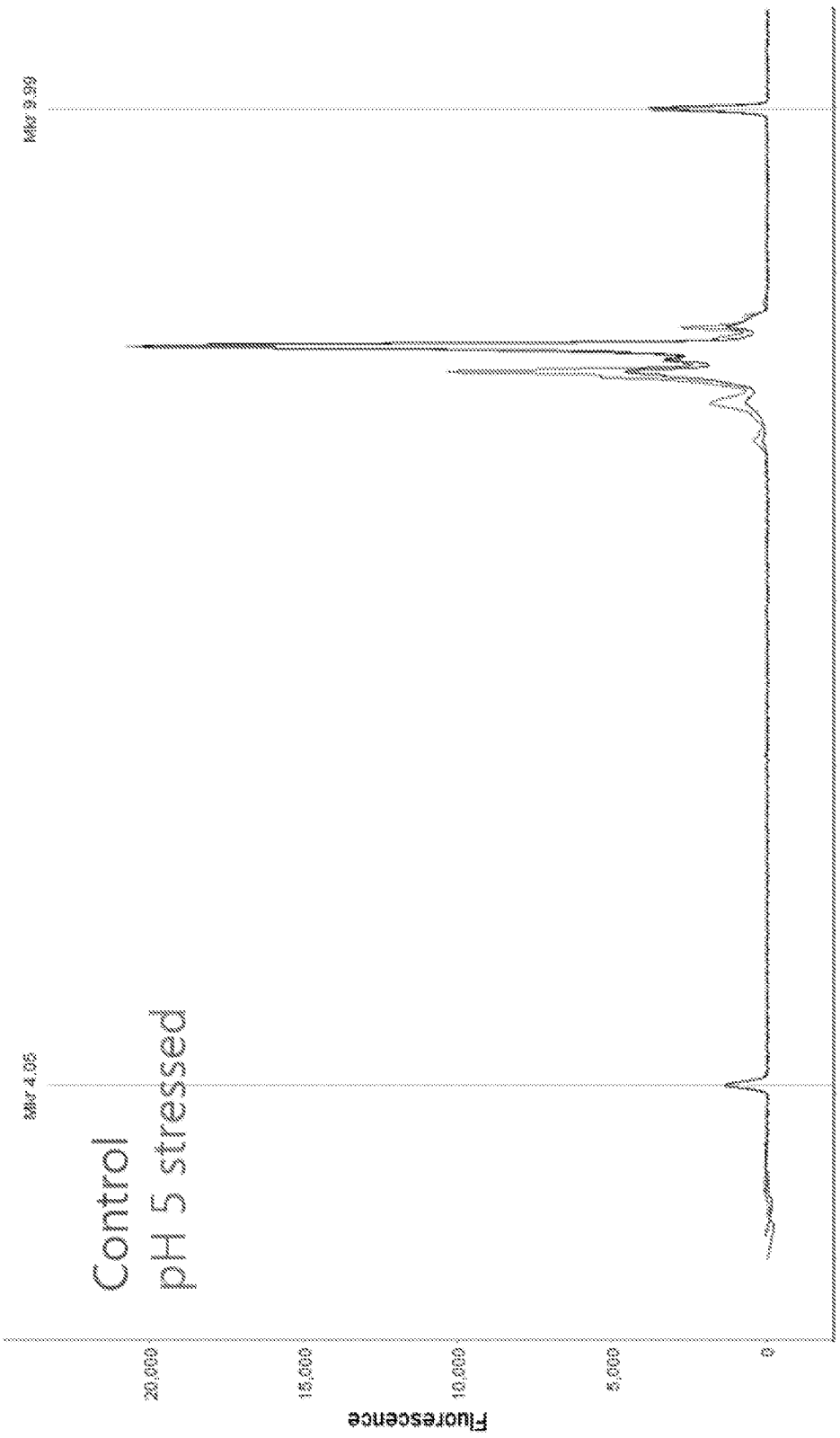
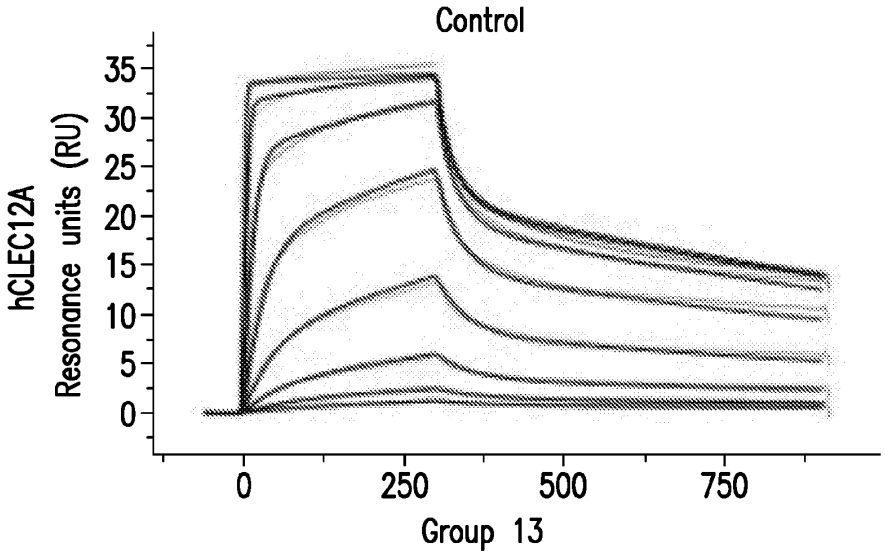
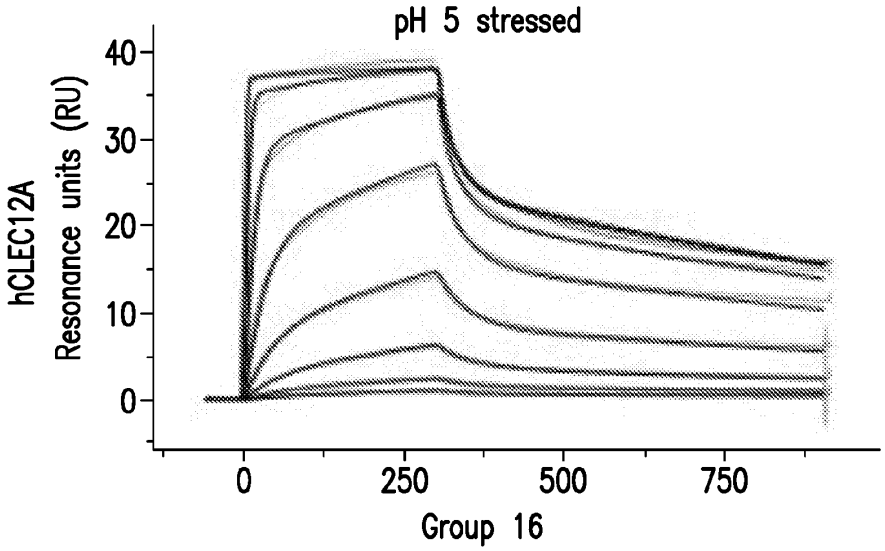

Control
pH 5 stressed
Mkr 4.06
Mkr 9.99
Fluorescence
20,000
15,000
10,000
5,000
0
3
4
5
6
7
8
9
10
pI

FIG. 179

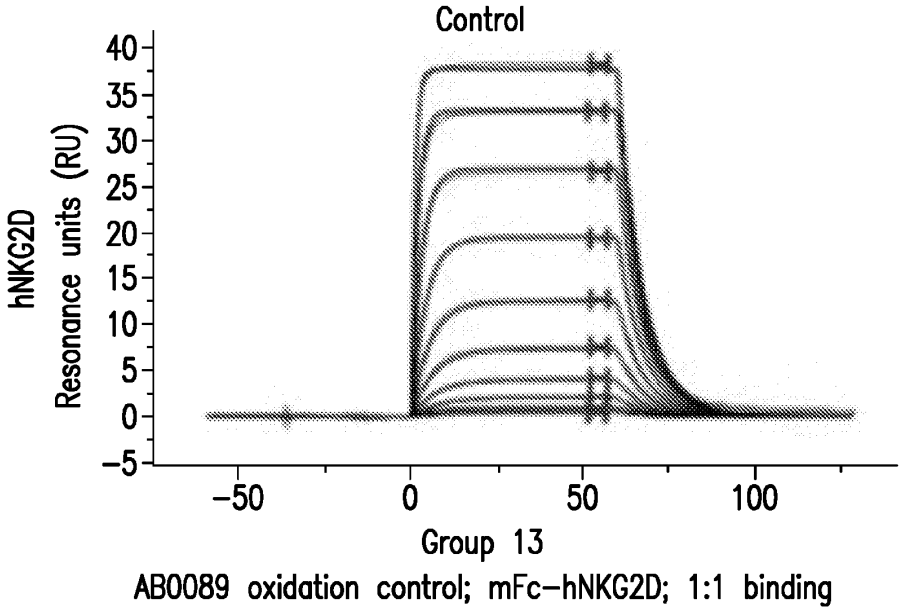


hCLEC12A–His; AB0089 Oxidation Control; Two state reaction



hCLEC12A–His; AB0089 pH 5 40C 2 Wks; Two state reaction

FIG. 180



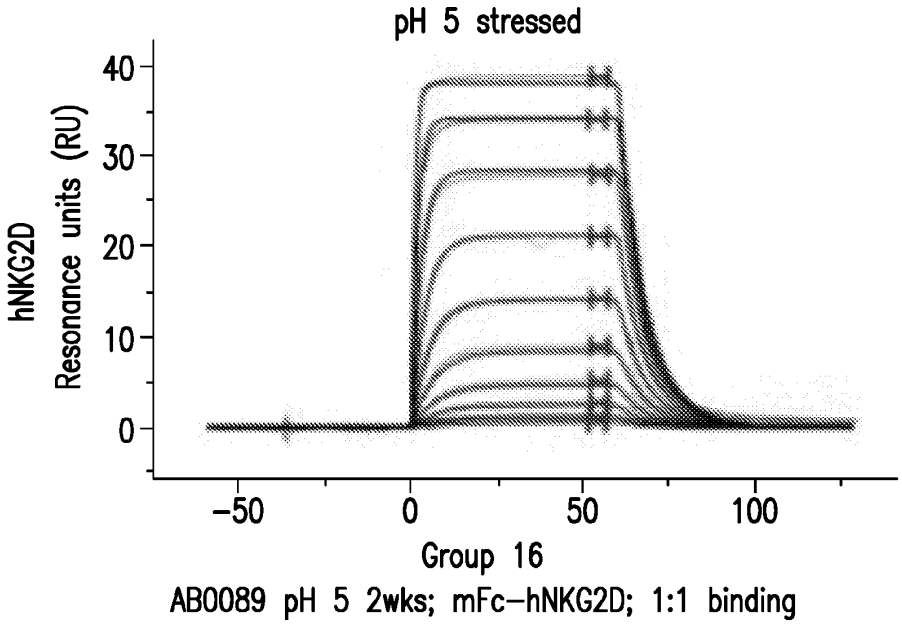
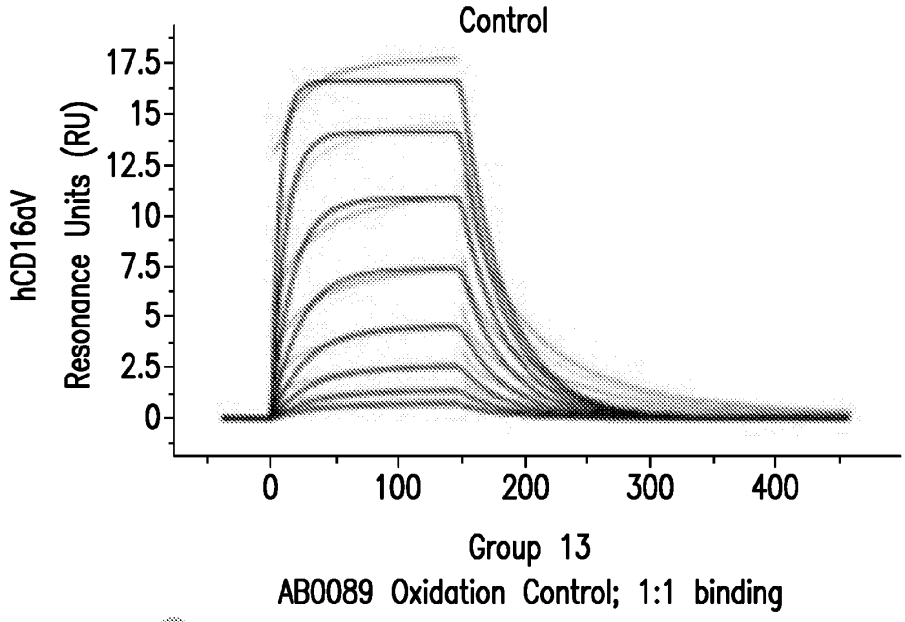


FIG. 180 Continued



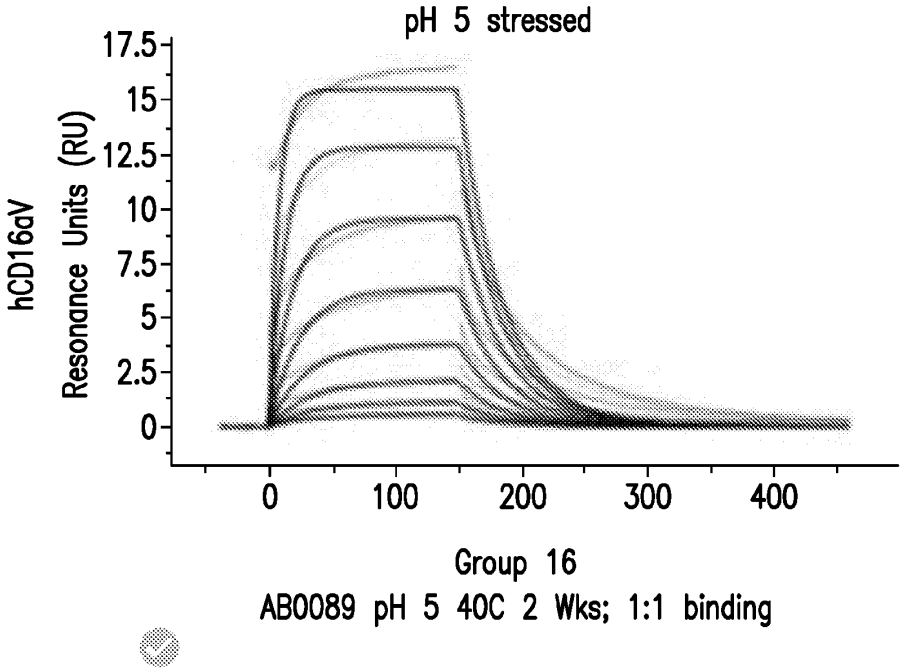
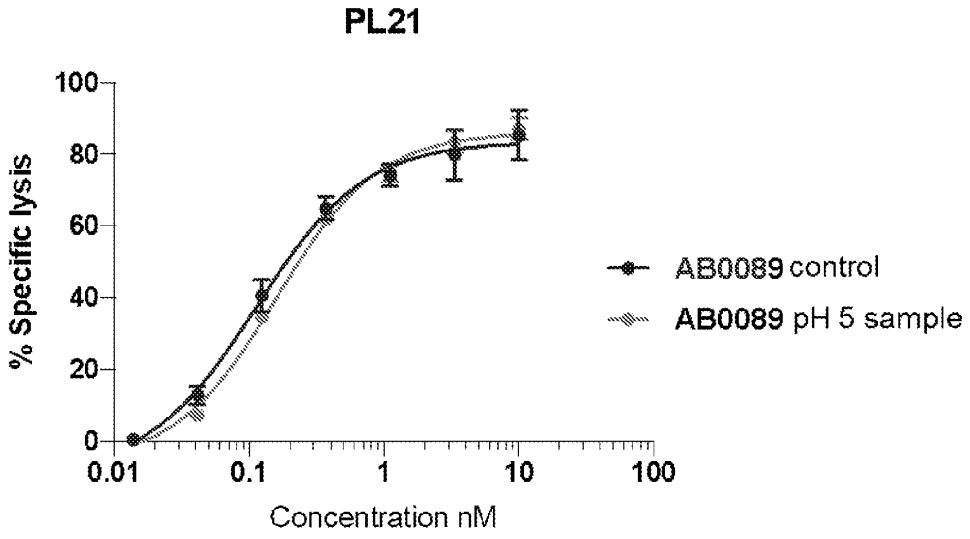


FIG. 180 Continued

FIG. 181


PL21
% Specific lysis
100
80
60
40
20
0
0.01
0.1
1
10
100
Concentration nM
AB0089 control
AB0089 pH 5 sample

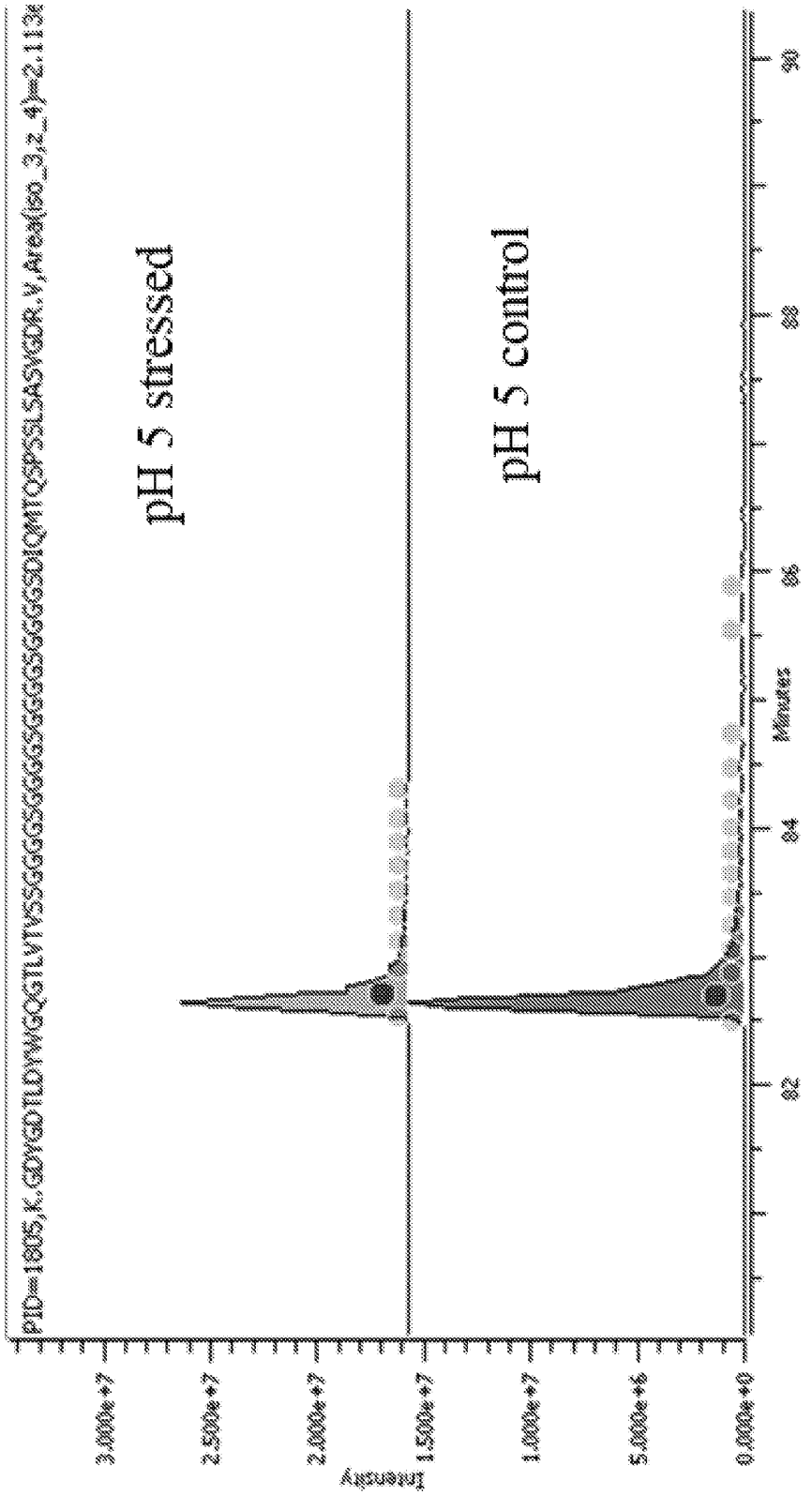

pH 5 stressed
pH 5 control
Intensity
Minutes
3.000e+7
2.500e+7
2.000e+7
1.500e+7
1.000e+7
5.000e+6
0.000e+0
82
84
86
88
90

FIG. 182

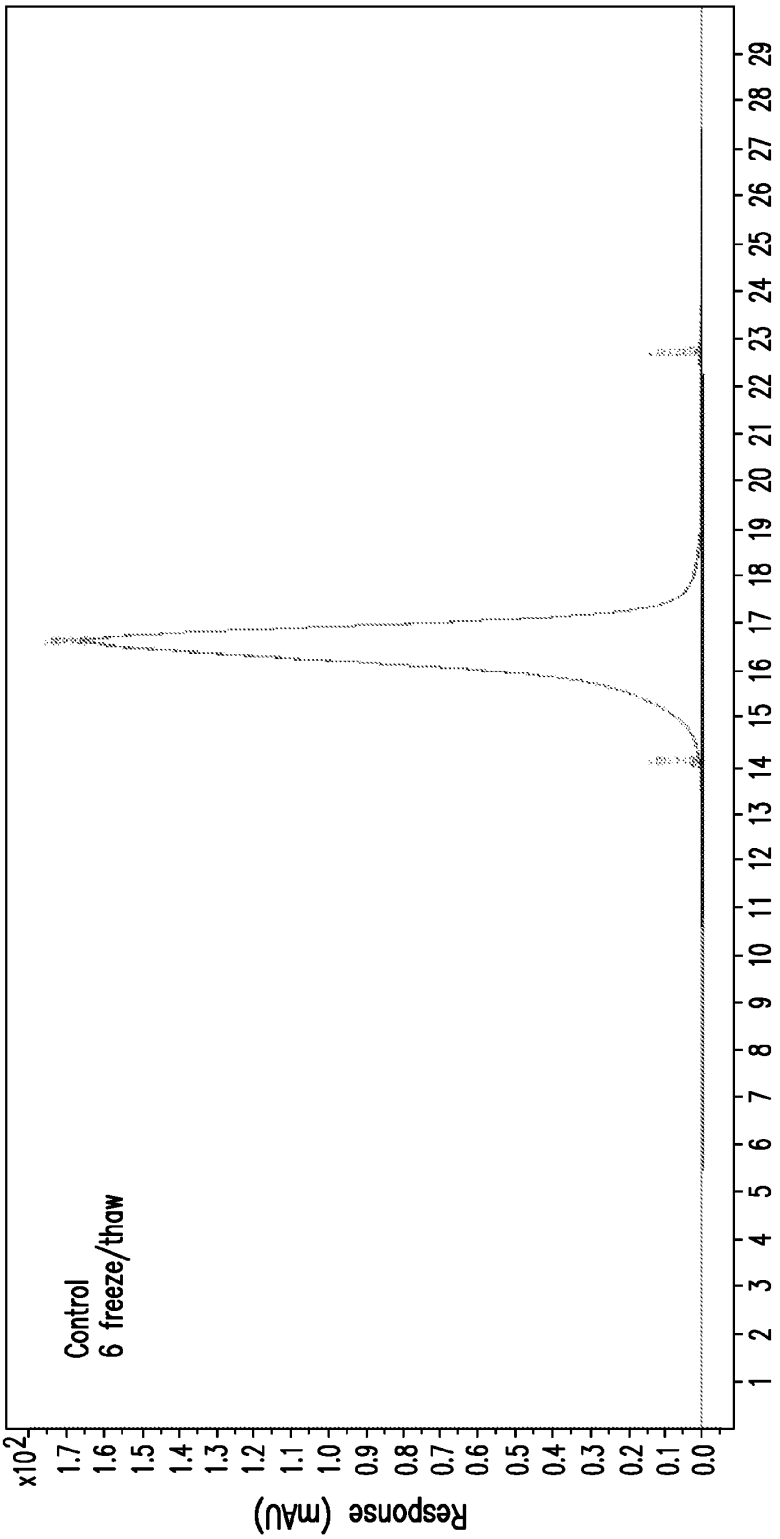

Control
6 freeze/thaw
Response (mAU)
x10^2
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29

FIG. 183

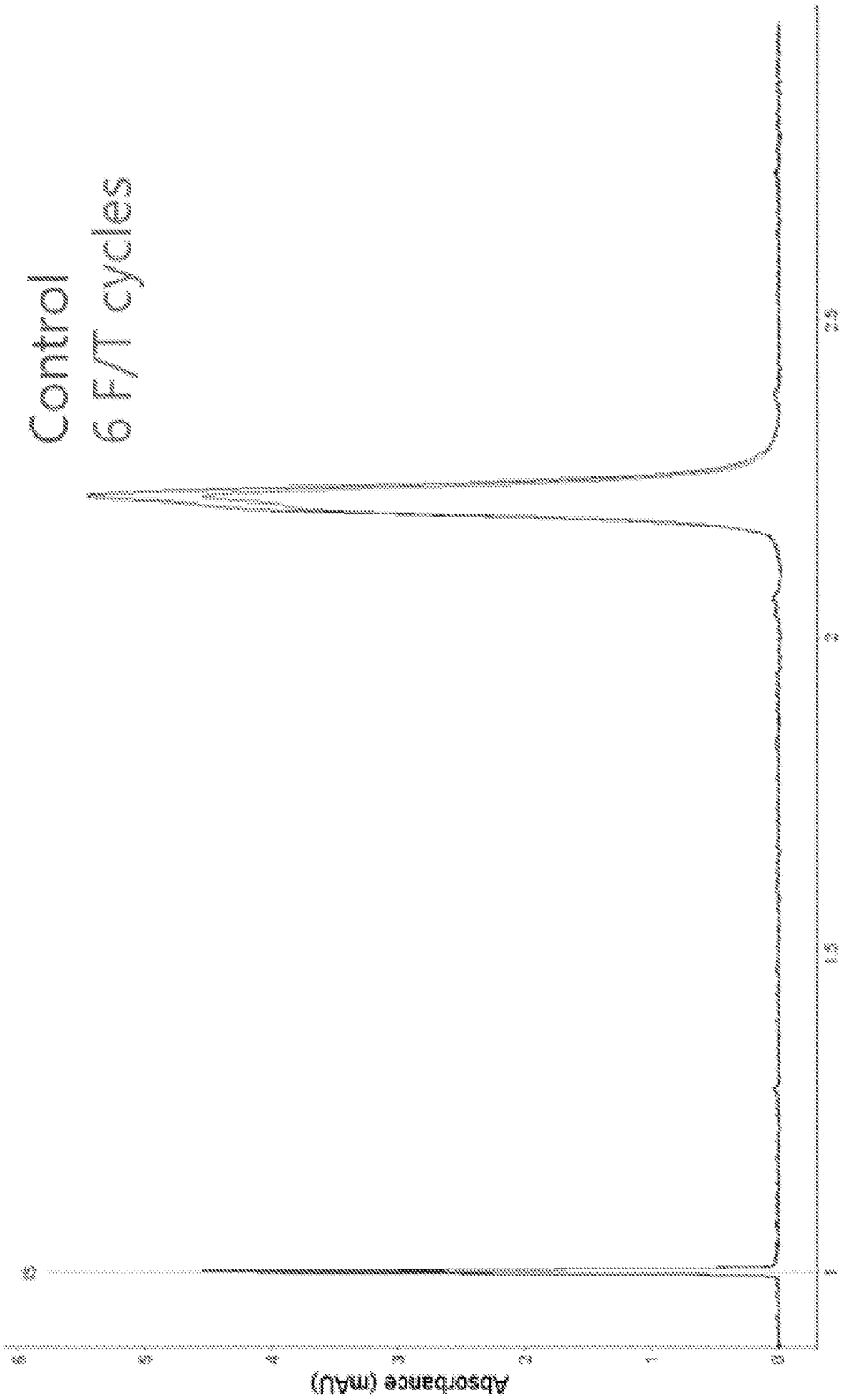

Control
6 F/T cycles
Absorbance (mAU)
Relative Migration Time

FIG. 184

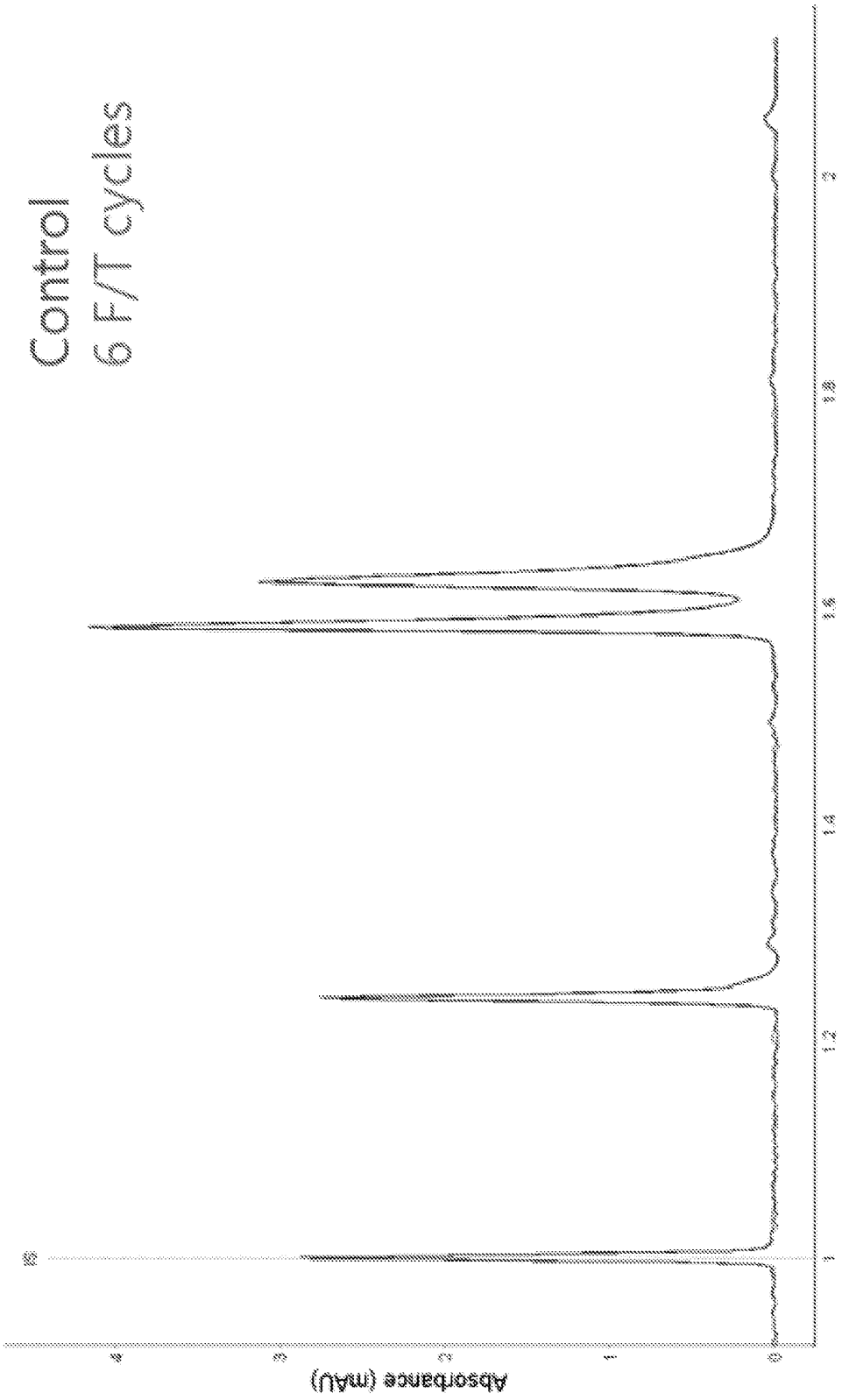


FIG. 184 Continued

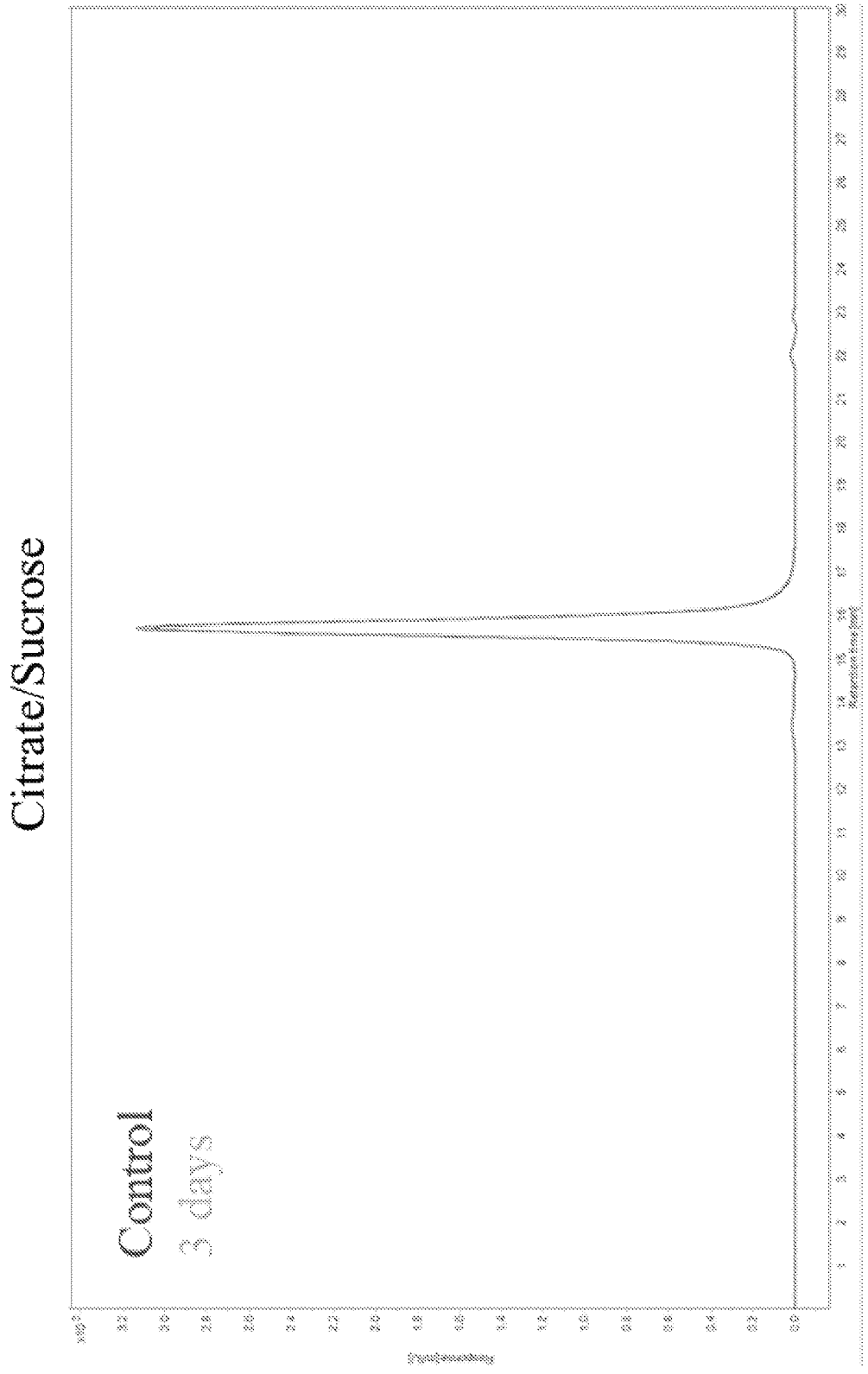


FIG. 185

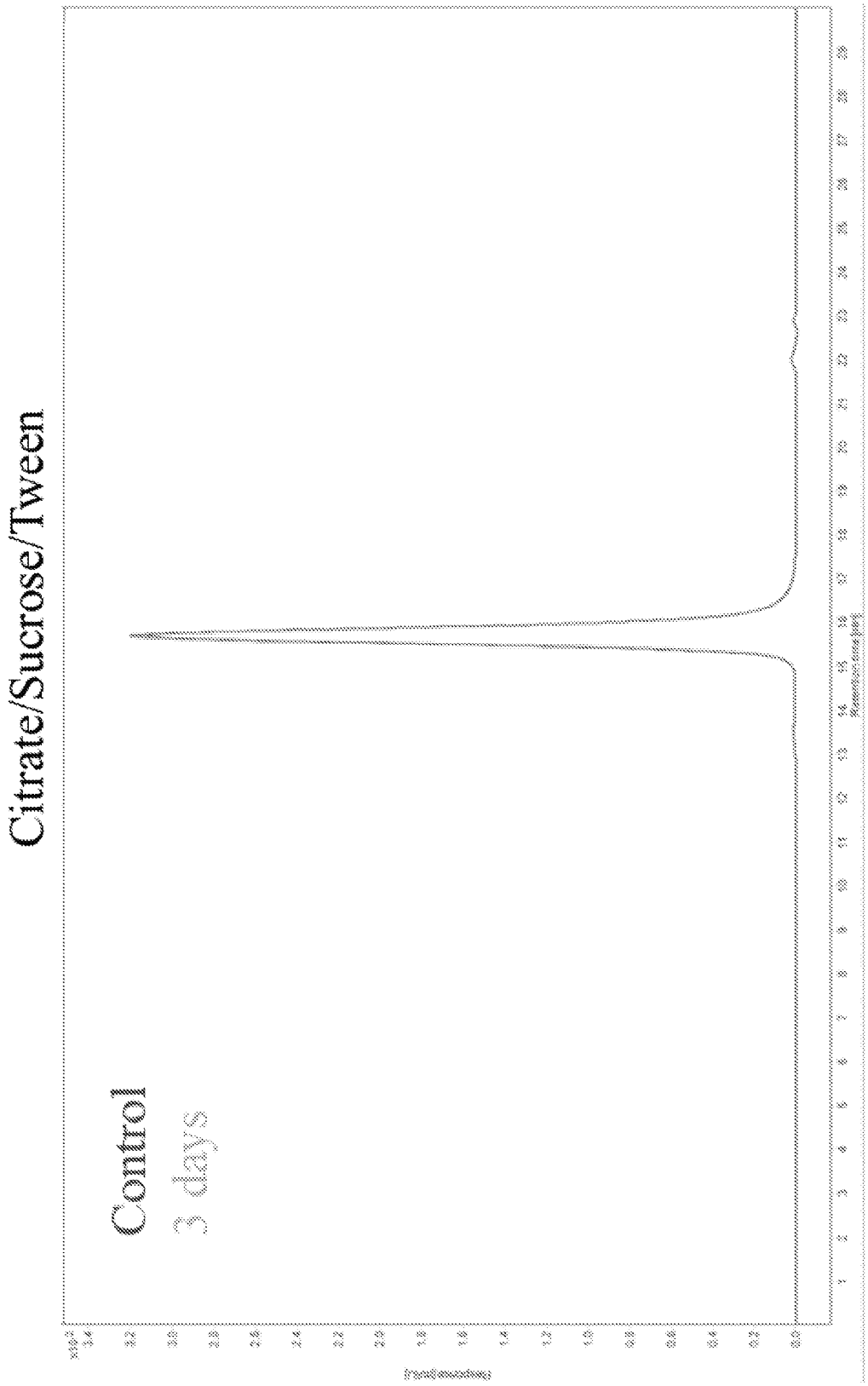


FIG. 185 Continued

FIG. 186

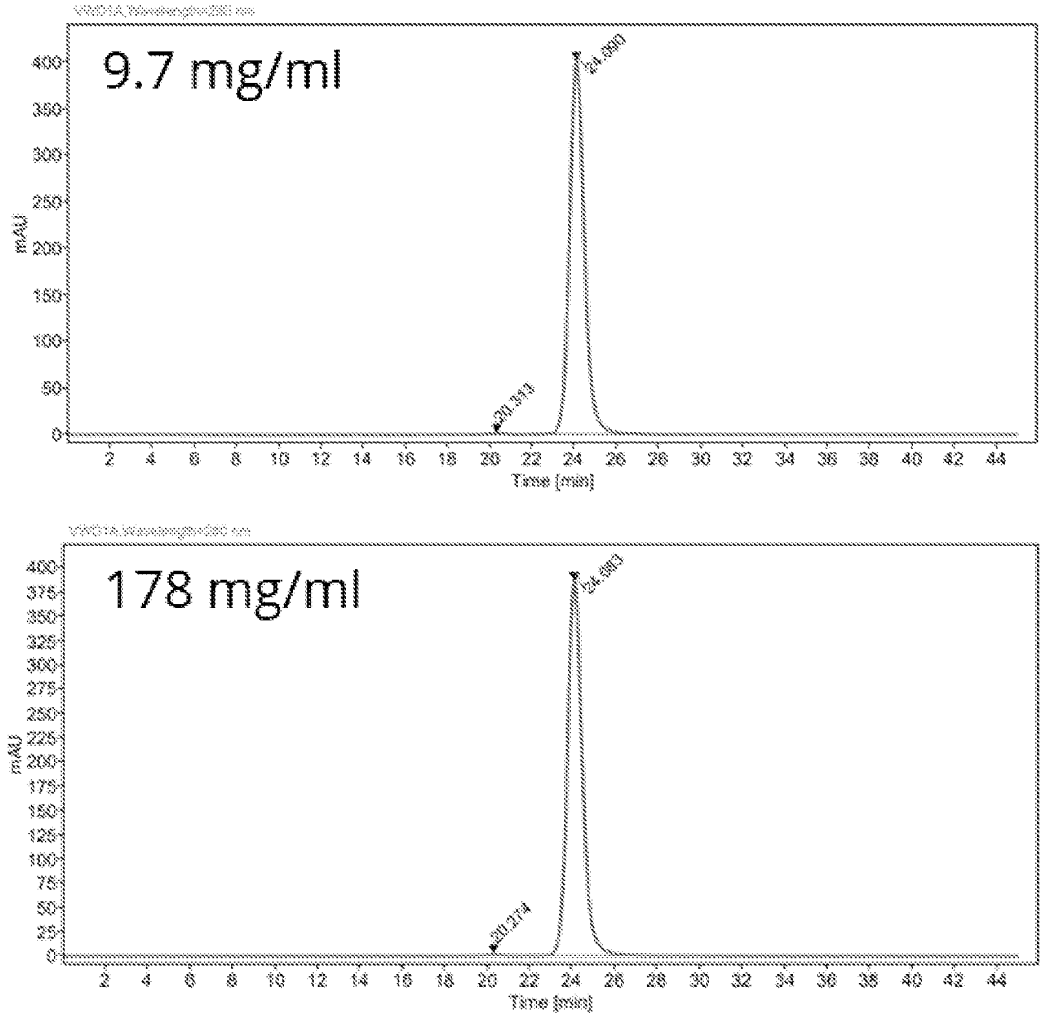

9.7 mg/ml
24.080
20.343
mAU
Time [min]
178 mg/ml
24.083
20.274
mAU
Time [min]

FIG. 187

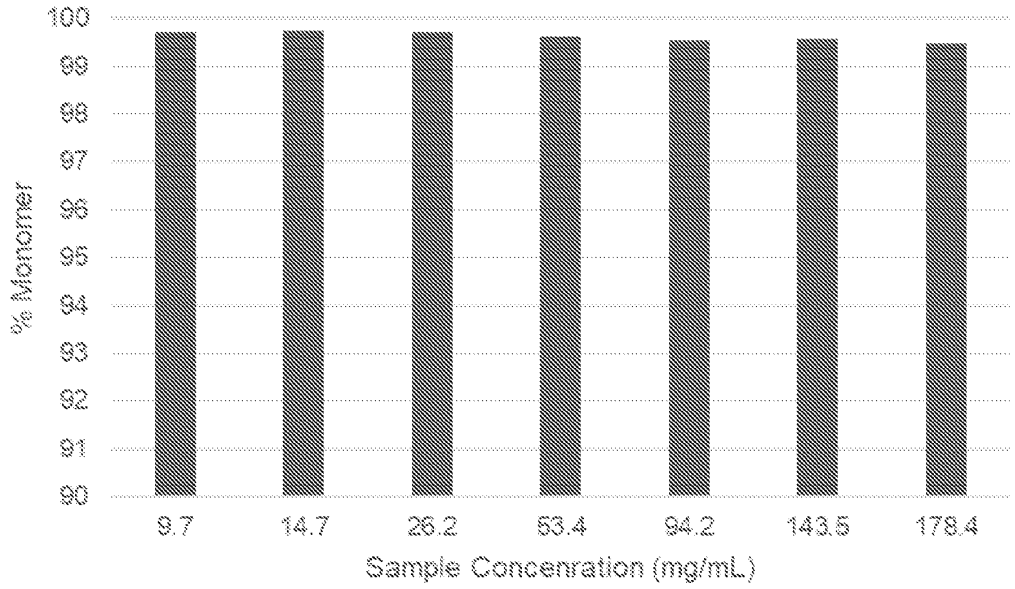
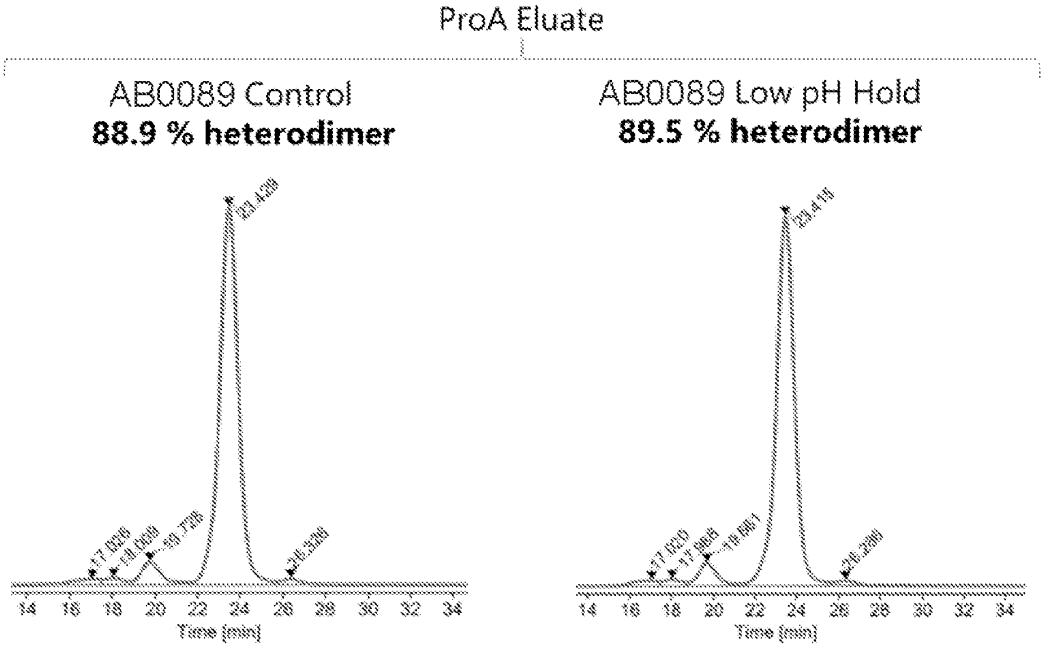


FIG. 188



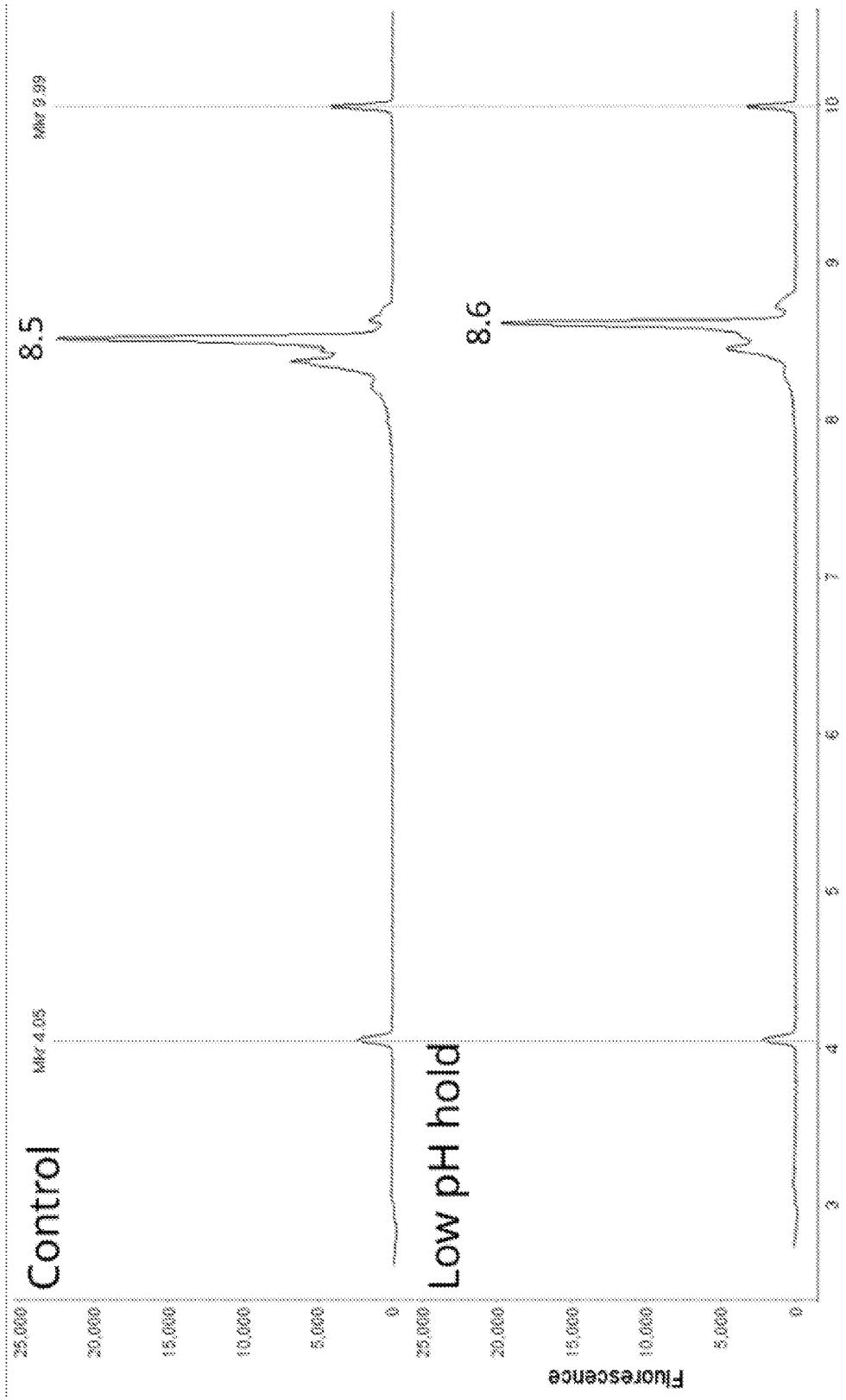
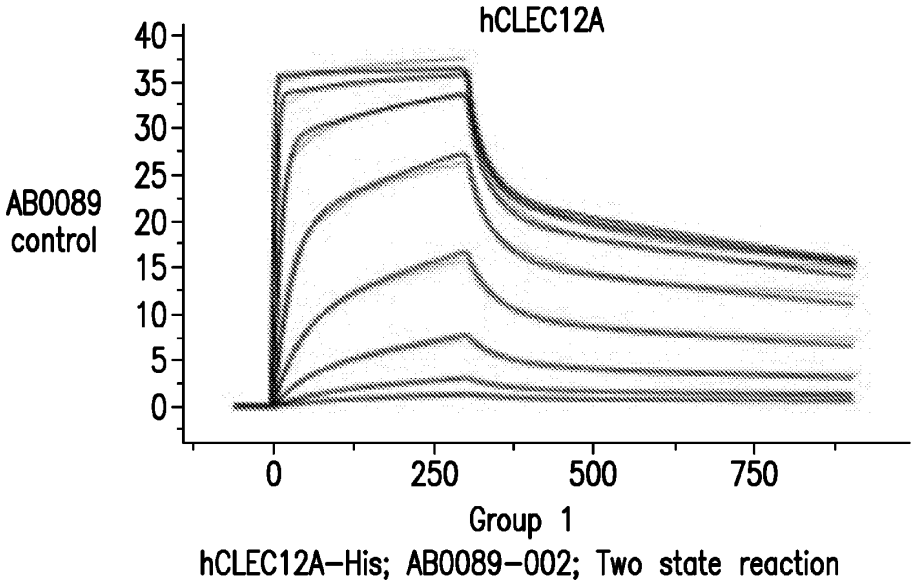

Control
Low pH hold
8.5
8.6
Mkr 4.05
Mkr 9.99
Fluorescence
pI
25,000
20,000
15,000
10,000
5,000
0
3
4
5
6
7
8
9
10

FIG. 189


hCLEC12A
AB0089
control
40
35
30
25
20
15
10
5
0
0
250
500
750
Group 1
hCLEC12A-His; AB0089-002; Two state reaction

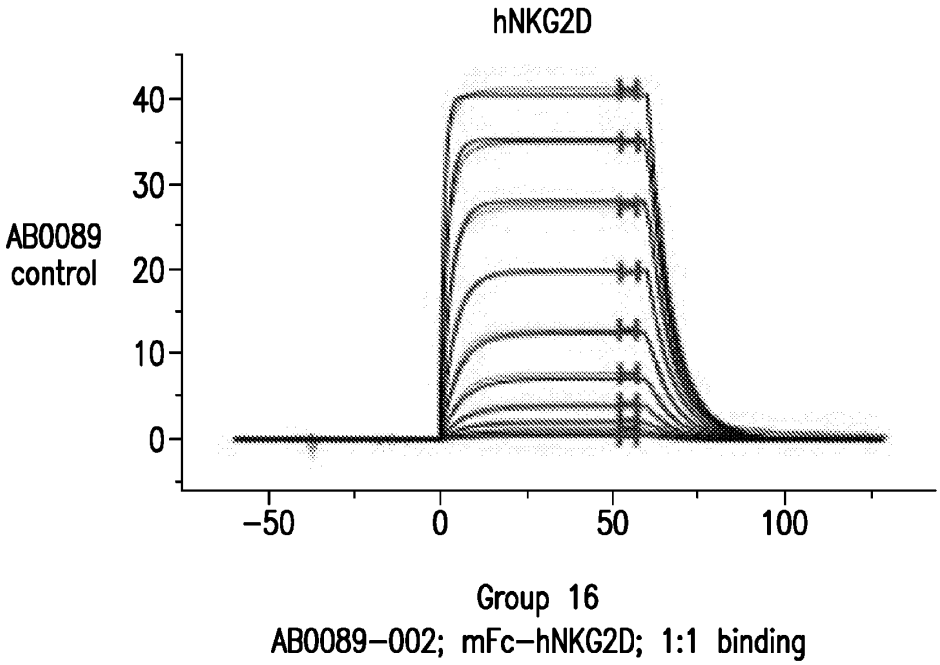
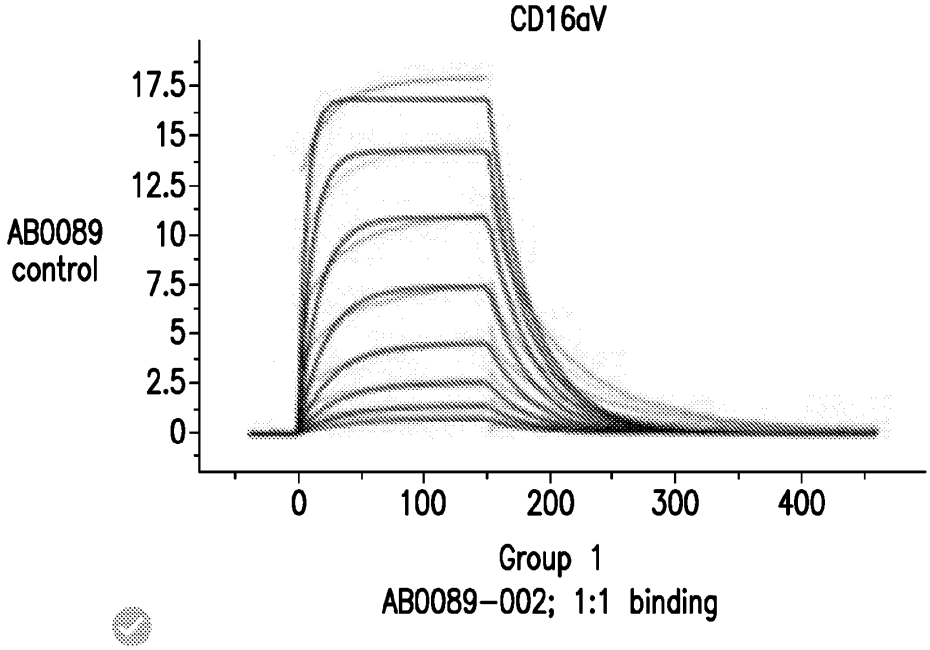

hNKG2D
AB0089
control
40
30
20
10
0
-50
0
50
100
Group 16
AB0089-002; mFc-hNKG2D; 1:1 binding

FIG. 190



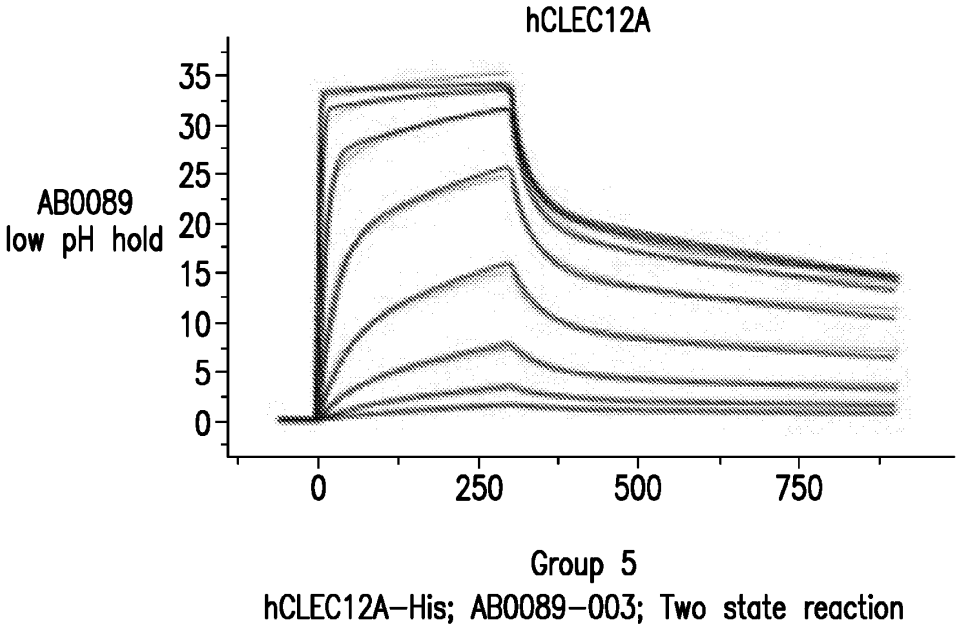
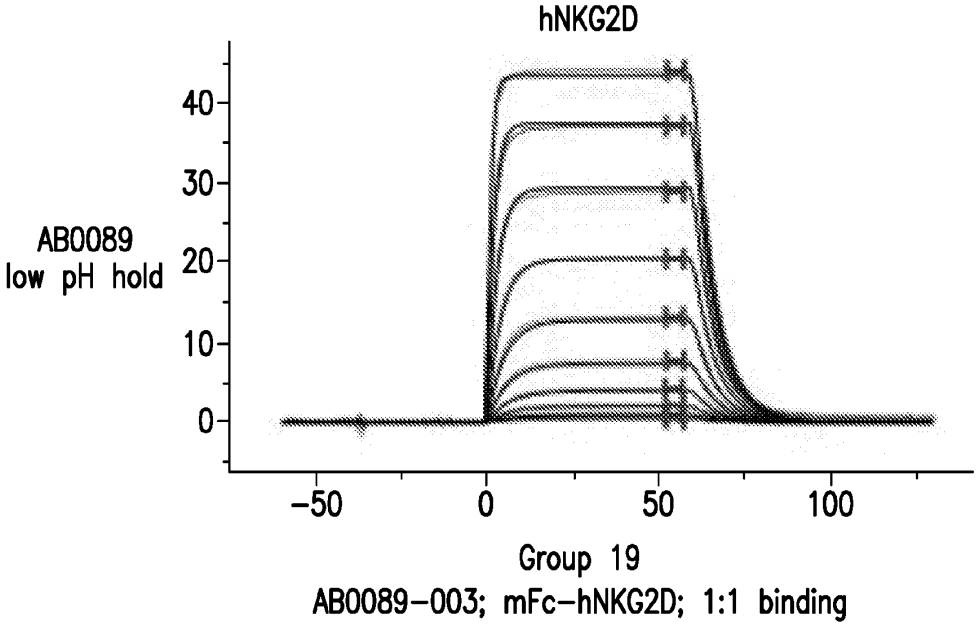


FIG. 190 Continued



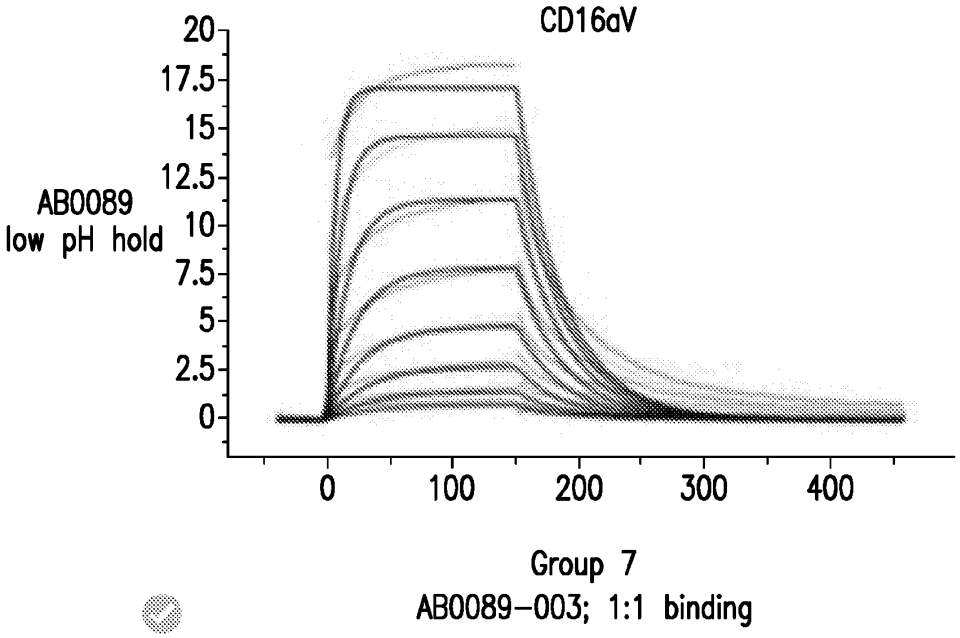
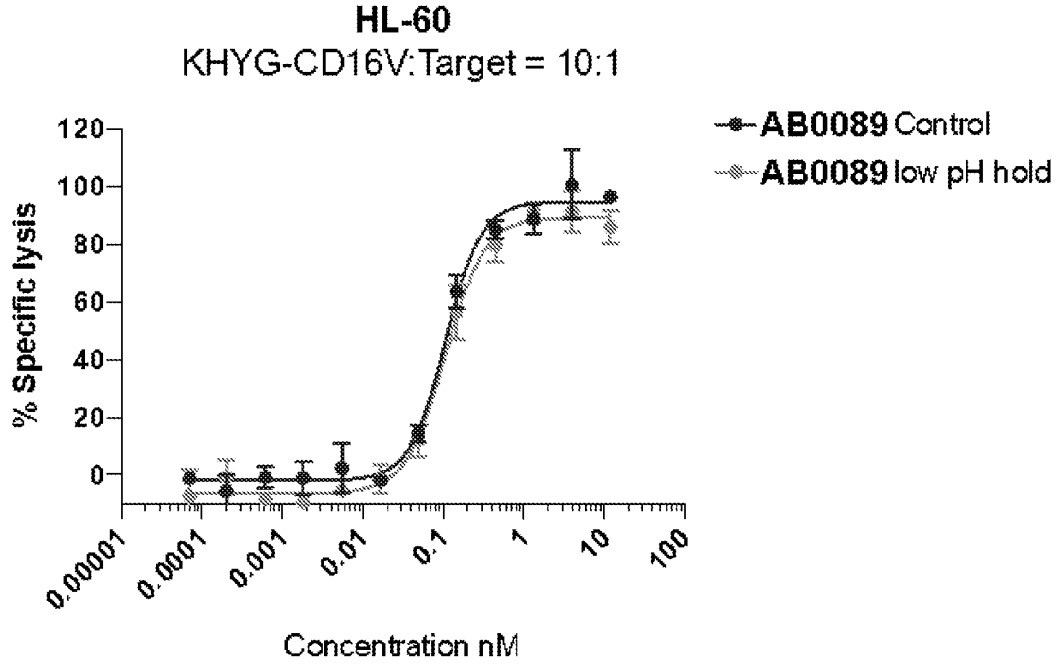


FIG. 190 Continued

FIG. 191


HL-60
KHYG-CD16V:Target = 10:1
% Specific lysis
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
Concentration nM
AB0089 Control
AB0089 low pH hold

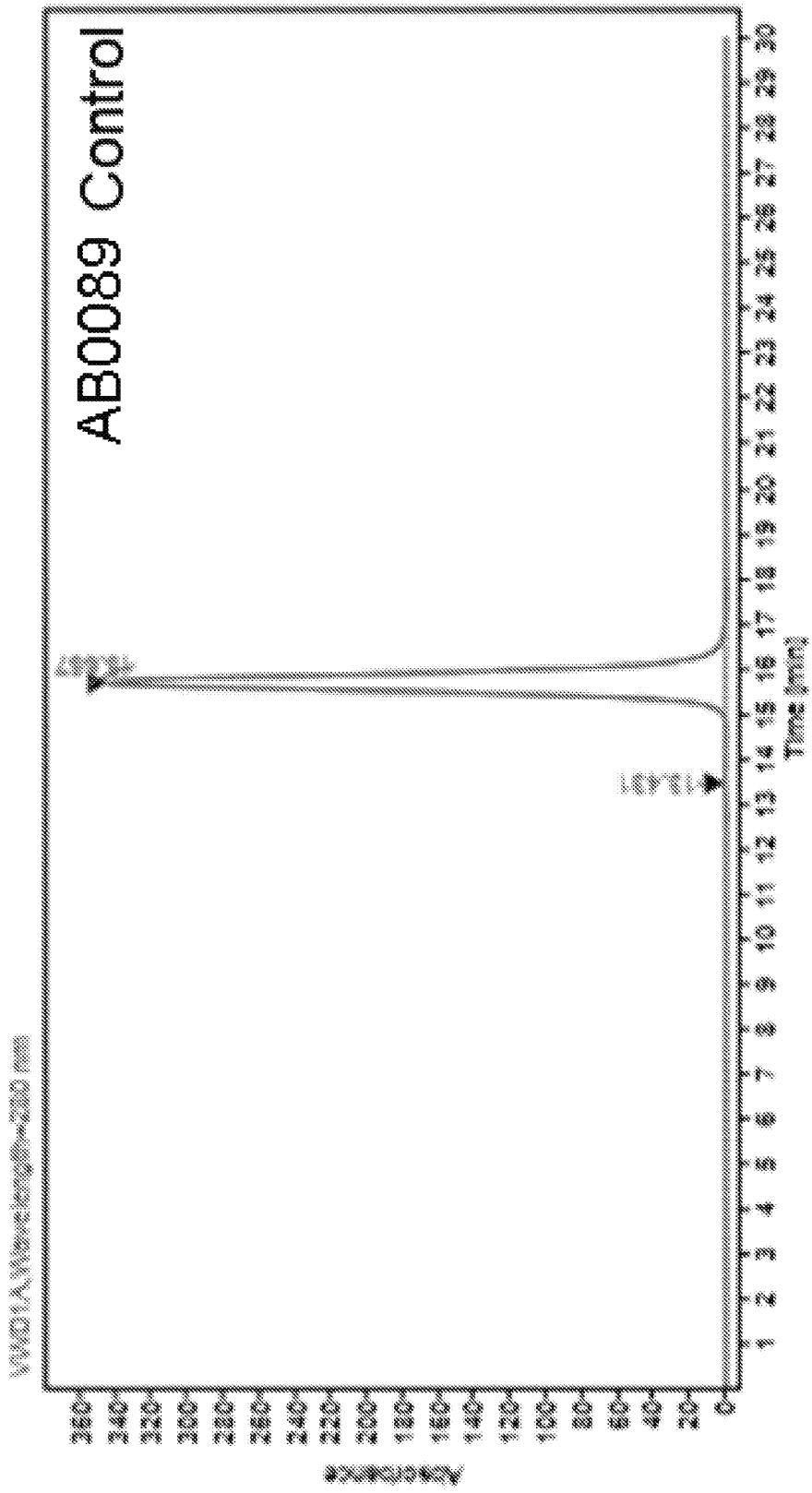


FIG. 192

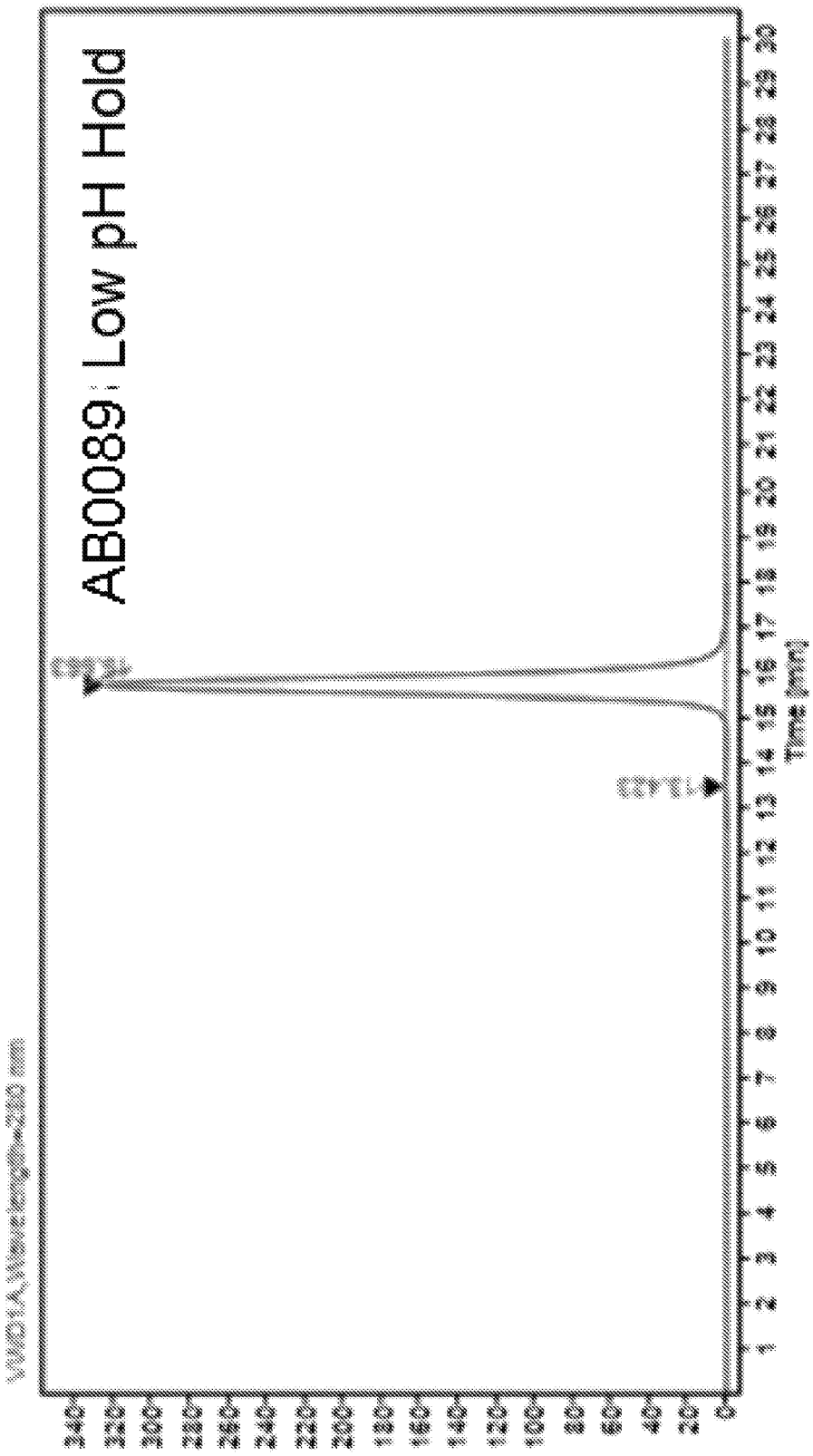


FIG. 192 Continued

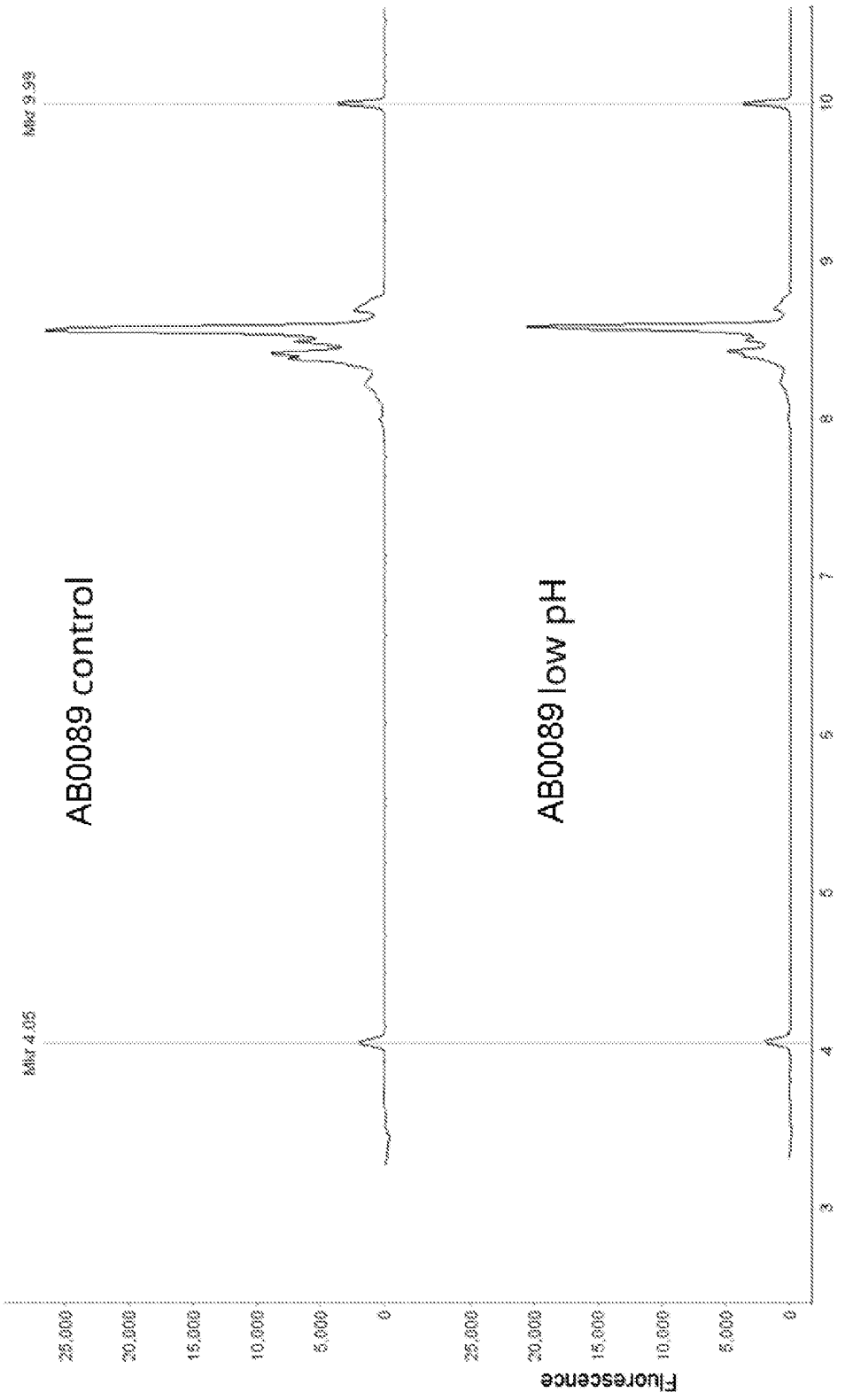

AB0089 control
AB0089 low pH
Mkr 4.05
Mkr 9.38
Fluorescence
pI
25,000
20,000
15,000
10,000
5,000
0
3
4
5
6
7
8
9
10

FIG. 193

FIG. 194

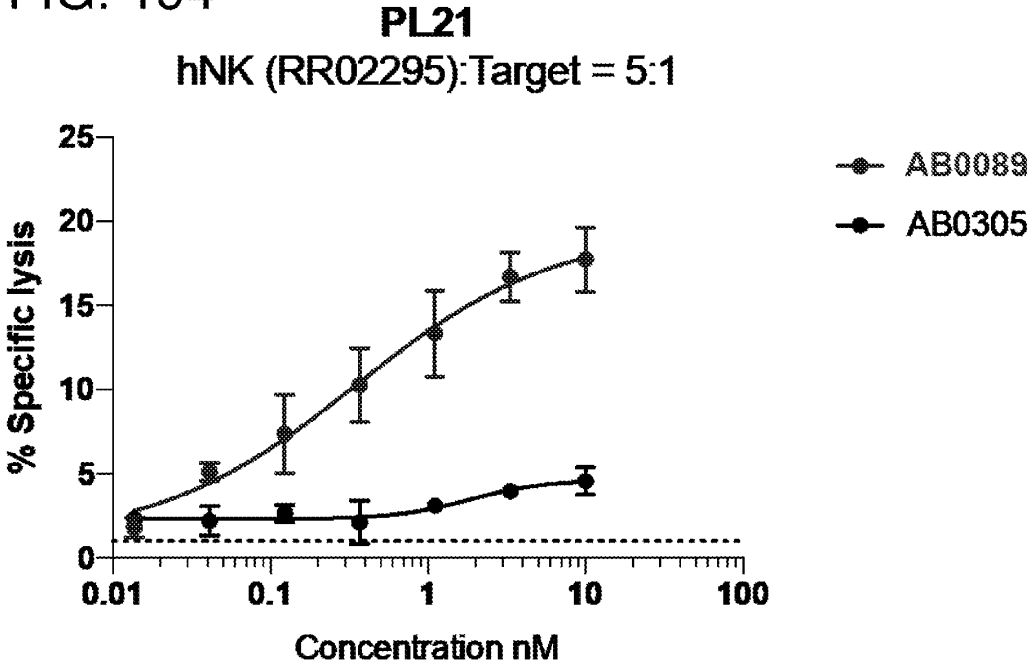


FIG. 195

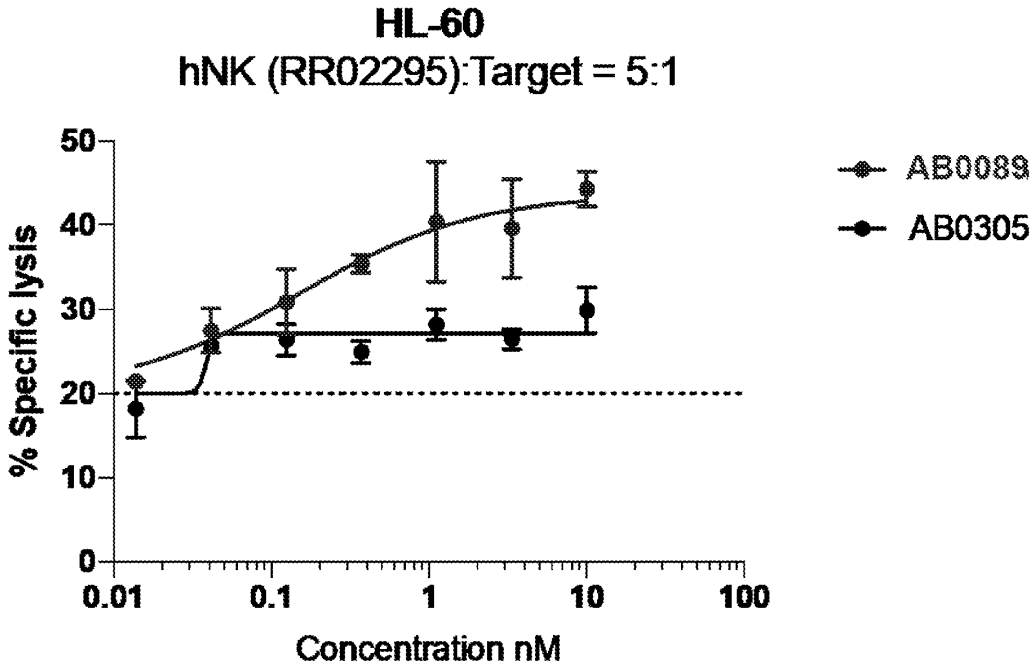


FIG. 196

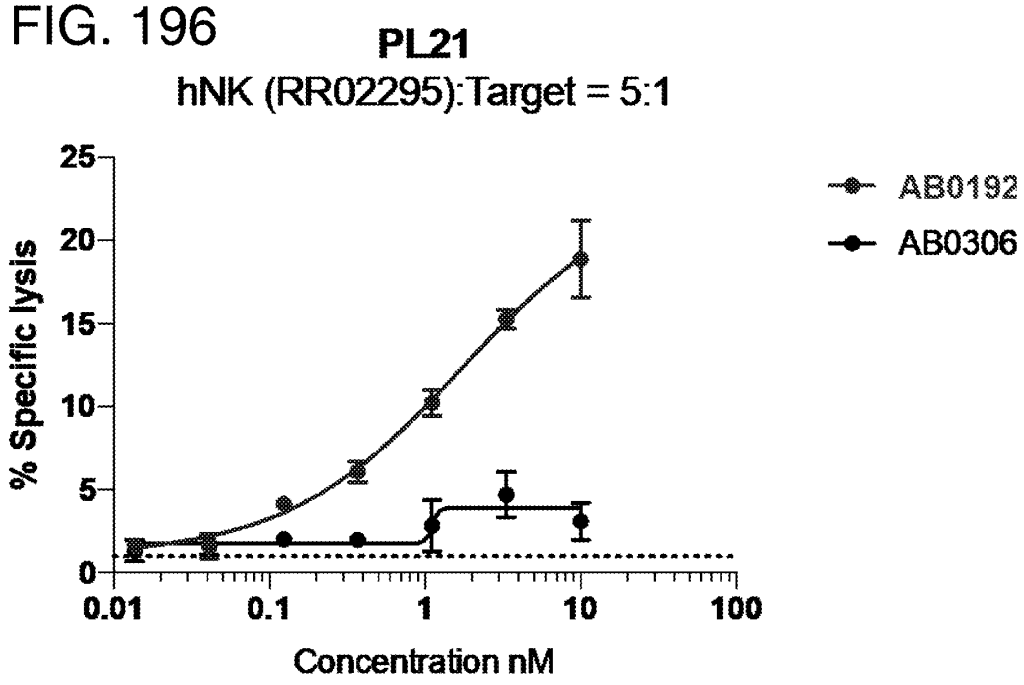


FIG. 197

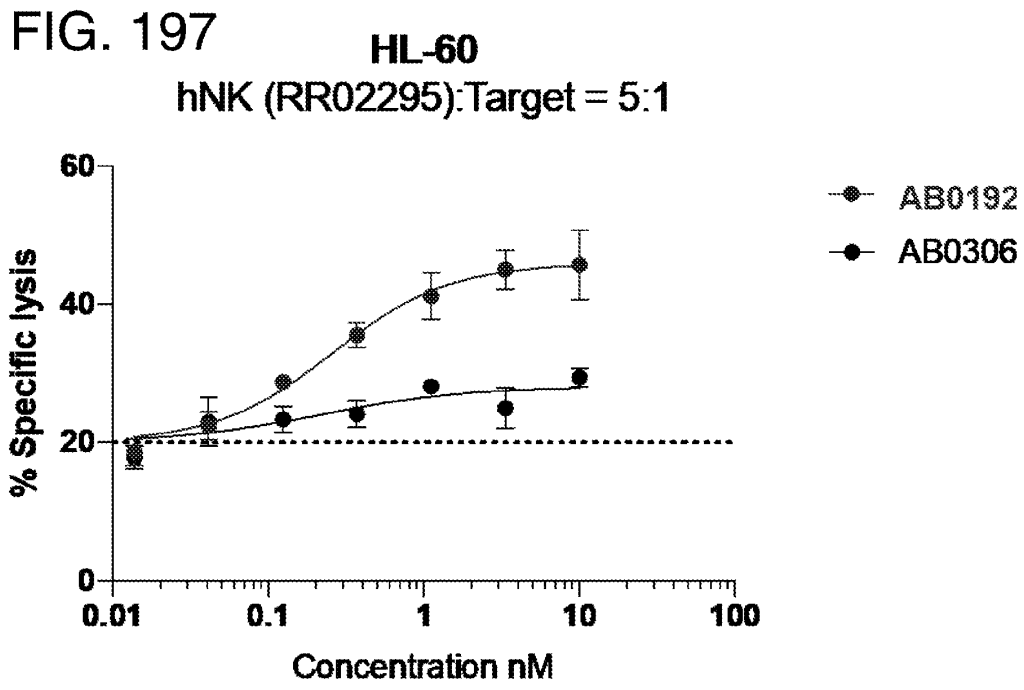


FIG. 198

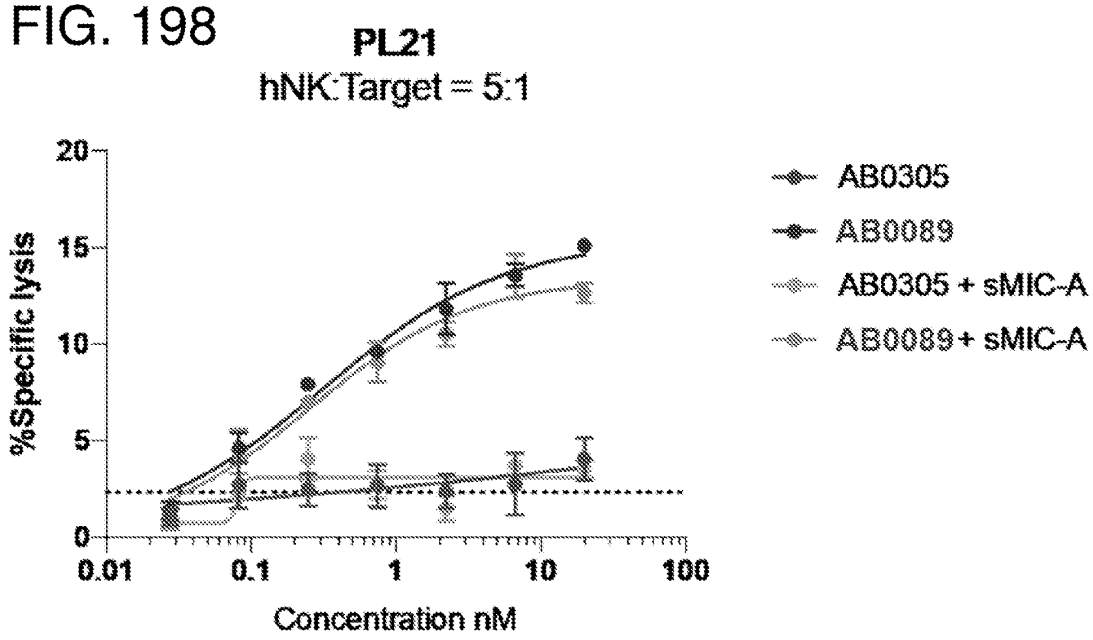


FIG. 199

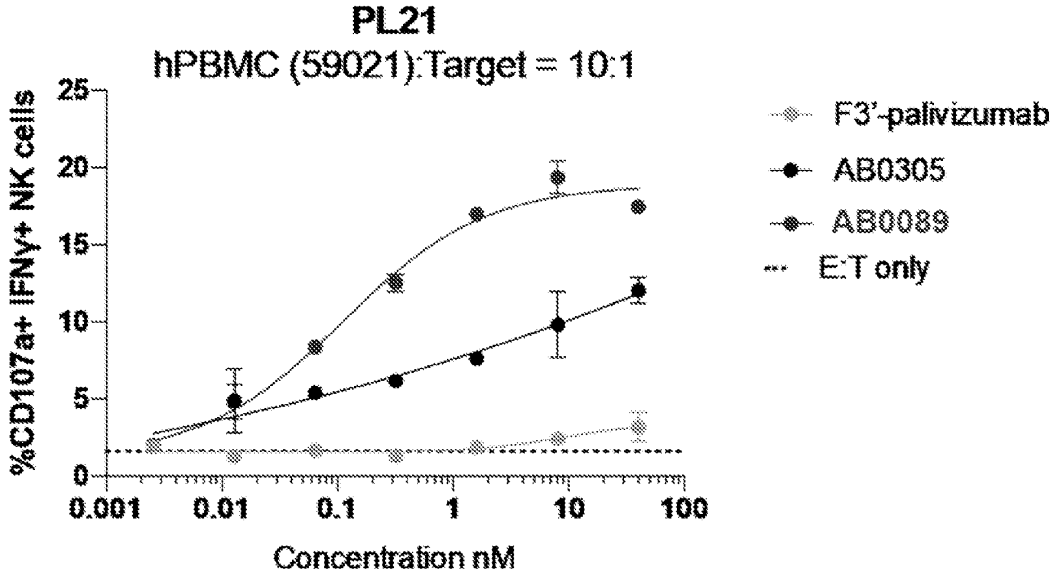


FIG. 200

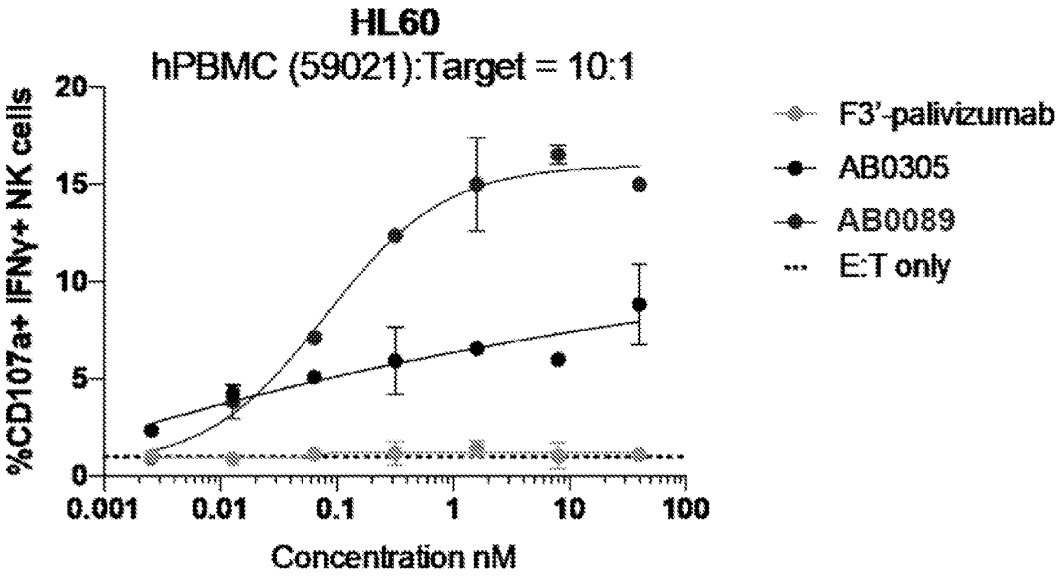


FIG. 201

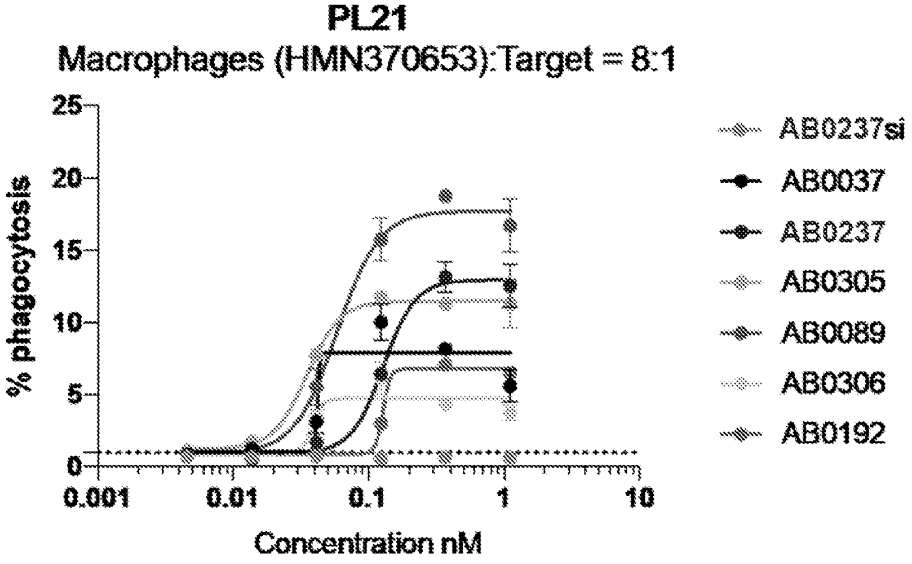


FIG. 202

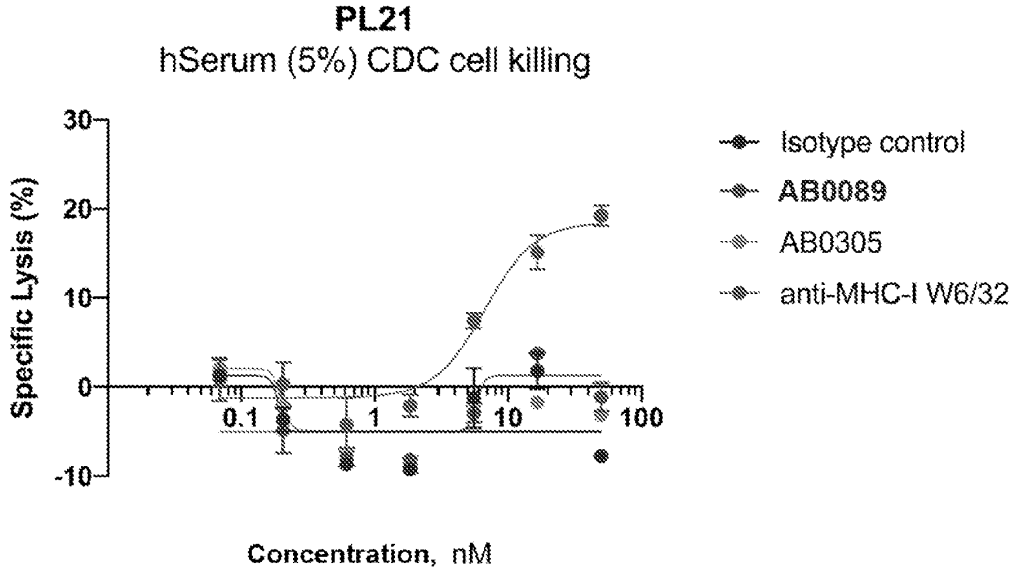


FIG. 203A

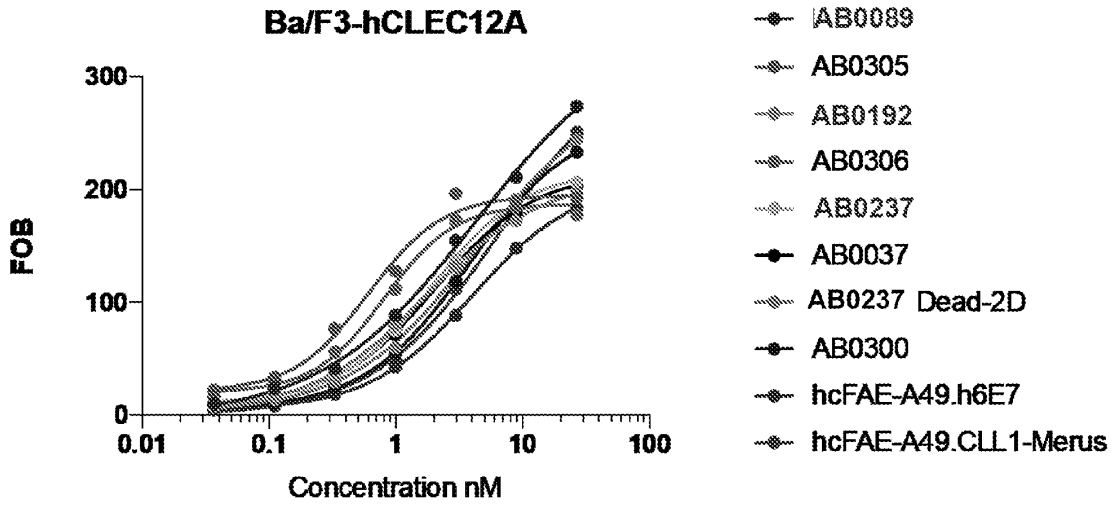


FIG. 203B

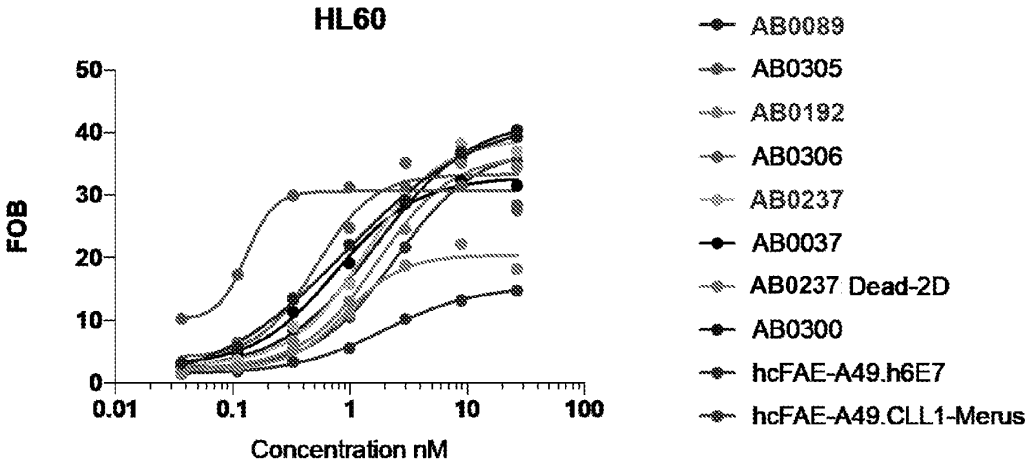


FIG. 203C

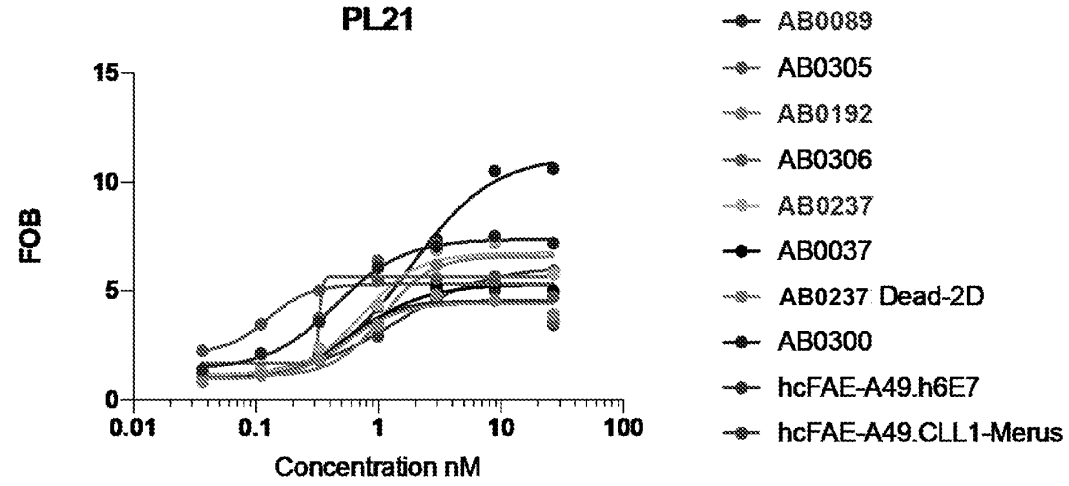


FIG. 204A

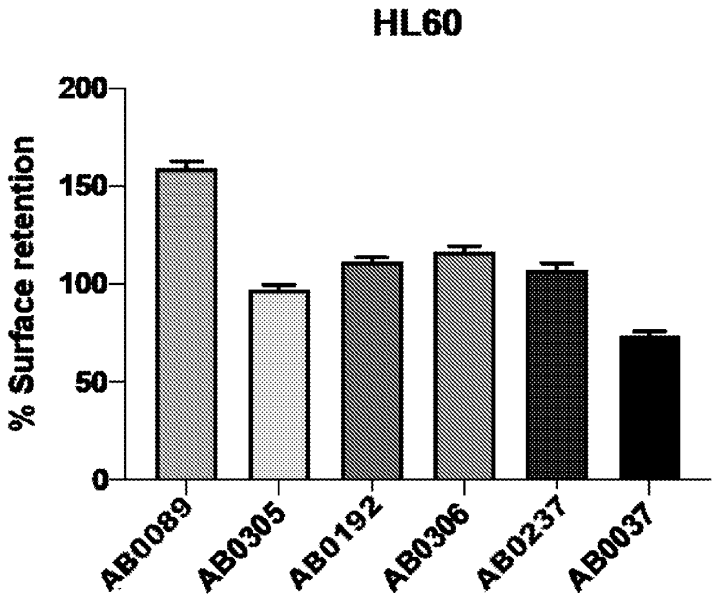


FIG. 204B

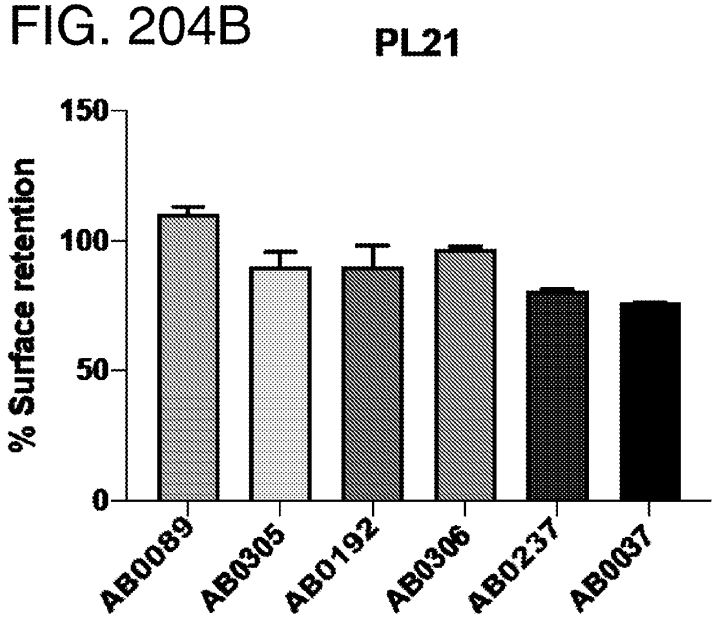


FIG. 205

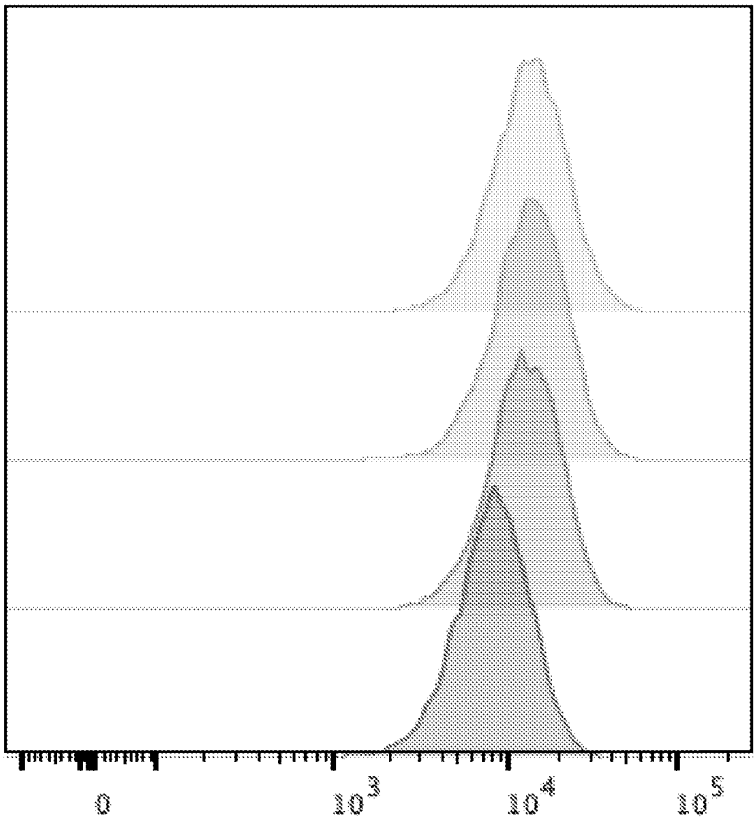


FIG. 206

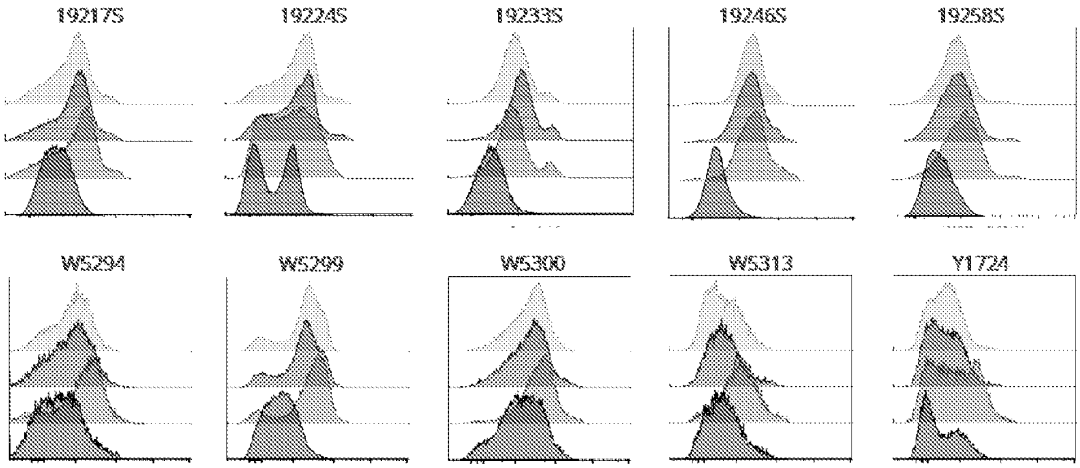
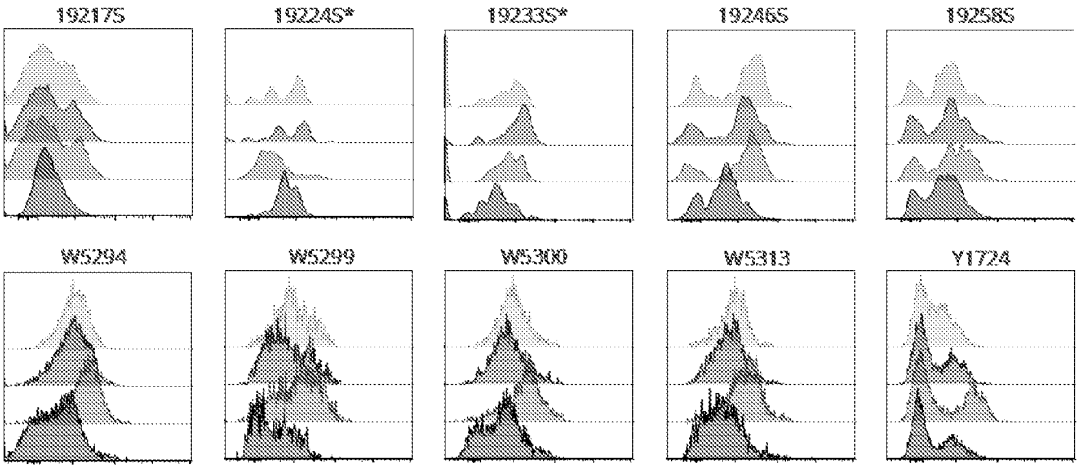


FIG. 207



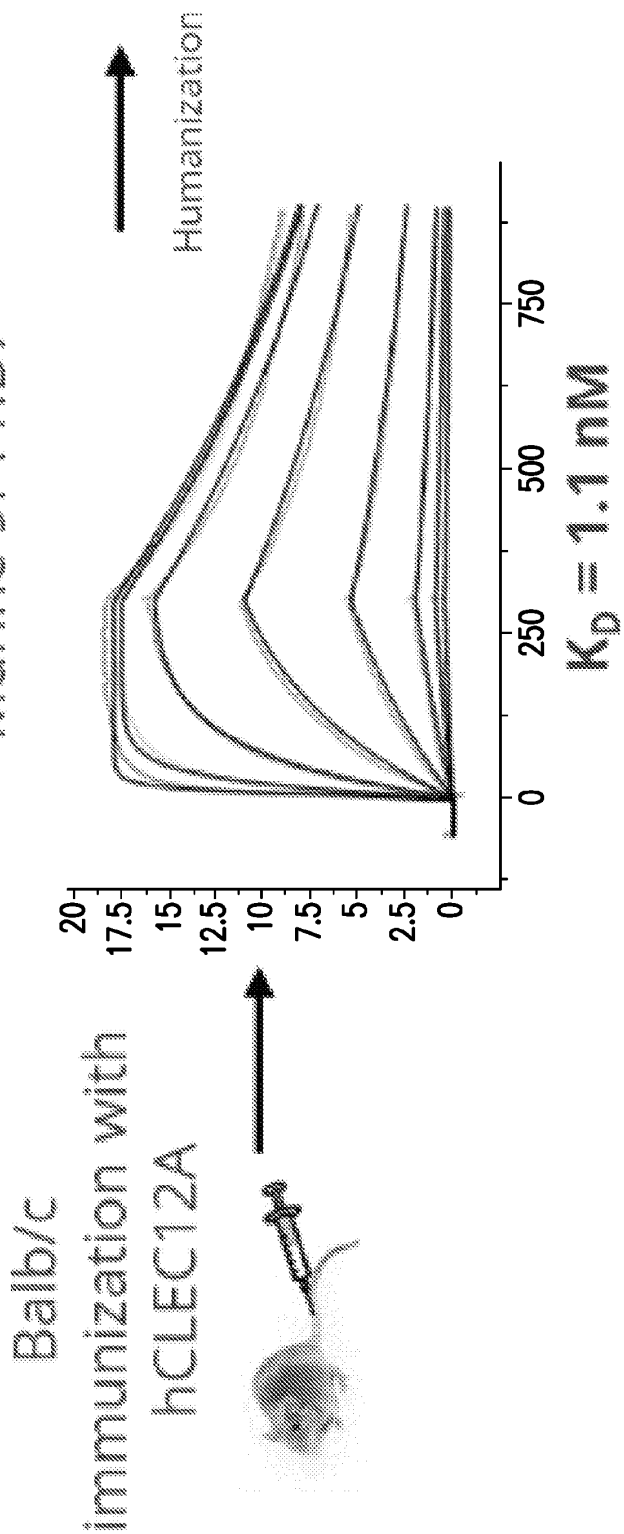
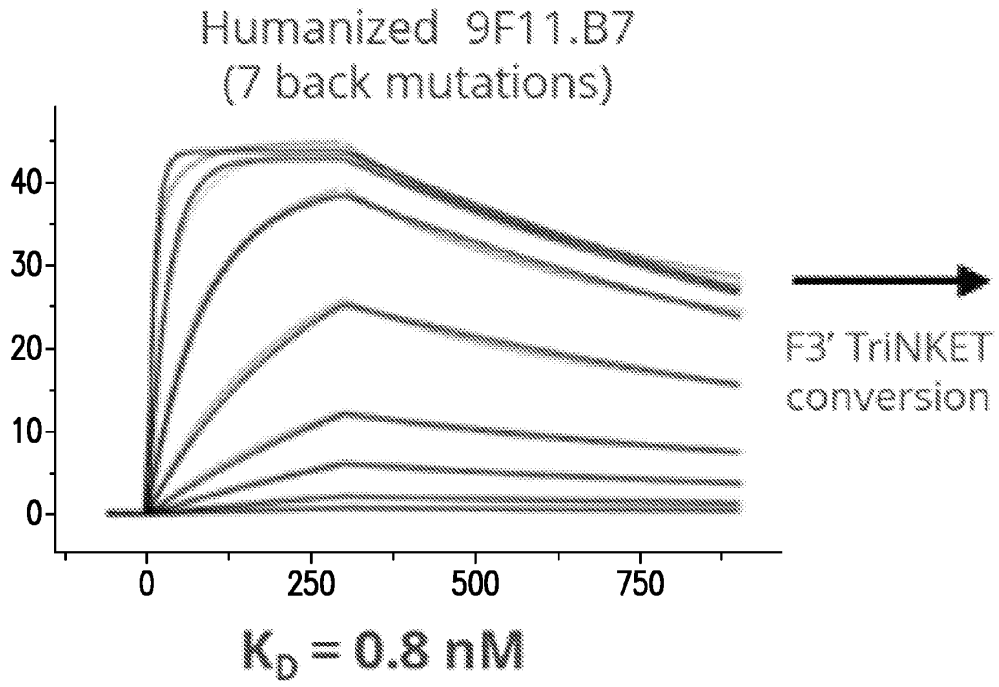

Balb/c
immunization with
hCLEC12A
Murine 9F11.B7
Humanization
20
17.5
15
12.5
10
7.5
5
2.5
0
0
250
500
750
K_D = 1.1 nM

FIG. 208



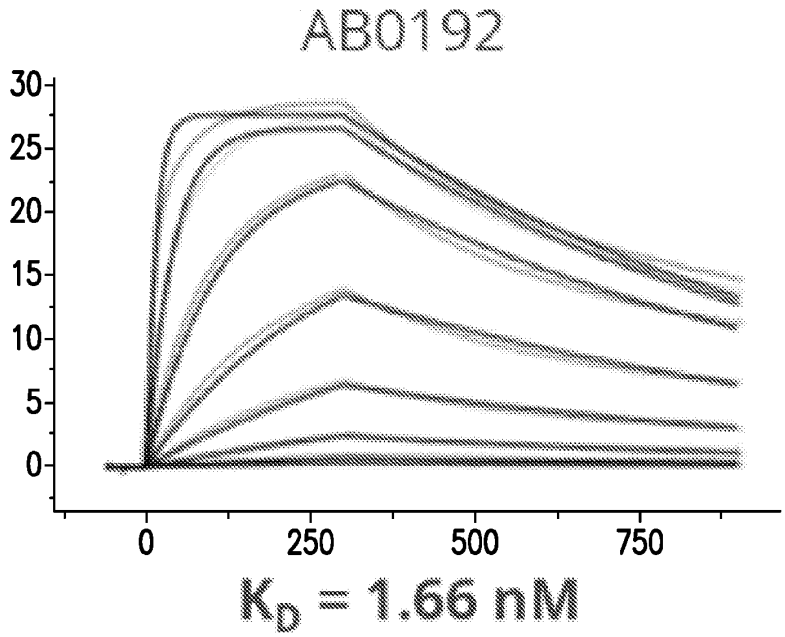
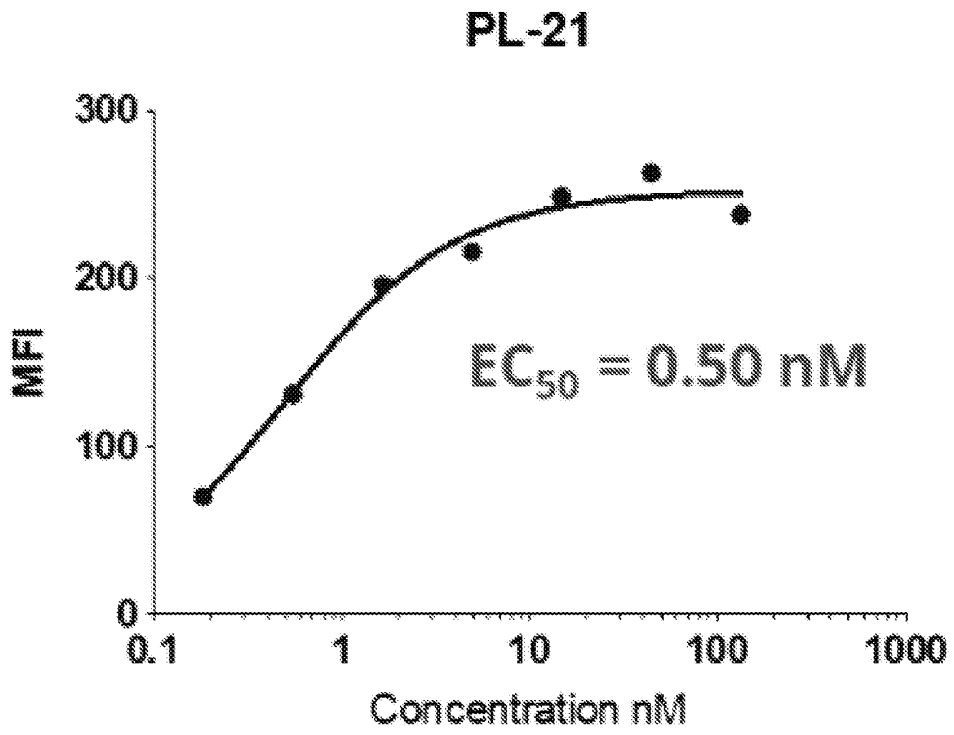


FIG. 208 Continued



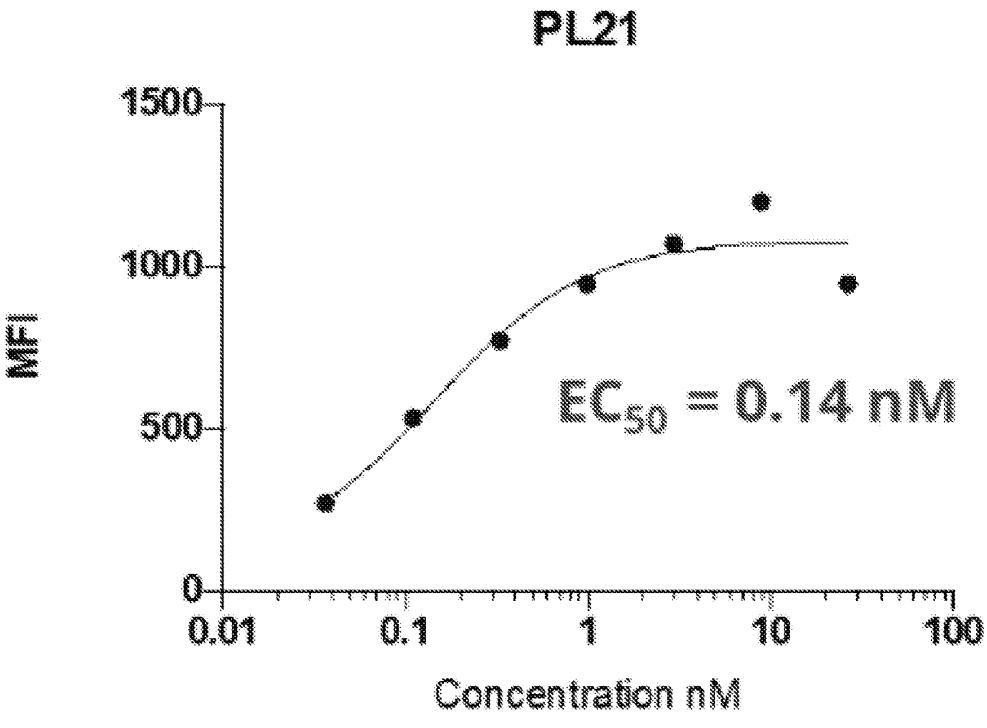


FIG. 208 Continued

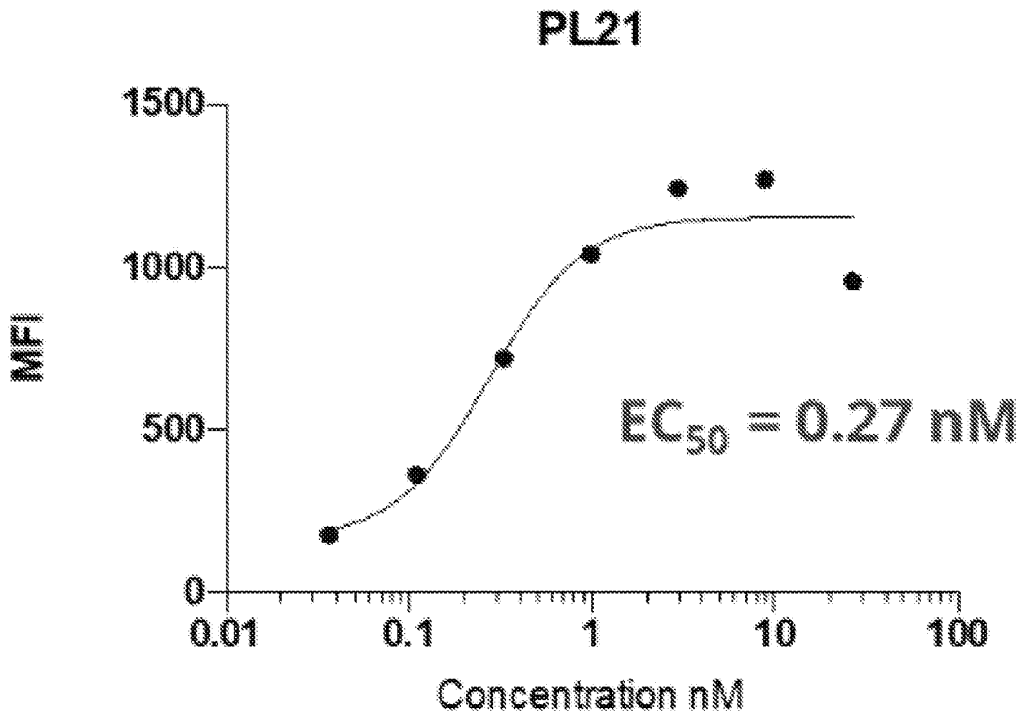


FIG. 208 Continued

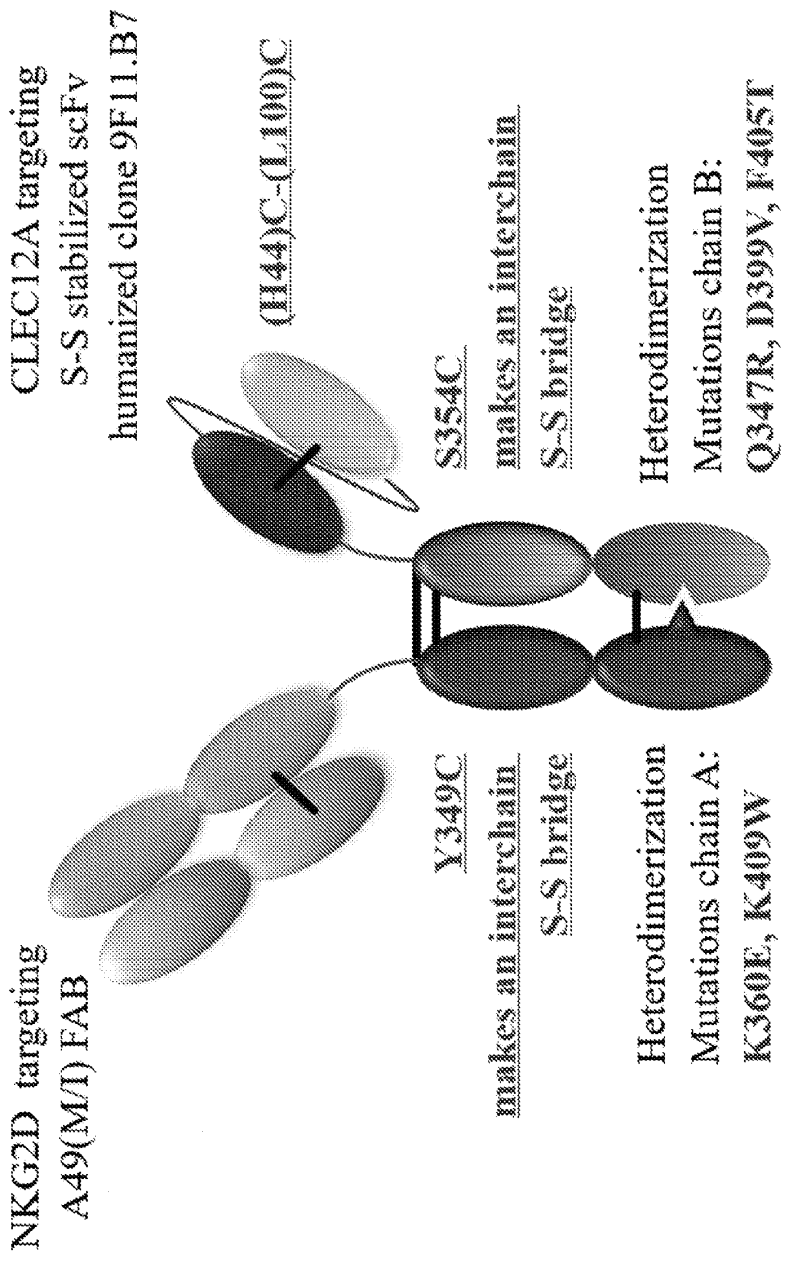

NKG2D targeting
A49(M/I) FAB
CLEC12A targeting
S-S stabilized scFv
humanized clone 9F11.B7
(H44)C-(L100)C
S354C
makes an interchain
S-S bridge
Heterodimerization
Mutations chain B:
Q347R, D399V, F405T
Y349C
makes an interchain
S-S bridge
Heterodimerization
Mutations chain A:
K360E, K409W

FIG. 209

FIG. 210

Chain S: anti-CLEC12A scFv-CH2-CH3 (humanized sequence, CDRs are bold and not underlined, back mutations are bold and underlined, engineered scFv disulfide in italic and bold, and Fc mutations for heterodimerization and the engineered CH3 disulfide are underlined and not bold).

EVQLVESGGGVVQPGGSLRLSCAAS**GFTFNAFGM**HWVRQAPGK***C***LEWVAFIS**SGSTSI**YYYANT VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA**RDGYPTGGAMDY**WGQGTSVTVSSGGGGS GGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITC**KASQDIYNYLS**WFQQKPGKAPK**PLIY RANILVS**GVPSRFSGSGSG**QDYTFTISSLQPEDIATYYC**LQFDAFPFT**FG***C***GTKLEIKGSDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPRVYTLPPCR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLVSDGSFTLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:278)

Chain H: anti-NKGD VH-CH1-CH2-CH3 (fully human, CDRs are bold and not underlined, Fc mutations for heterodimerization and the engineered CH3 disulfide are underlined and not bold)

EVQLVESGGGLVKPGGSLRLSCAAS**GFTFSSY**SMNWVRQAPGKGLEWVSS**ISSSSSY**IYYADSVK GRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR**GAPIGAAAGWFDP**WGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTENQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSWLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:260)

Chain L: anti-NKGD VL-CL (fully human, CDRs are bold and not underlined)

DIQMTQSPSSVSASVGDRVTITC**RASQGISSWLA**WYQQKPGKAPKLLIY**AASSLQS**GVPSRFSGS GSGTDFTLTISSLQPEDFATYYC**QQGVSFPRT**FGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC EVTHQGLSSPVTKSFNRGEC (SEQ ID NO:261)

FIG. 211

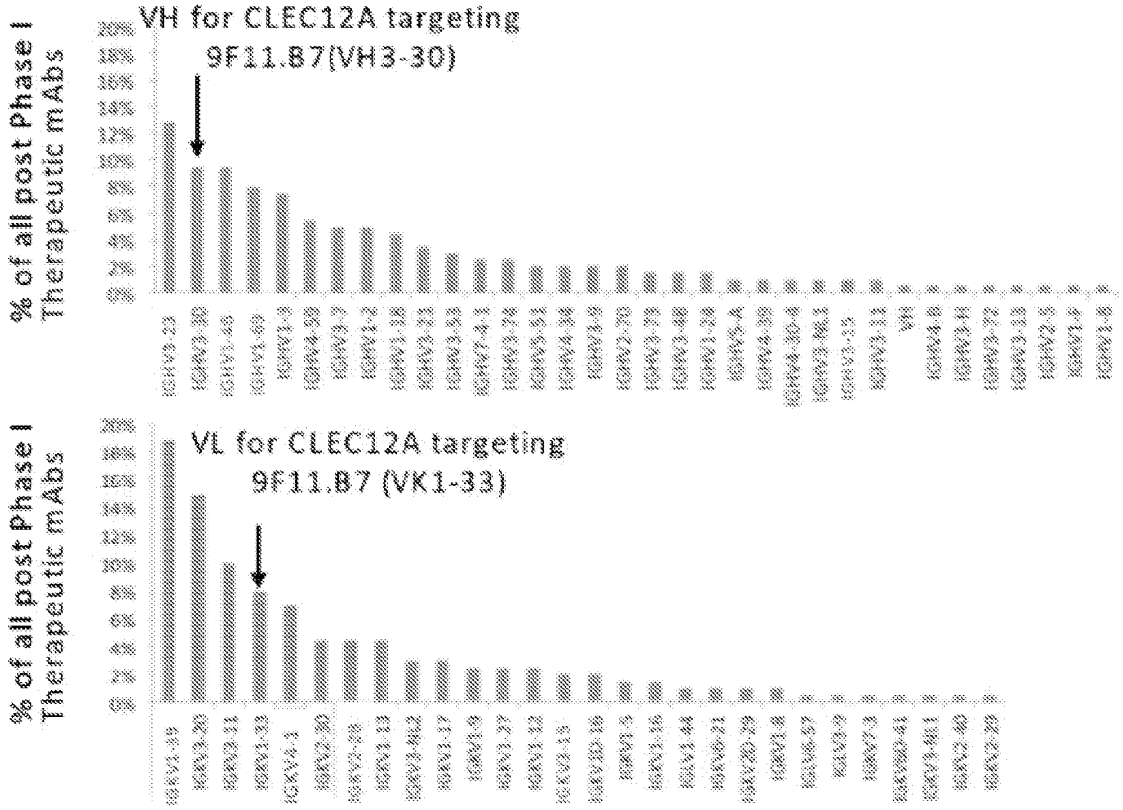
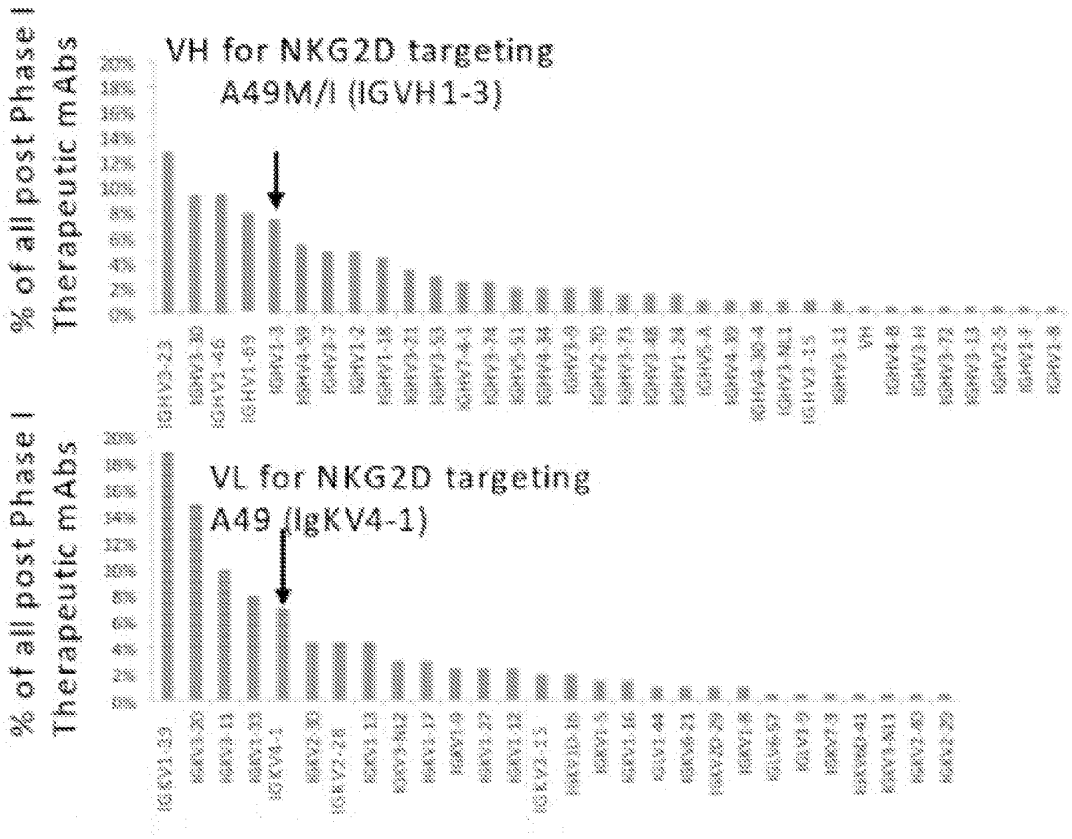

% of all post Phase I Therapeutic mAbs
VH for CLEC12A targeting
9F11.B7(VH3-30)
% of all post Phase I Therapeutic mAbs
VL for CLEC12A targeting
9F11.B7 (VK1-33)

FIG. 211 continued



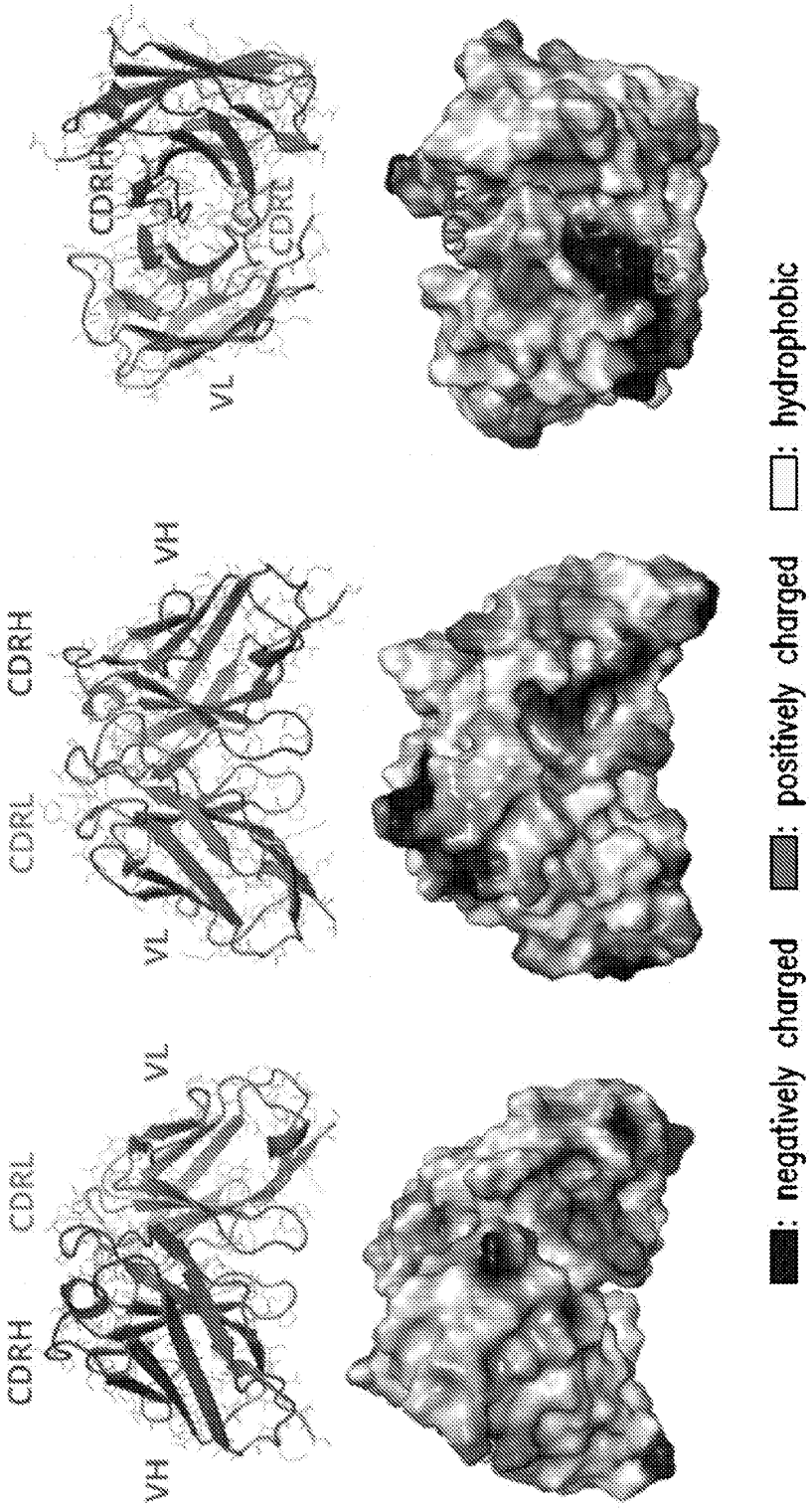

CDRH
CDRL
VL
CDRH
CDRL
VH
CDRH
CDRL
VL
VH
: negatively charged
: positively charged
: hydrophobic

FIG. 212

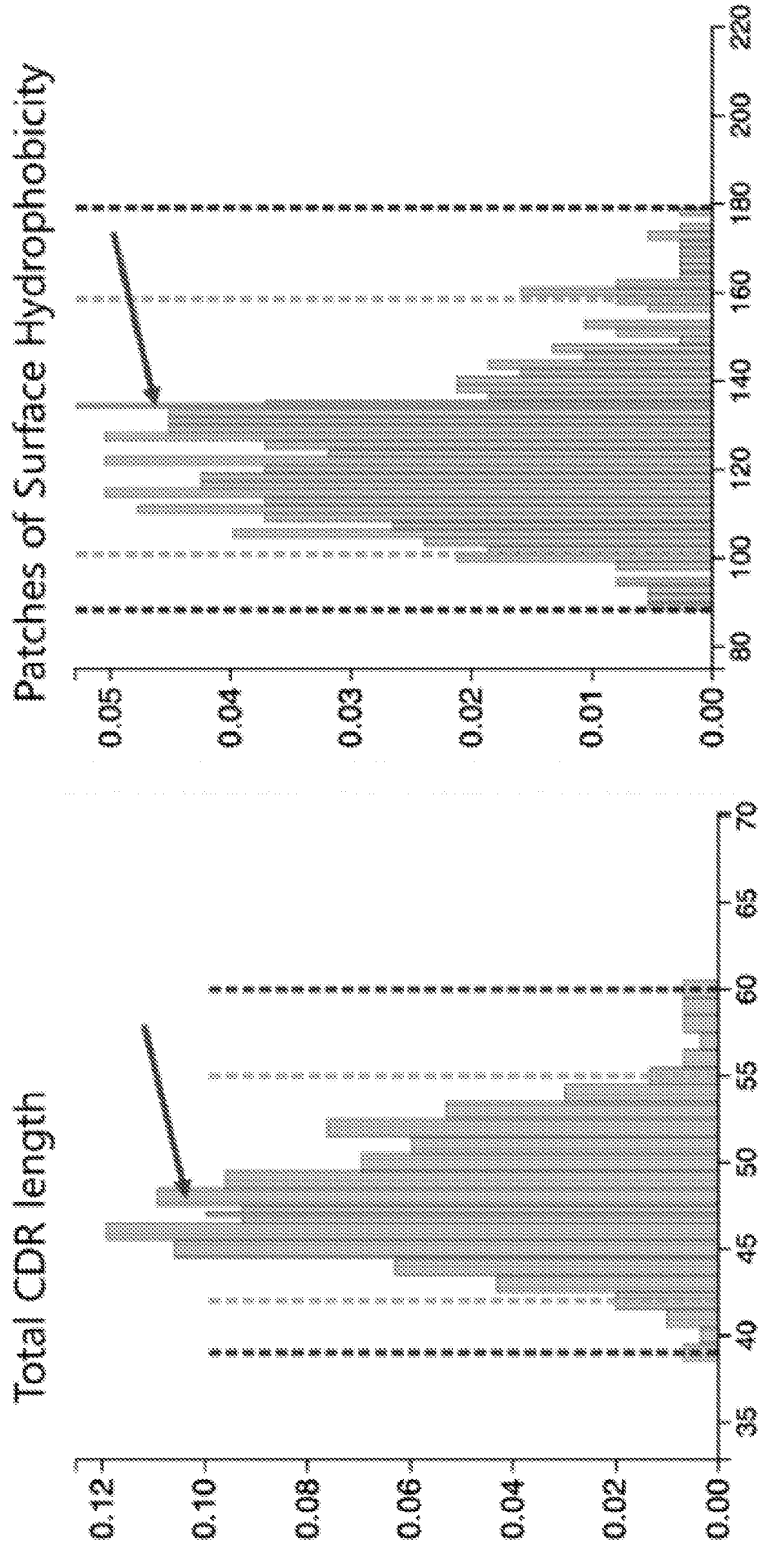

Patches of Surface Hydrophobicity
0.05
0.04
0.03
0.02
0.01
0.00
80
100
120
140
160
180
200
220
Total CDR length
0.12
0.10
0.08
0.06
0.04
0.02
0.00
35
40
45
50
55
60
65
70

FIG. 213

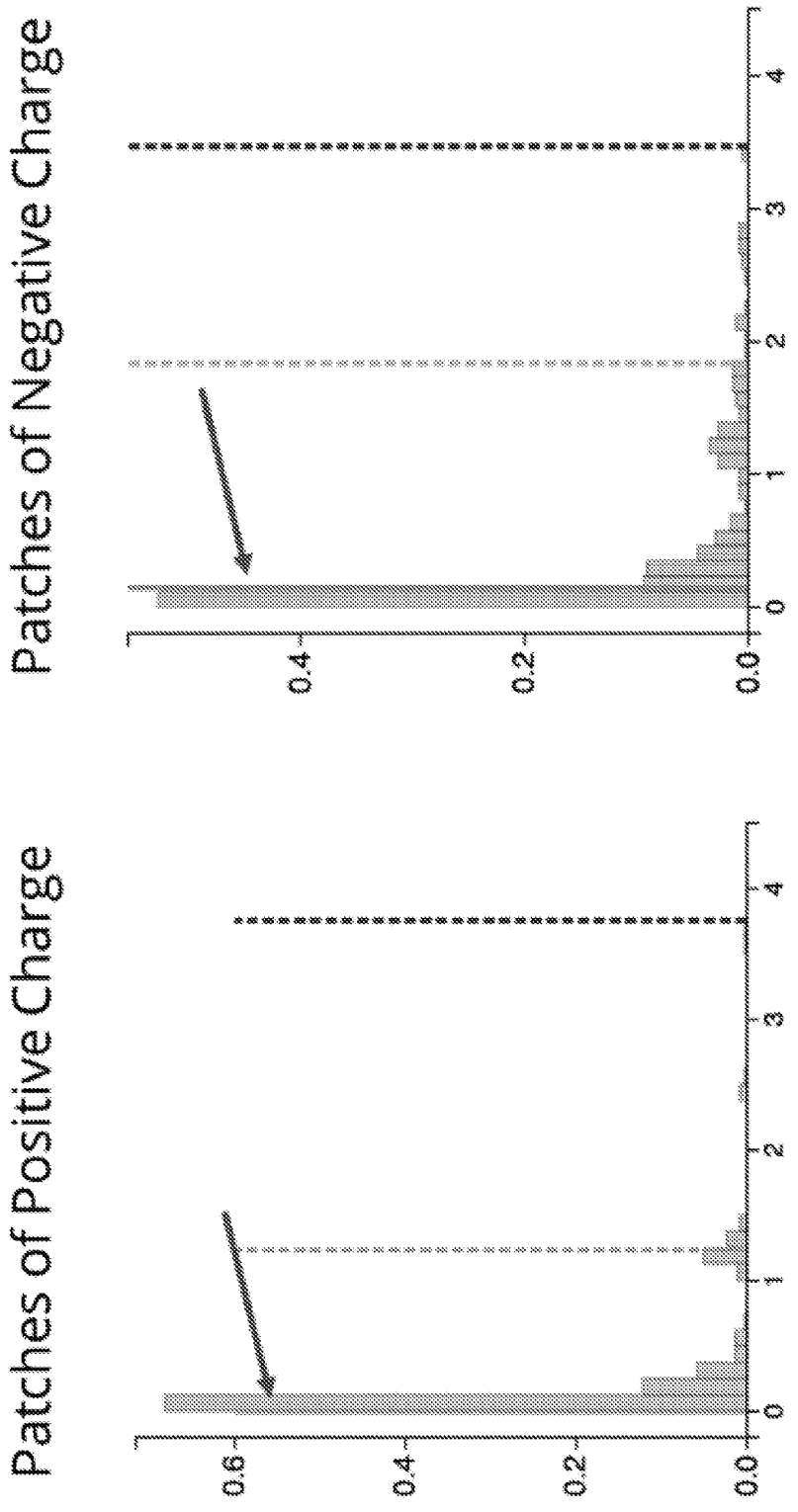

Patches of Negative Charge
0.0
0.2
0.4
0
1
2
3
4
Patches of Positive Charge
0.0
0.2
0.4
0.6
0
1
2
3
4

FIG. 214

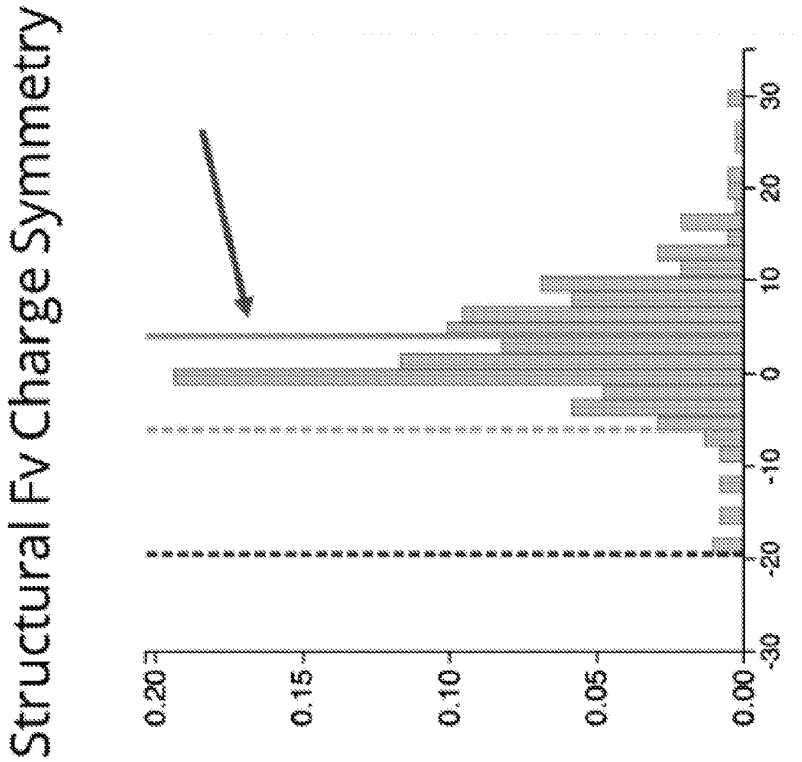


FIG. 214 Continued

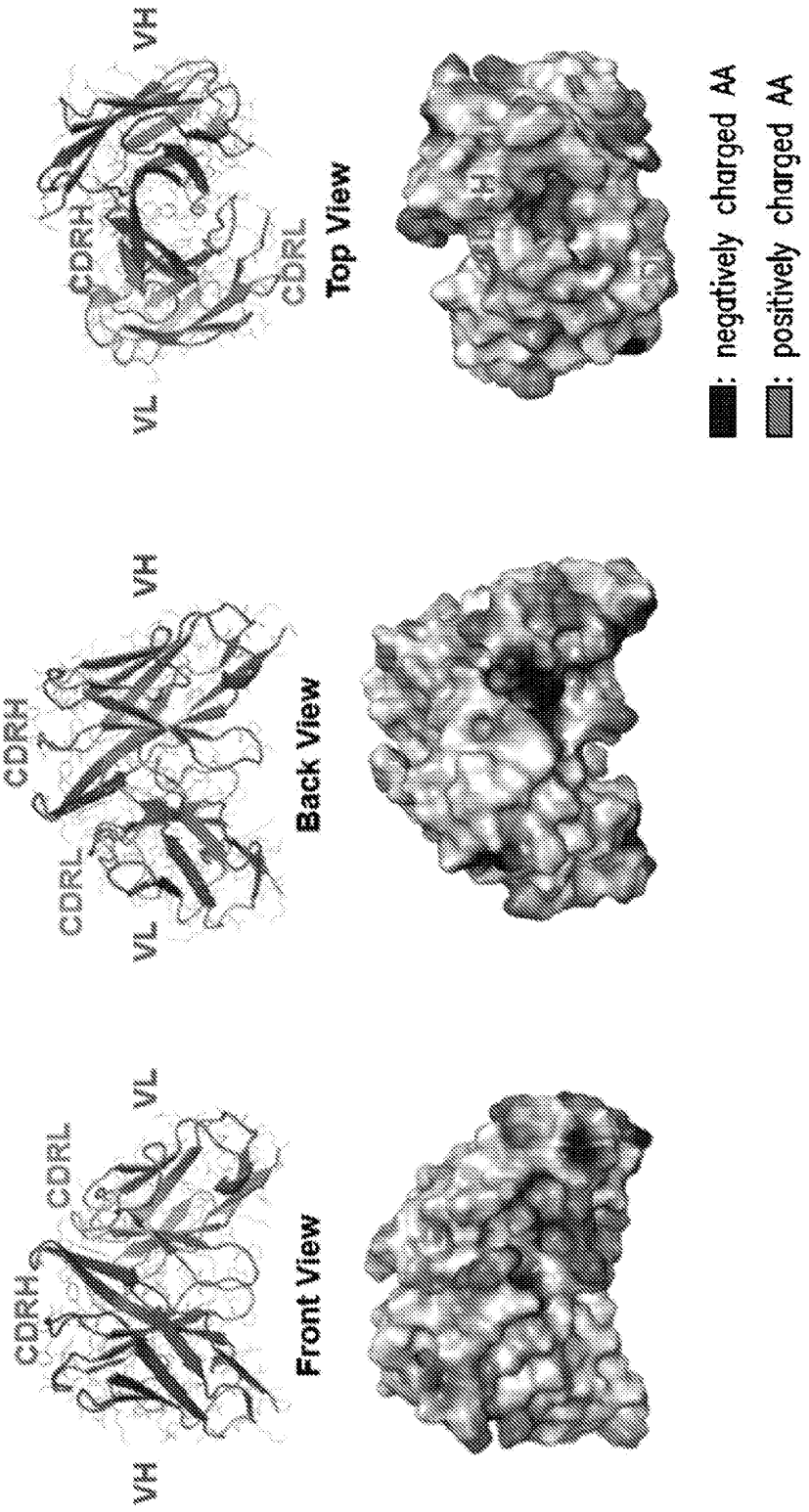

VH
CDRH
CDRL
VL
Top View
: negatively charged AA
: positively charged AA
VH
CDRH
CDRL
VL
Back View
VL
CDRL
CDRH
VH
Front View

FIG. 215

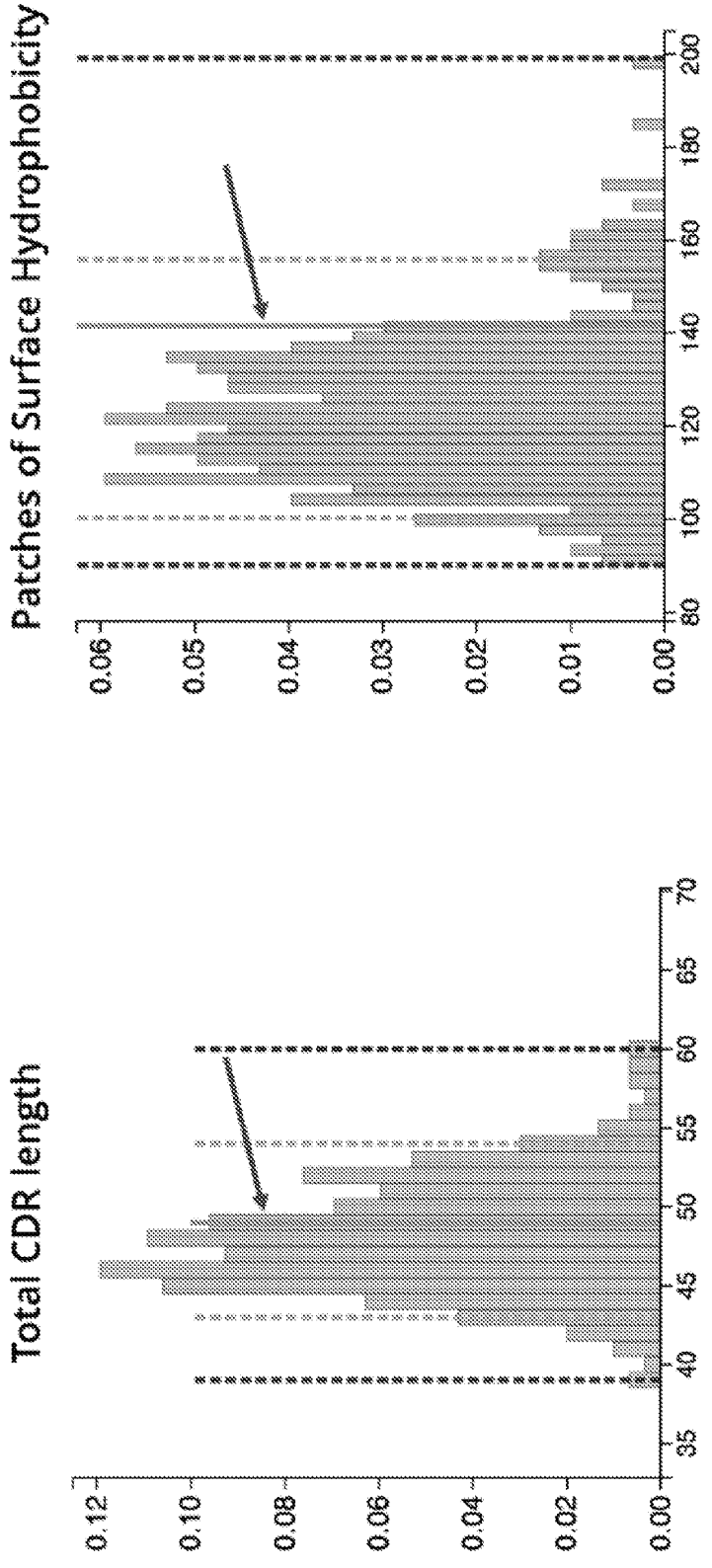

Patches of Surface Hydrophobicity
0.06
0.05
0.04
0.03
0.02
0.01
0.00
80
100
120
140
160
180
200
Total CDR length
0.12
0.10
0.08
0.06
0.04
0.02
0.00
35
40
45
50
55
60
65
70

FIG. 216

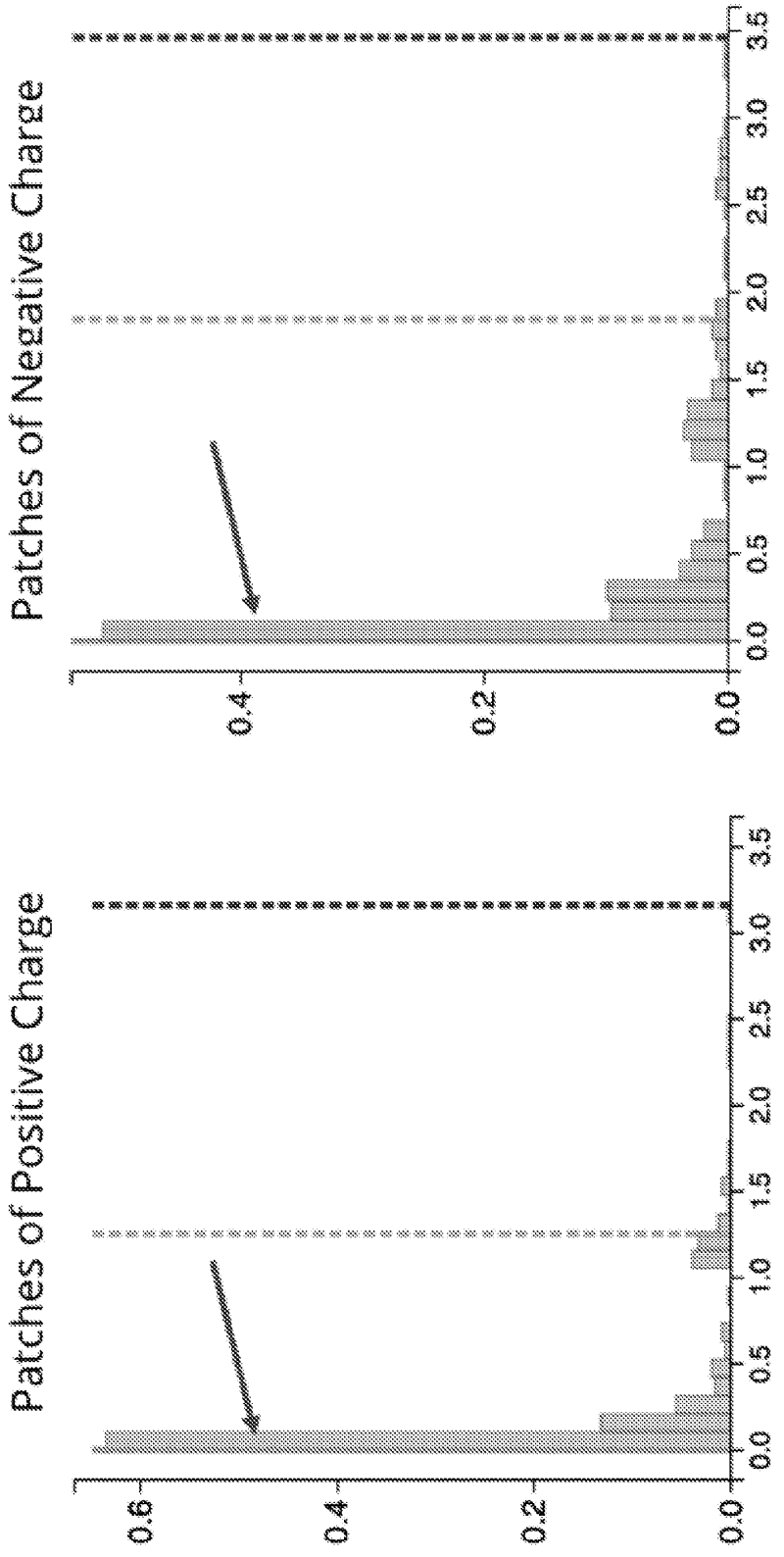

Patches of Negative Charge
0.0
0.2
0.4
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Patches of Positive Charge
0.0
0.2
0.4
0.6
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5

FIG. 217

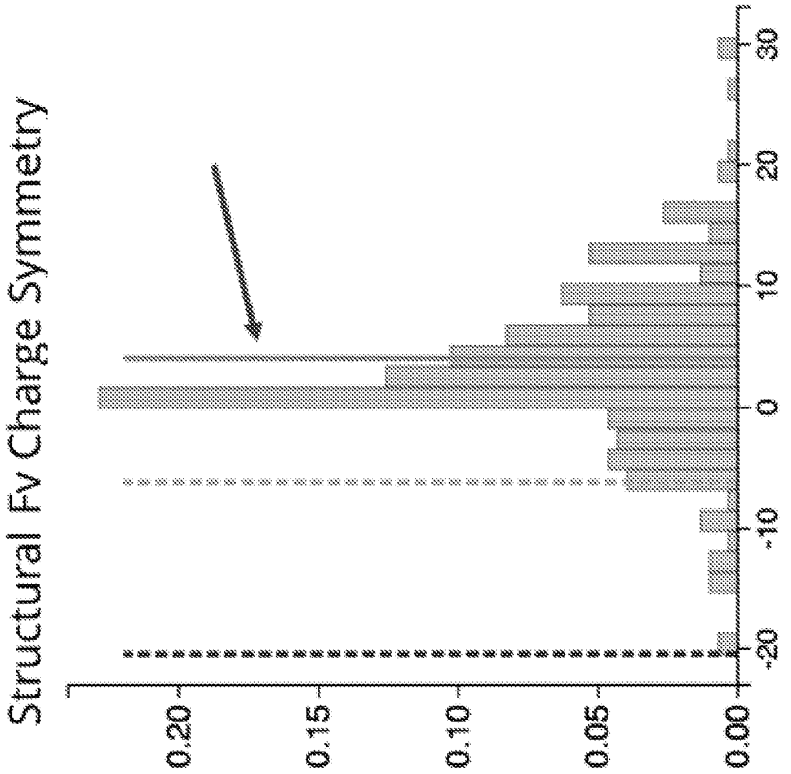


FIG. 217 Continued

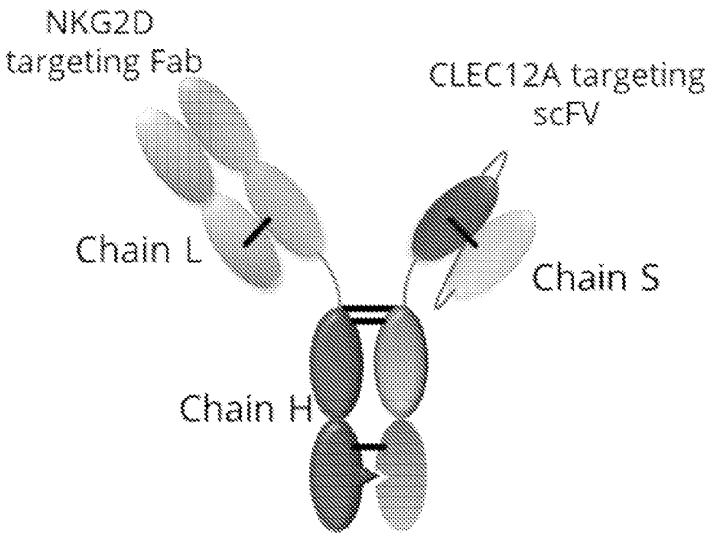

NKG2D
targeting Fab
CLEC12A targeting
scFV
Chain L
Chain S
Chain H

FIG. 218A

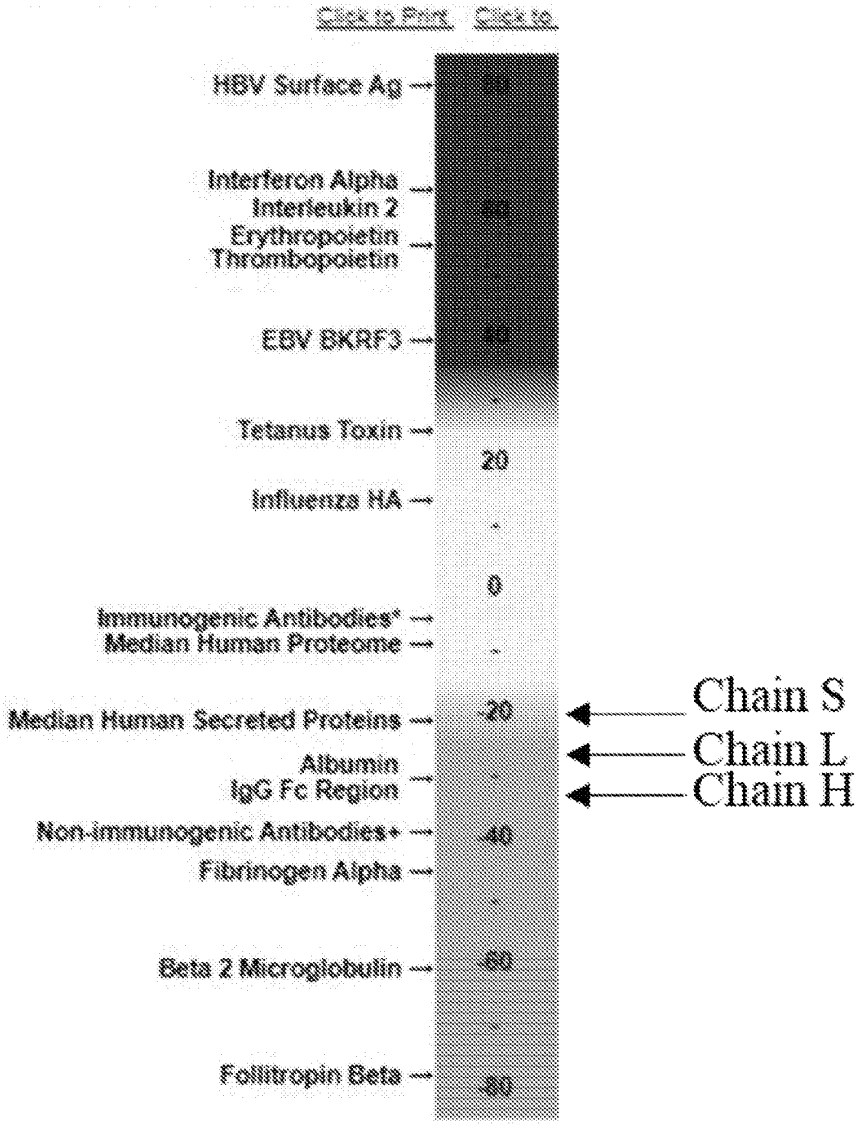

Click to Print
Click to
HBV Surface Ag
Interferon Alpha
Interleukin 2
Erythropoietin
Thrombopoietin
EBV BKRF3
Tetanus Toxin
Influenza HA
Immunogenic Antibodies*
Median Human Proteome
Median Human Secreted Proteins
Albumin
IgG Fc Region
Non-immunogenic Antibodies+
Fibrinogen Alpha
Beta 2 Microglobulin
Follitropin Beta
80
60
40
20
0
-20
-40
-60
-80
Chain S
Chain L
Chain H

FIG. 218B

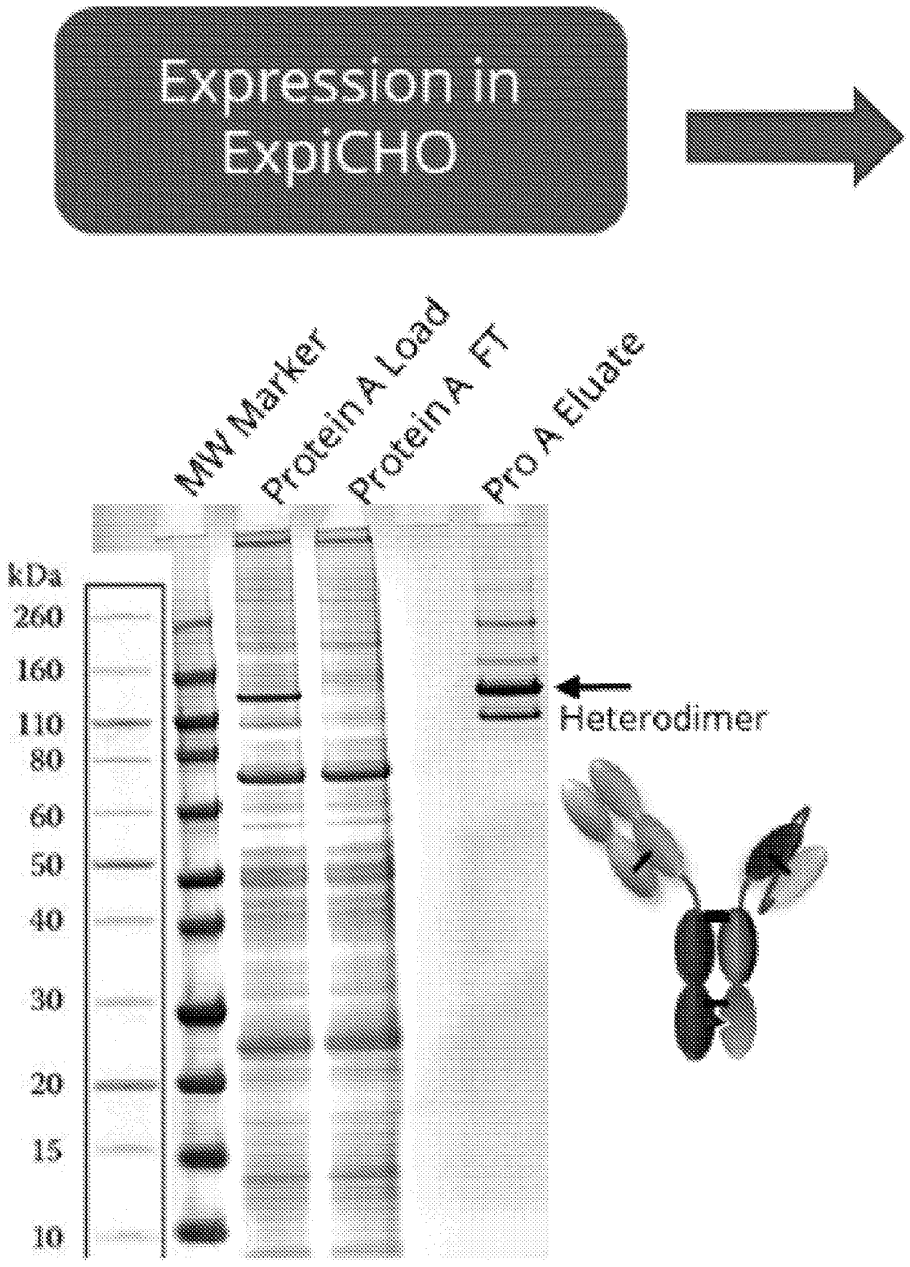
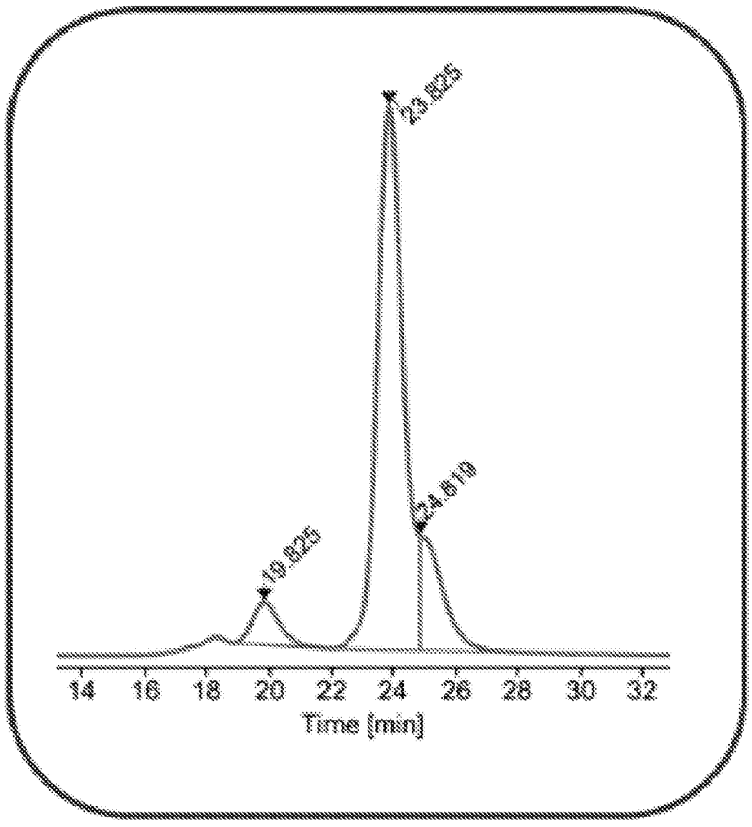

Expression in ExpiCHO
MW Marker
Protein A Load
Protein A FT
Pro A Eluate
kDa
260
160
110
80
60
50
40
30
20
15
10
Heterodimer
Plasmid ratio unoptimized.

FIG. 219

Protein A Capture Chromatography

SEC

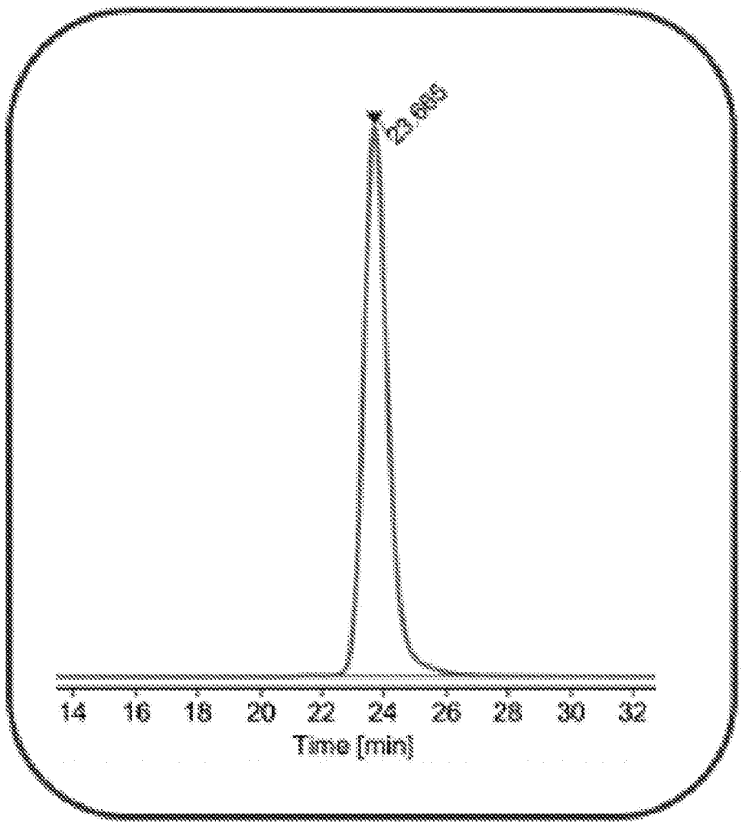


ProA Eluate:
~79% F3' heterodimer

FIG. 219 Continued

CEX Chromatography

SEC



CIEX Product:
>99% F3' heterodimer

FIG. 219 Continued

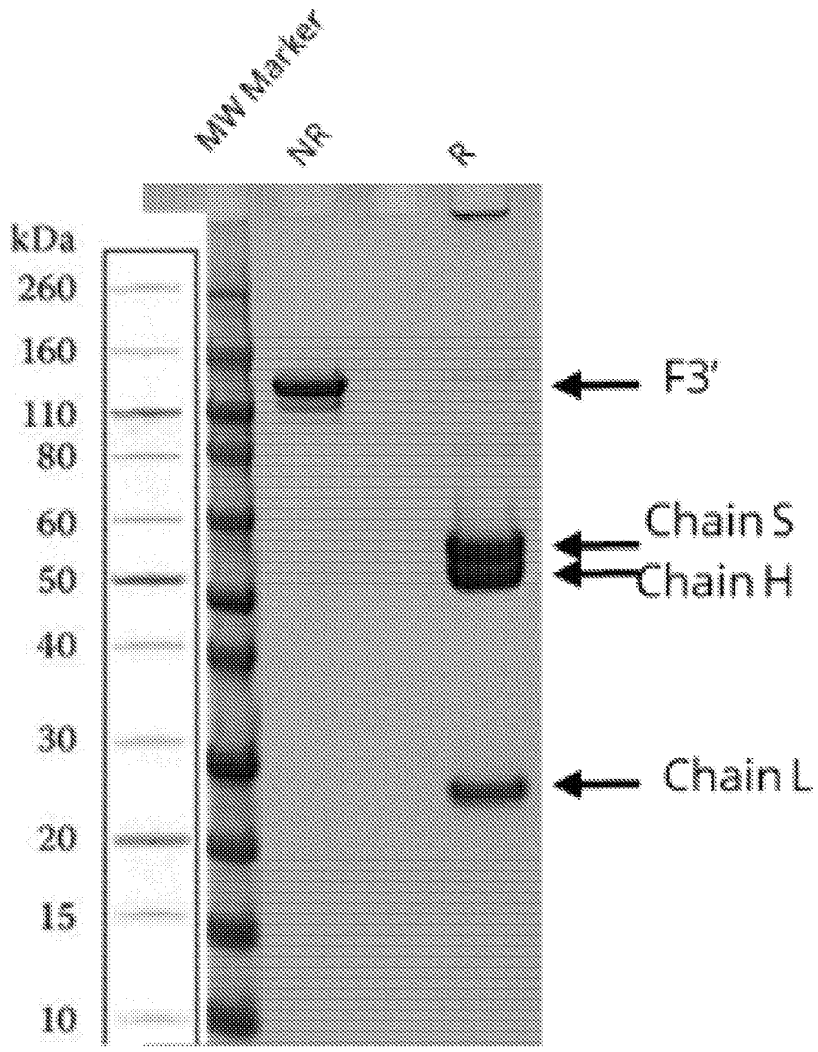
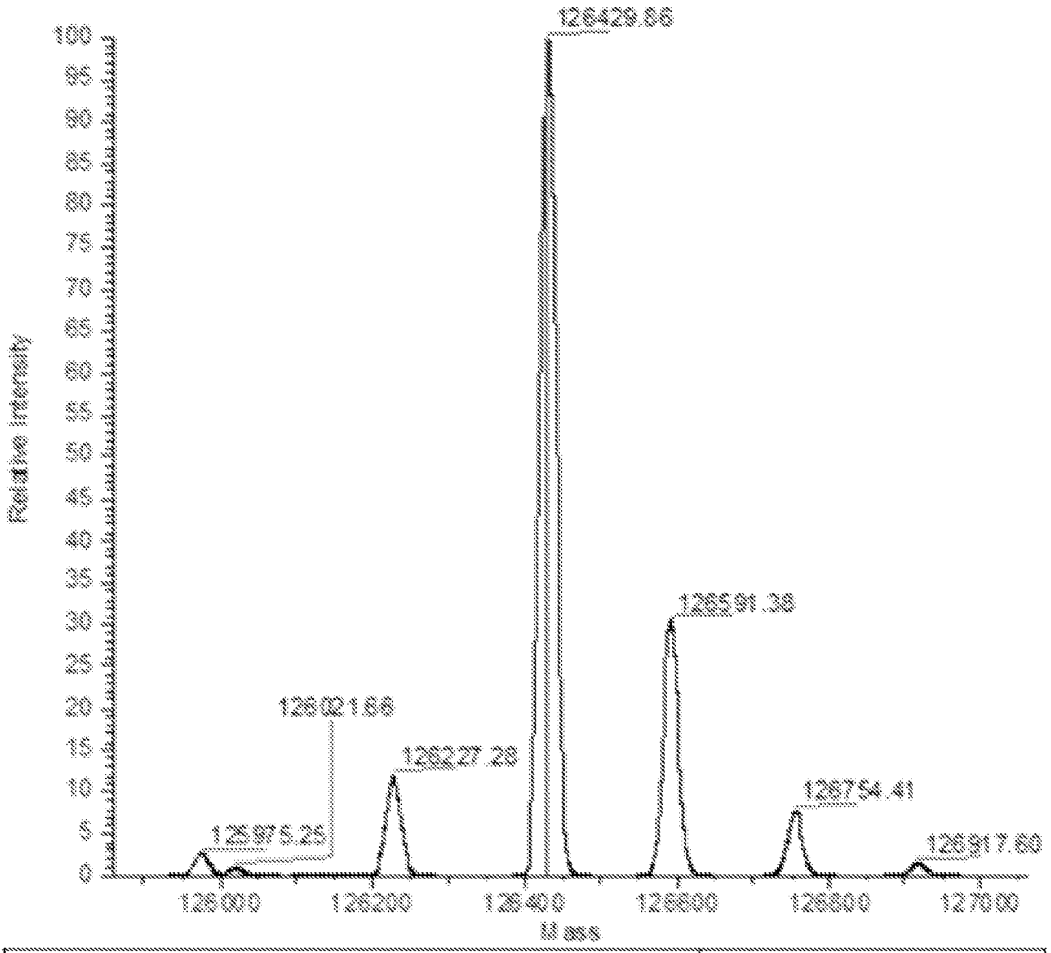


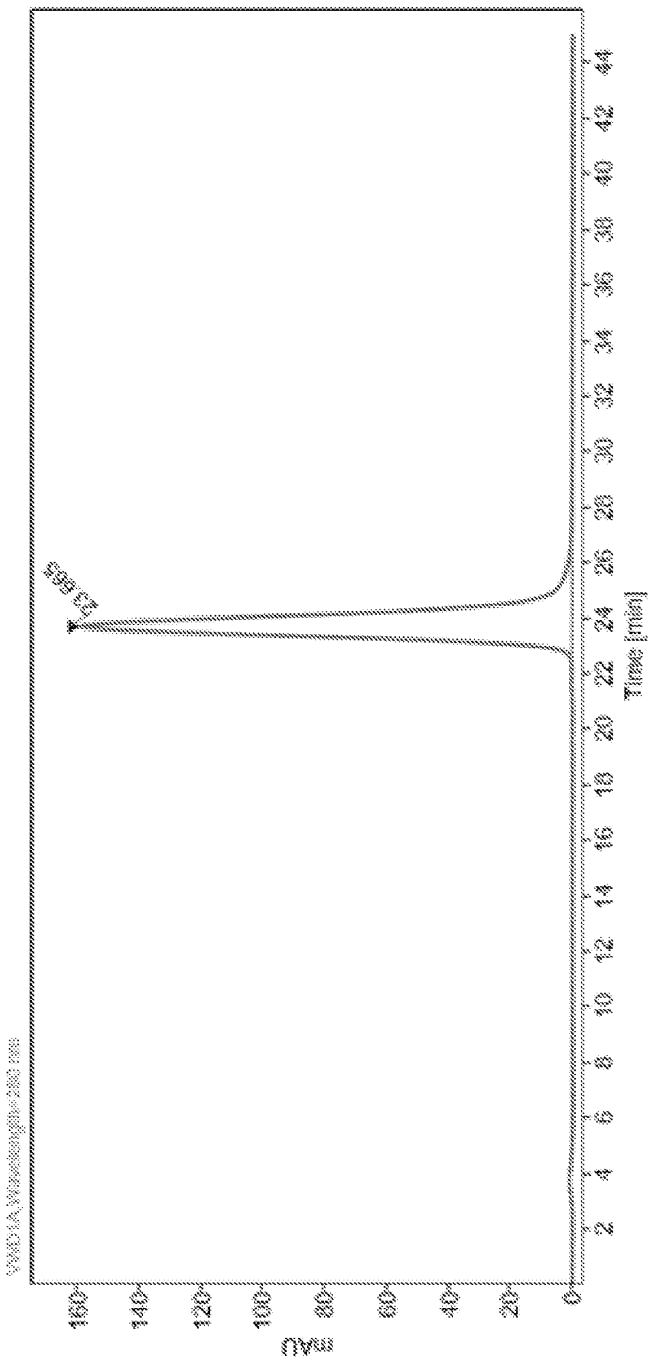
FIG. 219 Continued

FIG. 220



intact mass (theoretical)	126,429.5 Da
intact mass (experimental)	126,429.9 Da

FIG. 221



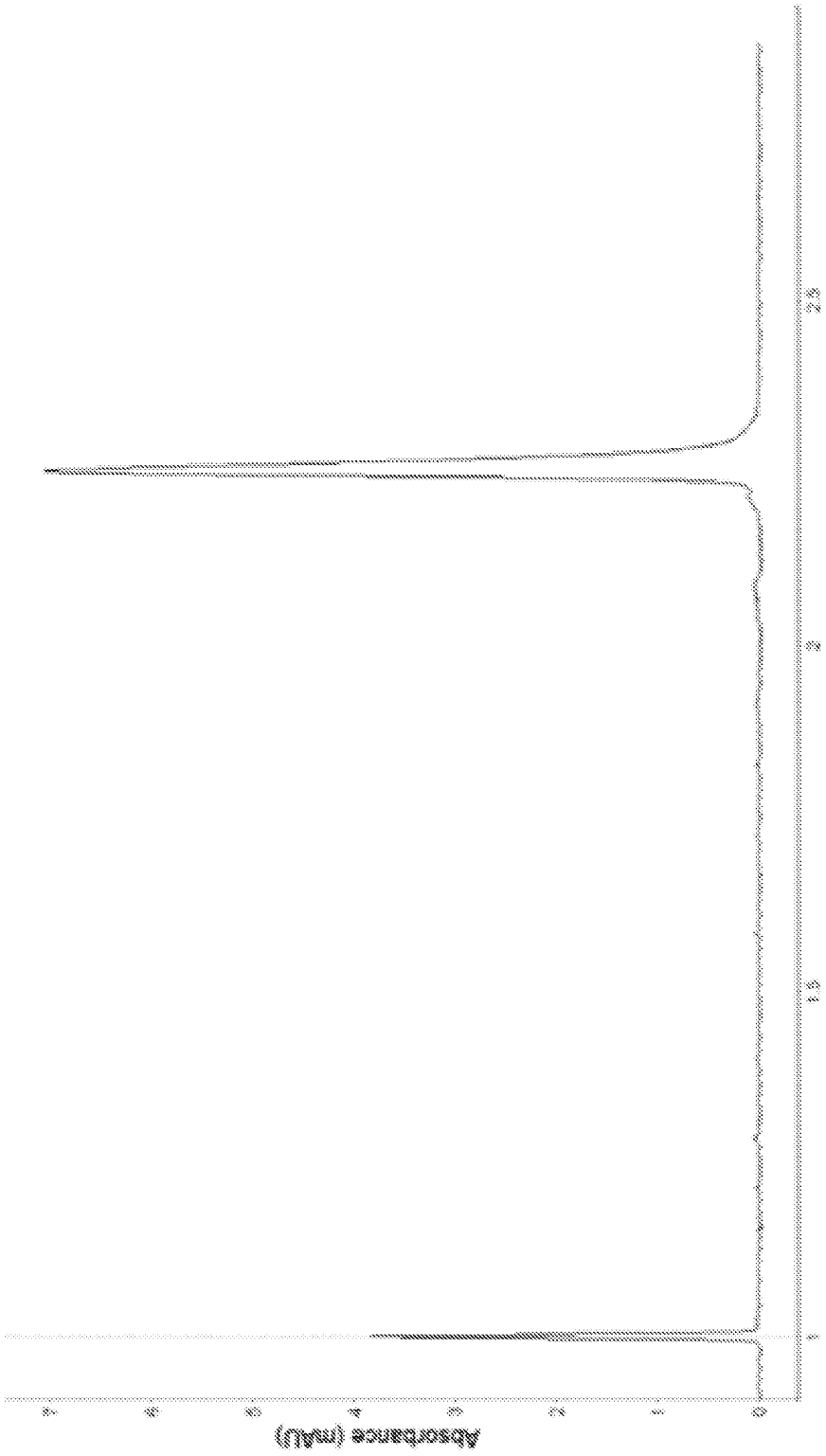


FIG. 222

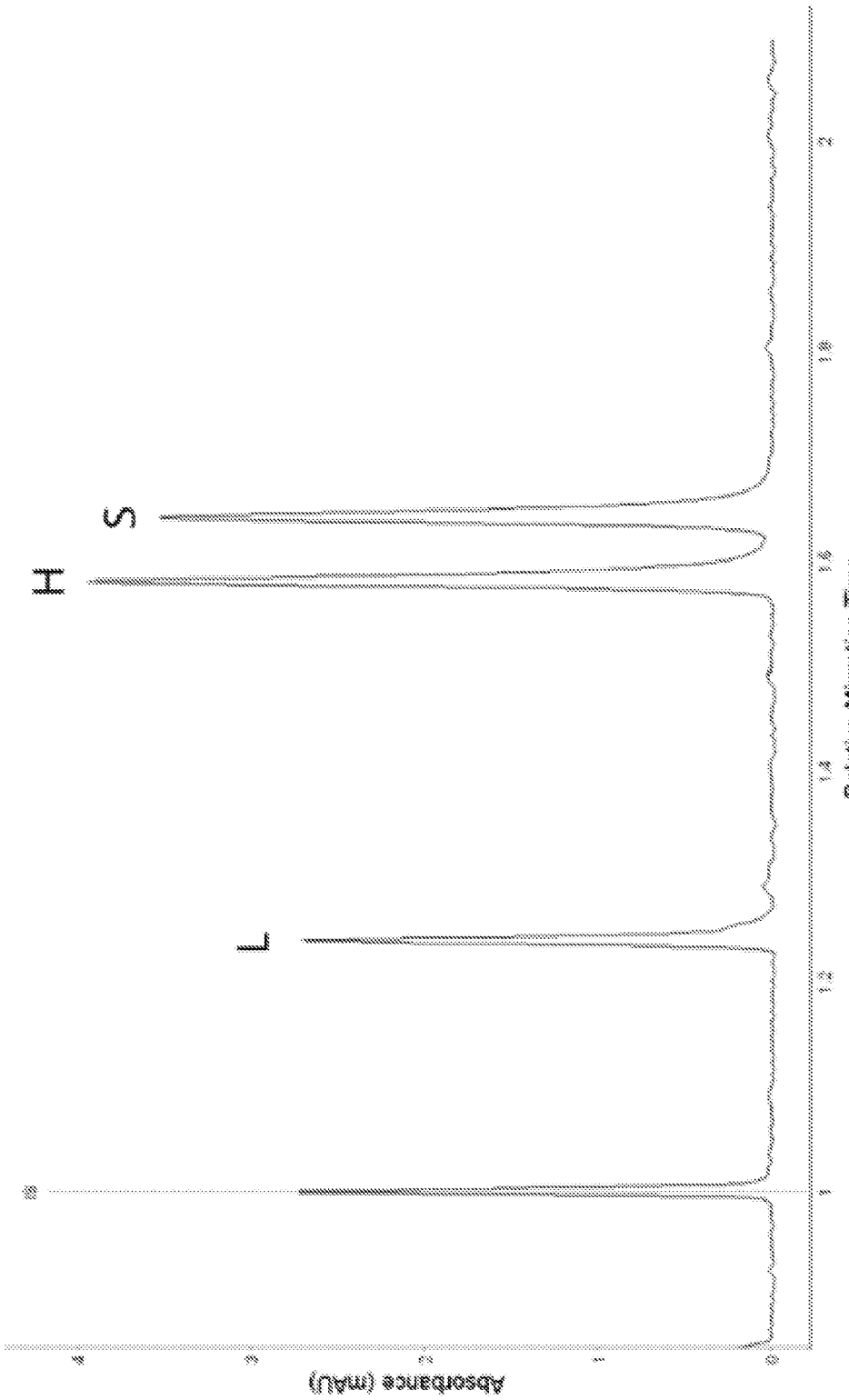


FIG. 222 Continued

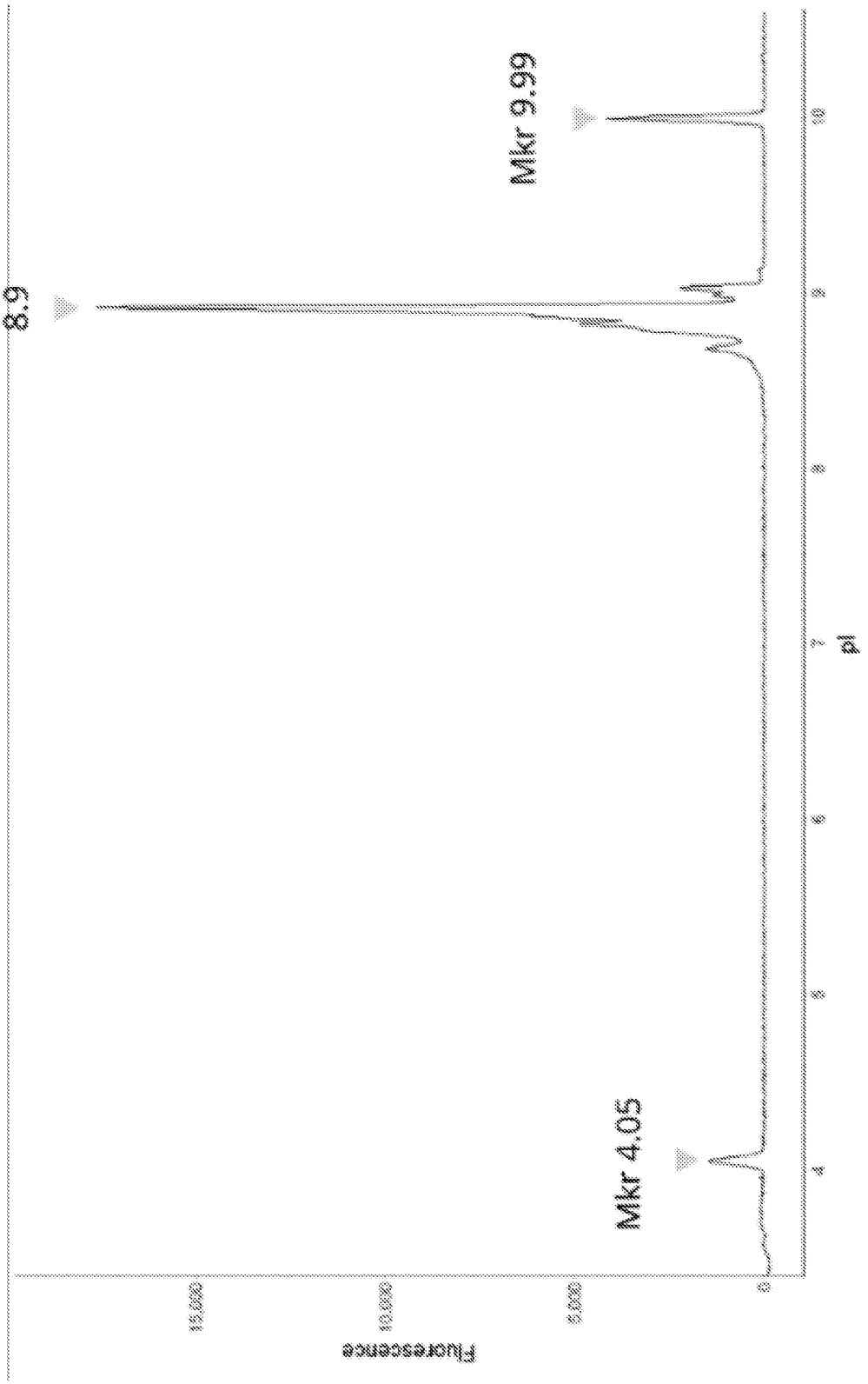

8.9
Mkr 9.99
Mkr 4.05
Fluorescence
15,000
10,000
5,000
0
4
5
6
7
8
9
10
pI

FIG. 223

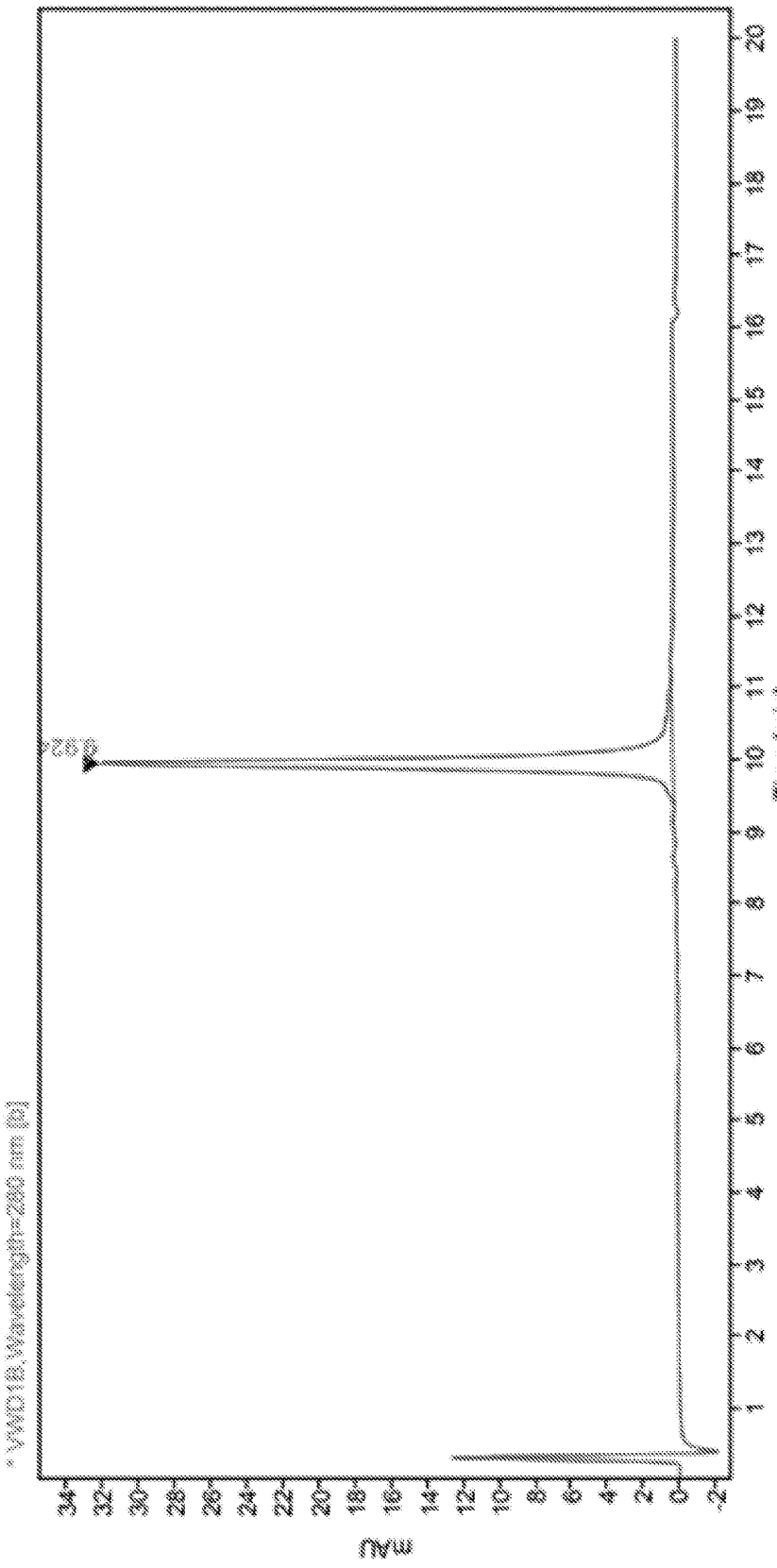


FIG. 224

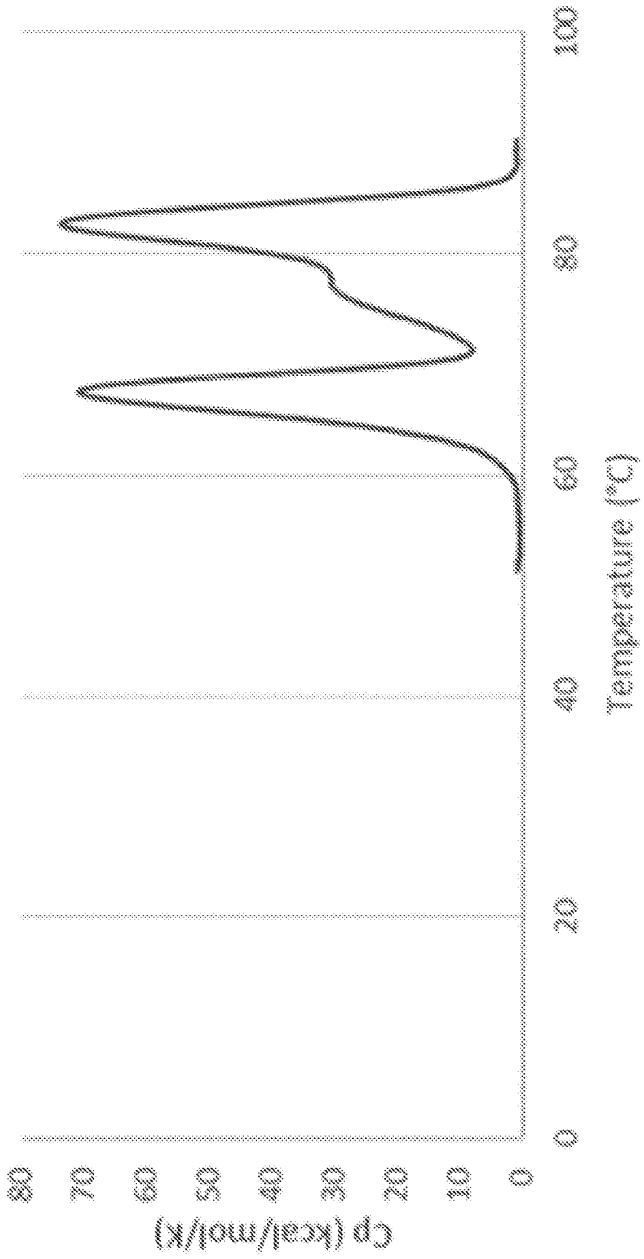

Cp (kcal/mol/K)
0
10
20
30
40
50
60
70
80
0
20
40
60
80
100
Temperature (°C)

FIG. 225A

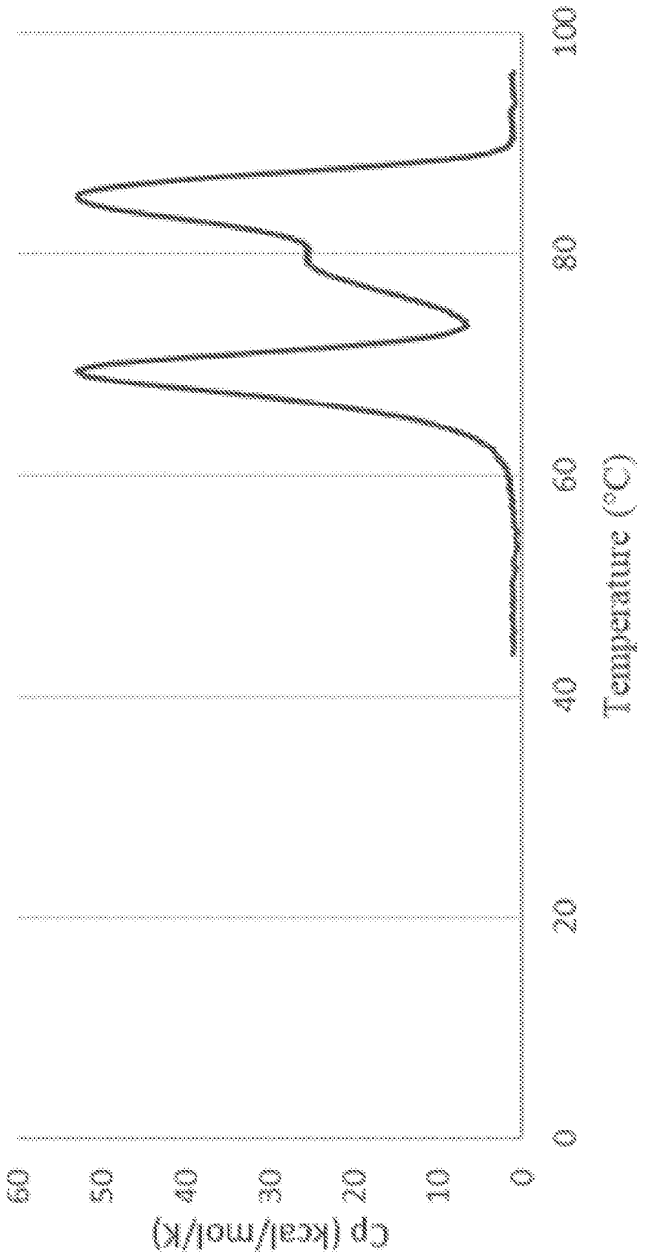


FIG. 225B

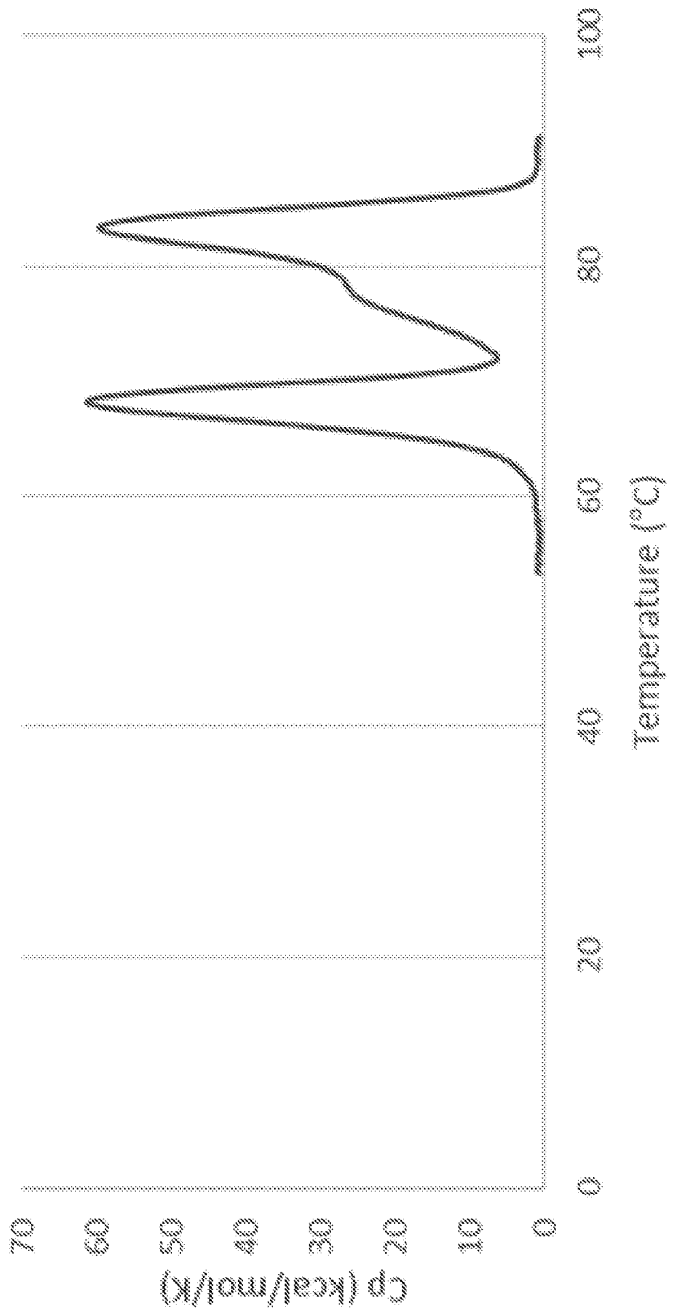
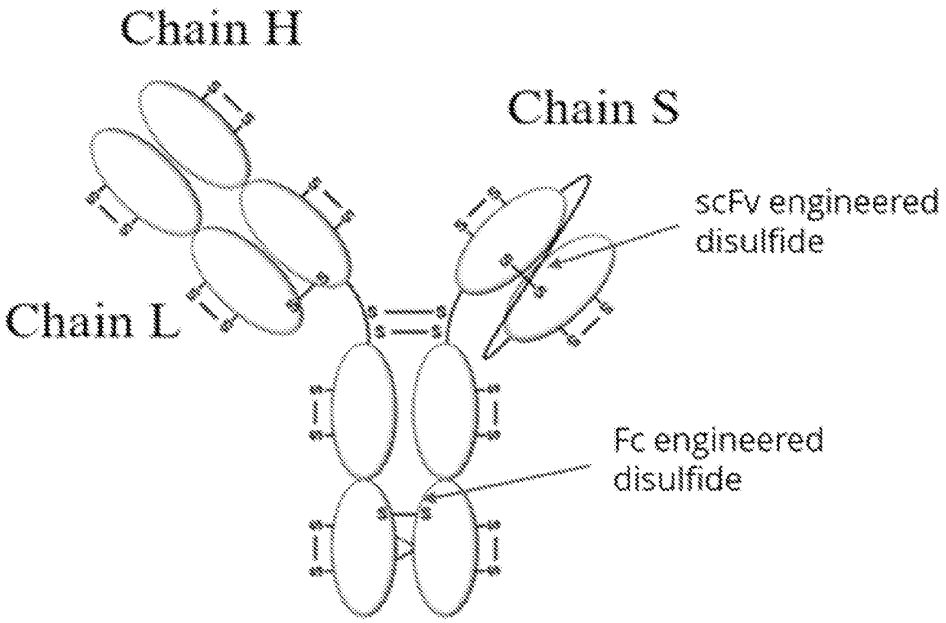


FIG. 225C

FIG. 226


Chain H
Chain S
scFv engineered disulfide
Chain L
Fc engineered disulfide

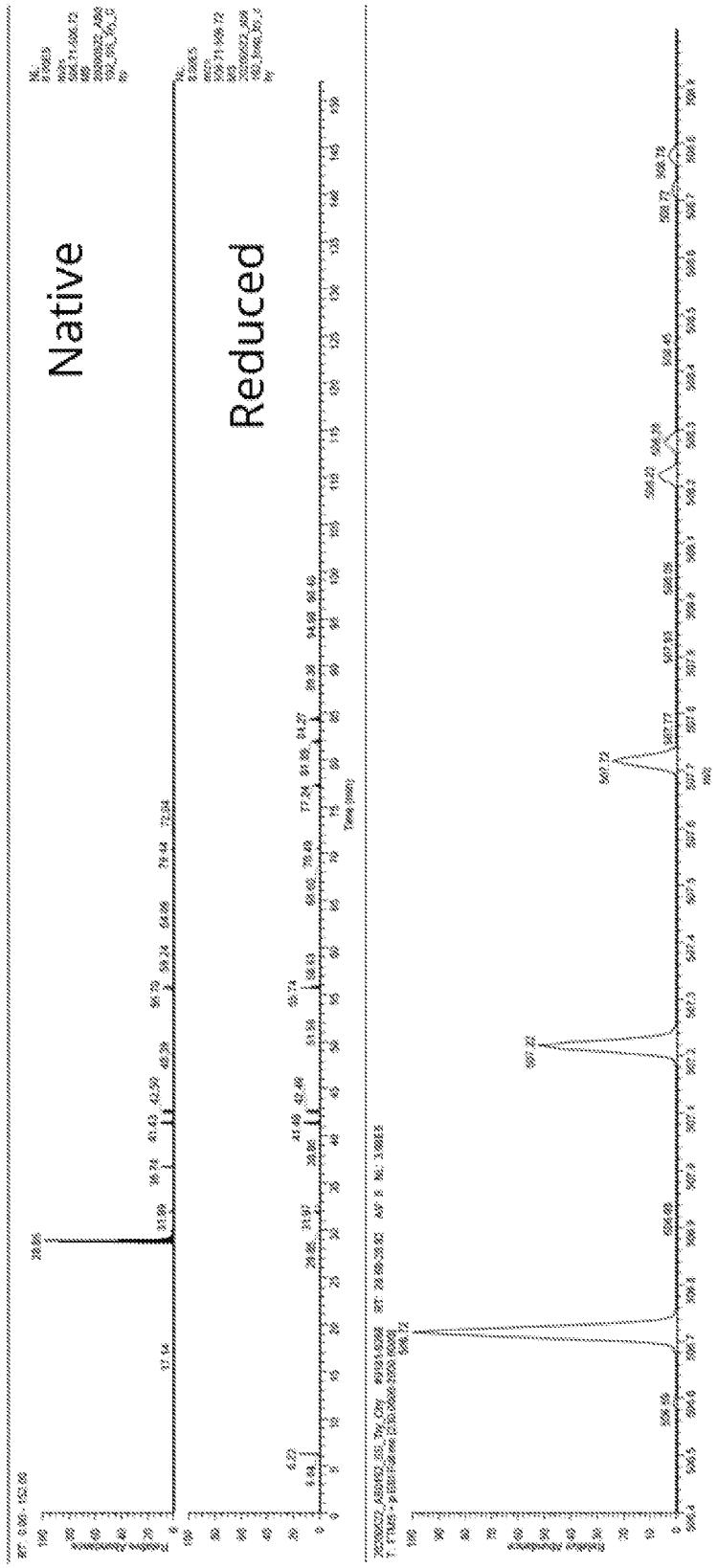


FIG. 227

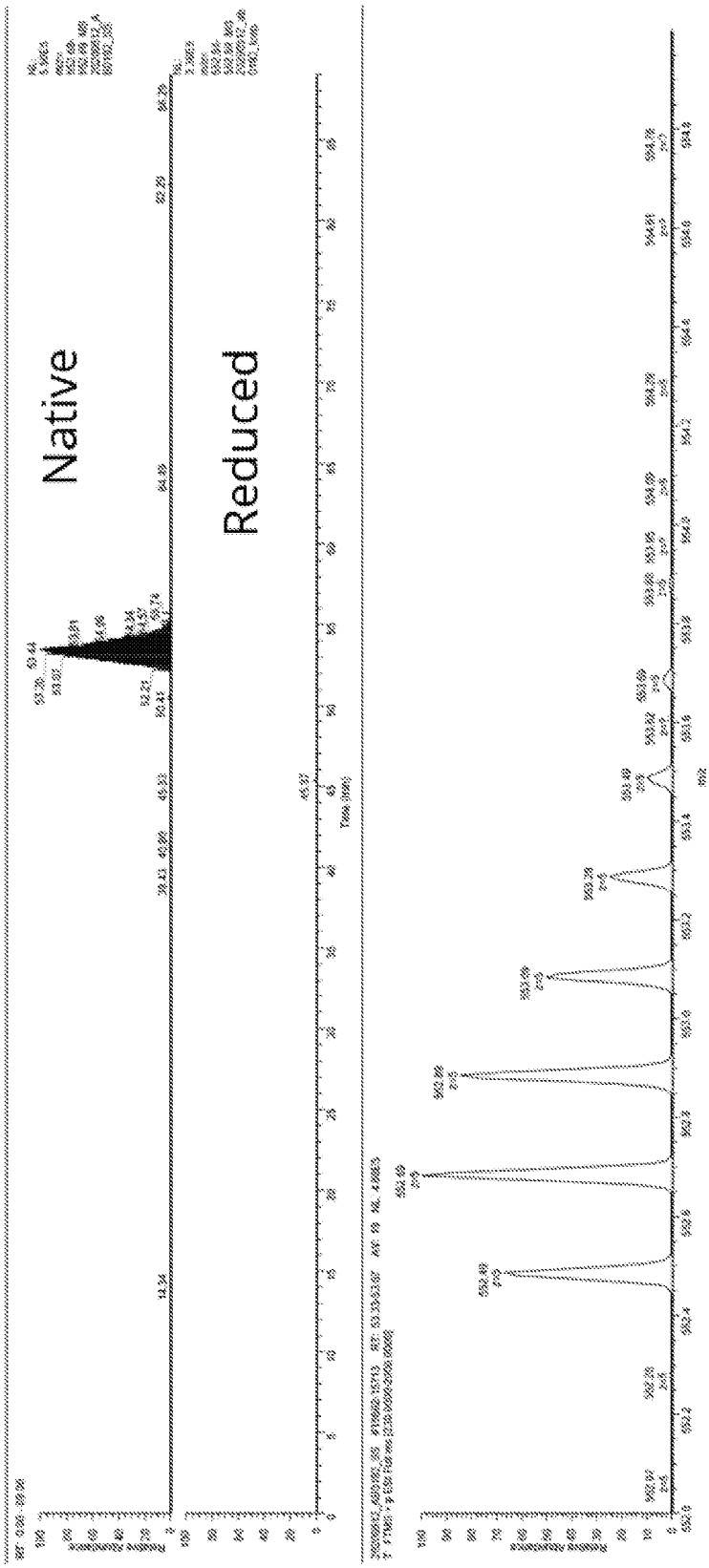


FIG. 228

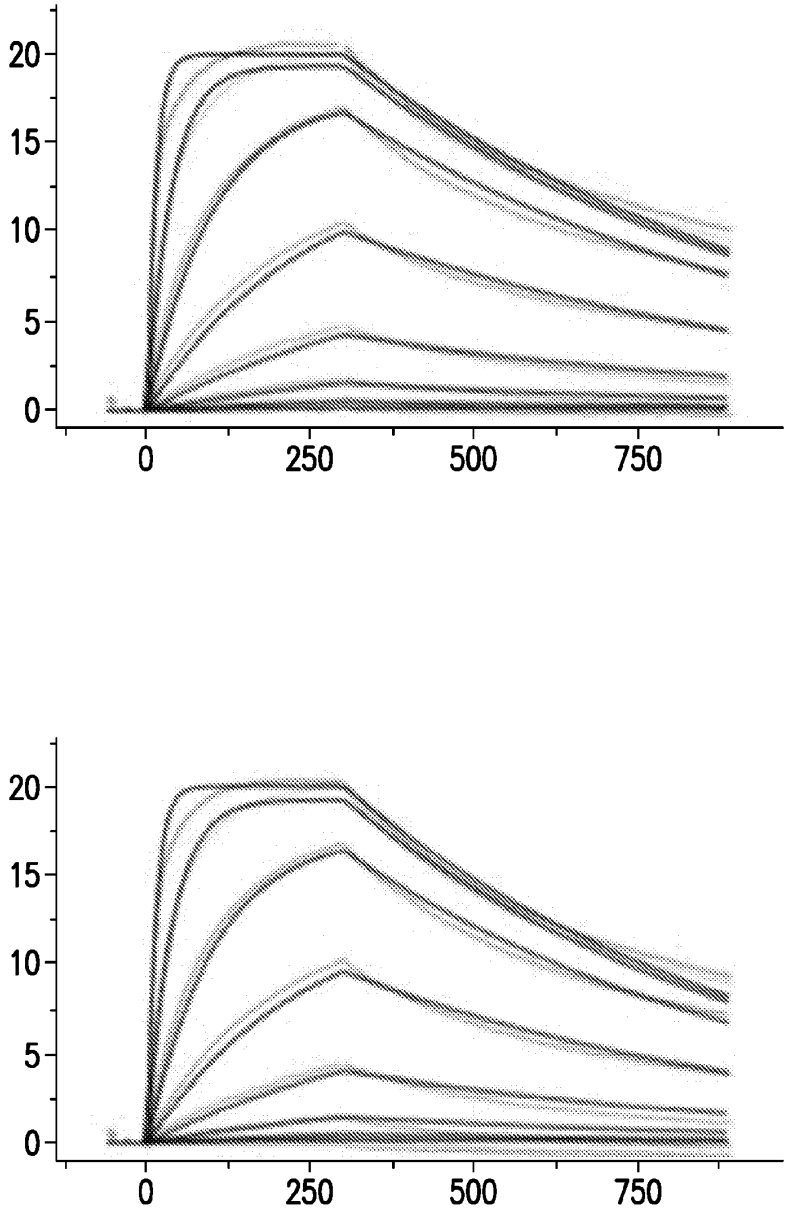


FIG. 229

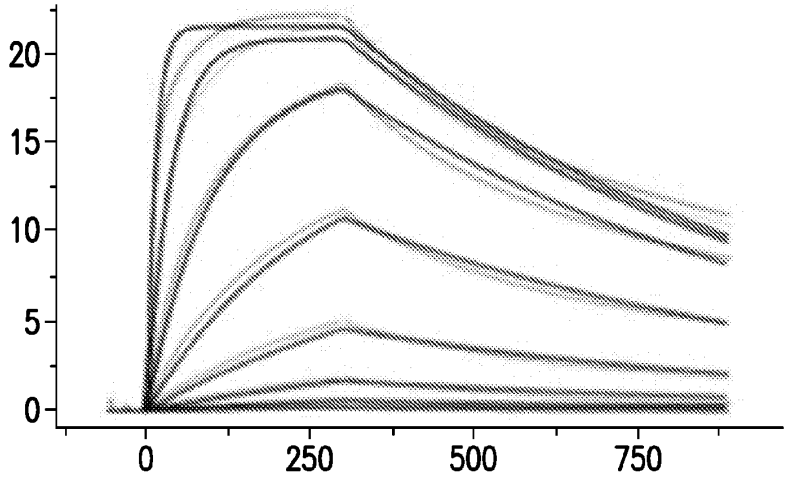
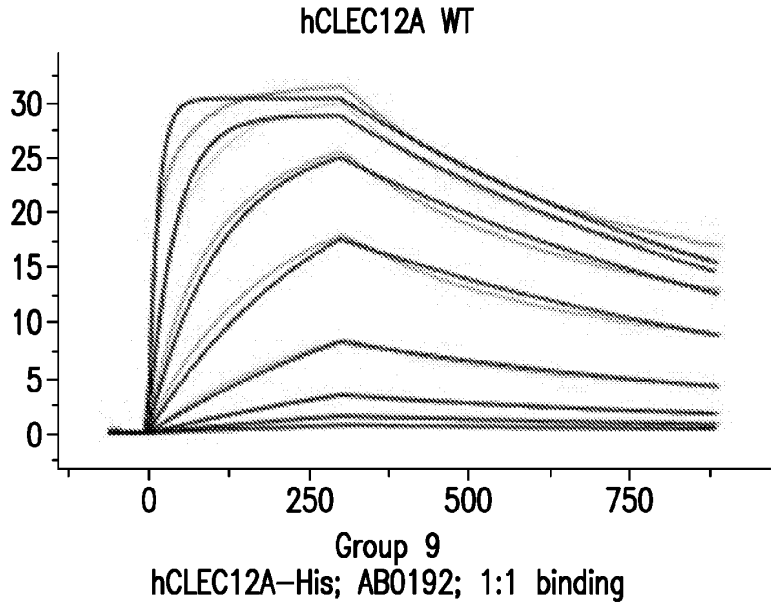


FIG. 229 Continued


hCLEC12A WT
30
25
20
15
10
5
0
0
250
500
750
Group 9
hCLEC12A–His; AB0192; 1:1 binding

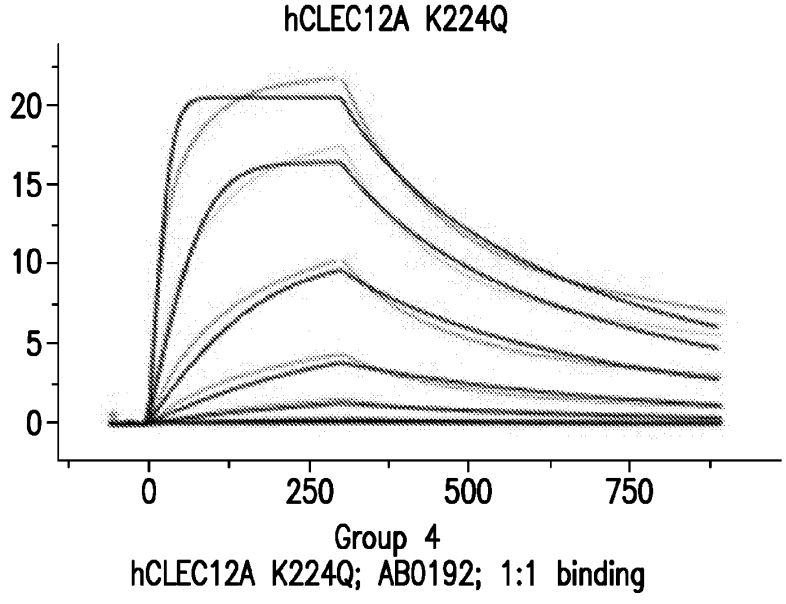

hCLEC12A K224Q
20
15
10
5
0
0
250
500
750
Group 4
hCLEC12A K224Q; AB0192; 1:1 binding

FIG. 230

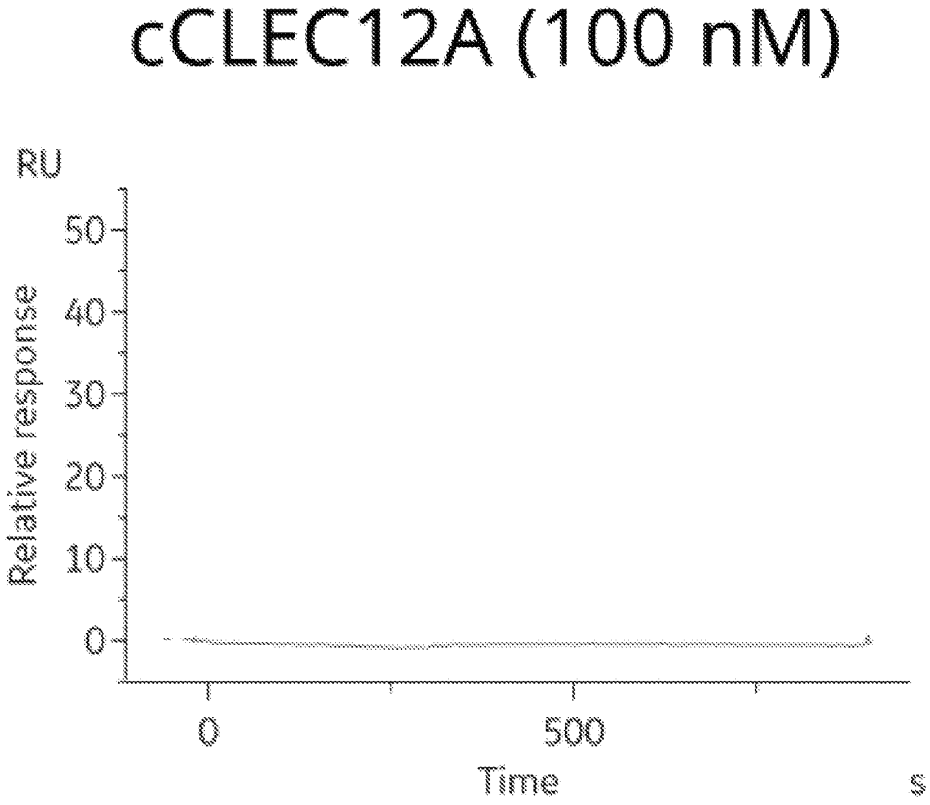
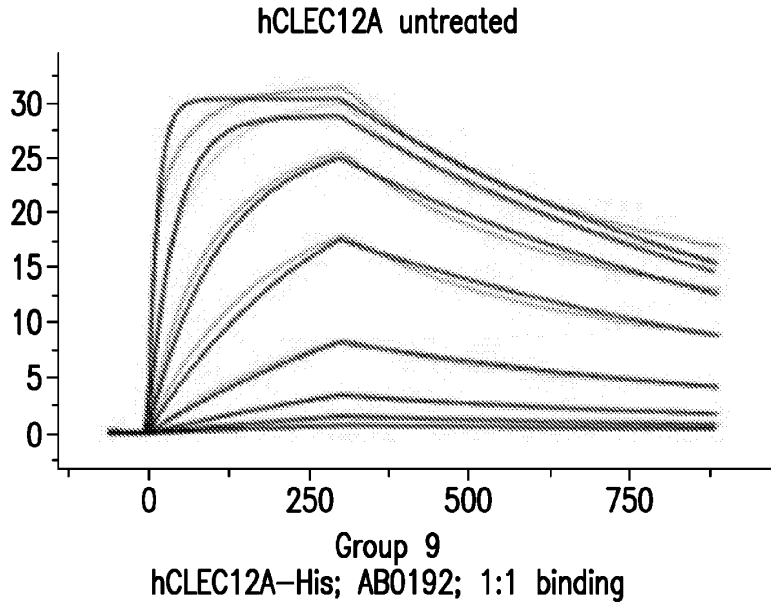


FIG. 230 Continued


hCLEC12A untreated
30
25
20
15
10
5
0
0
250
500
750
Group 9
hCLEC12A–His; AB0192; 1:1 binding

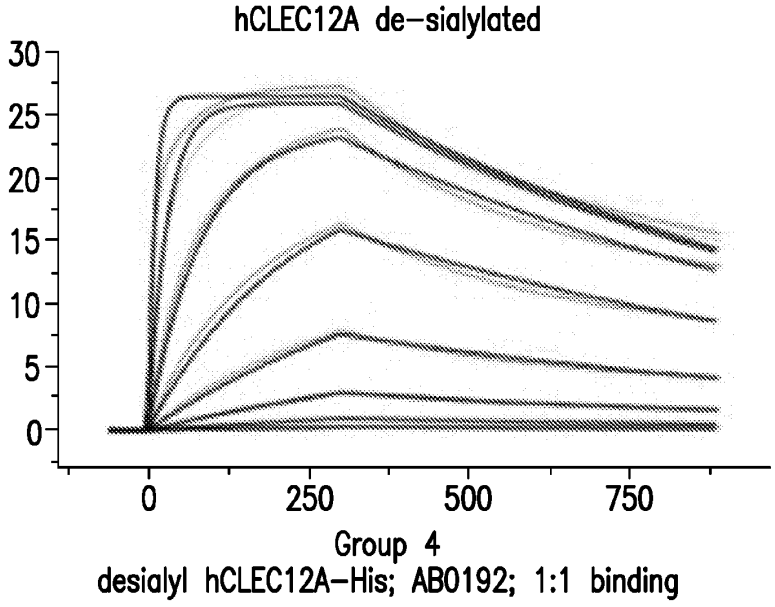

hCLEC12A de–sialylated
30
25
20
15
10
5
0
0
250
500
750
Group 4
desialyl hCLEC12A–His; AB0192; 1:1 binding

FIG. 231

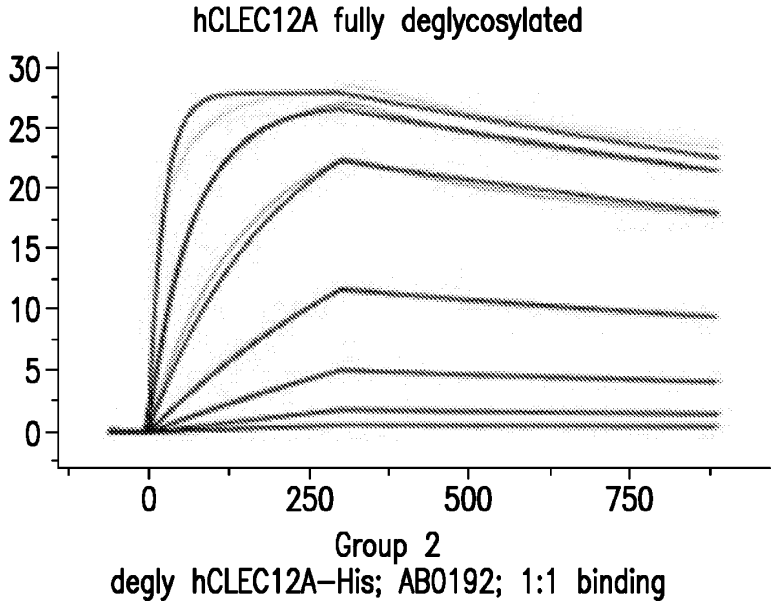
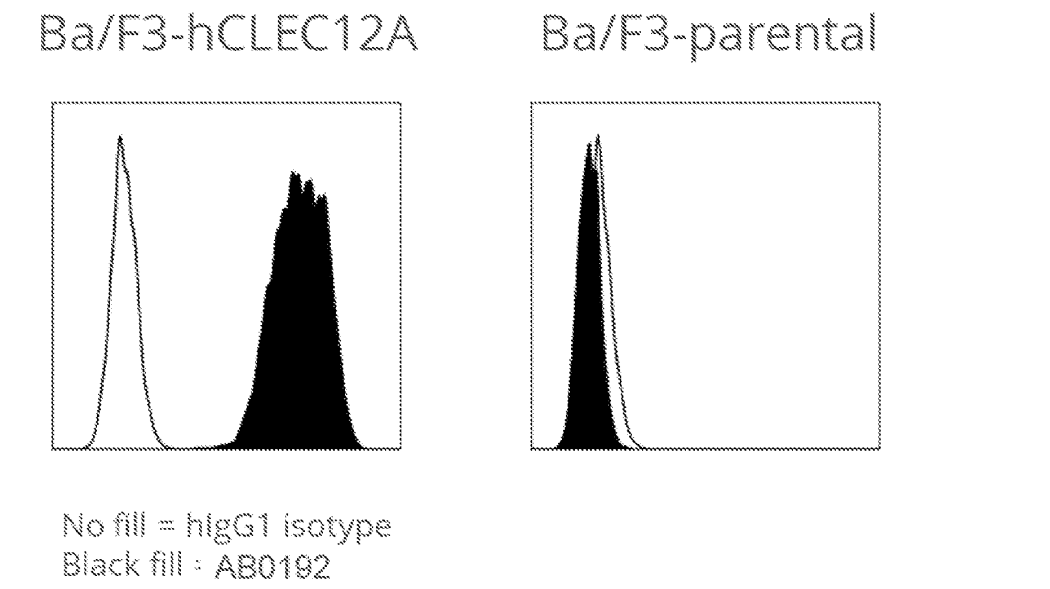


FIG. 231 Continued

FIG. 232


Ba/F3-hCLEC12A
Ba/F3-parental
No fill = hIgG1 isotype
Black fill = AB0192
PL-21
HL60
FOB
Concentration nM
0.01
0.1
1
10
100
0
5
10
15
2
4
6
8

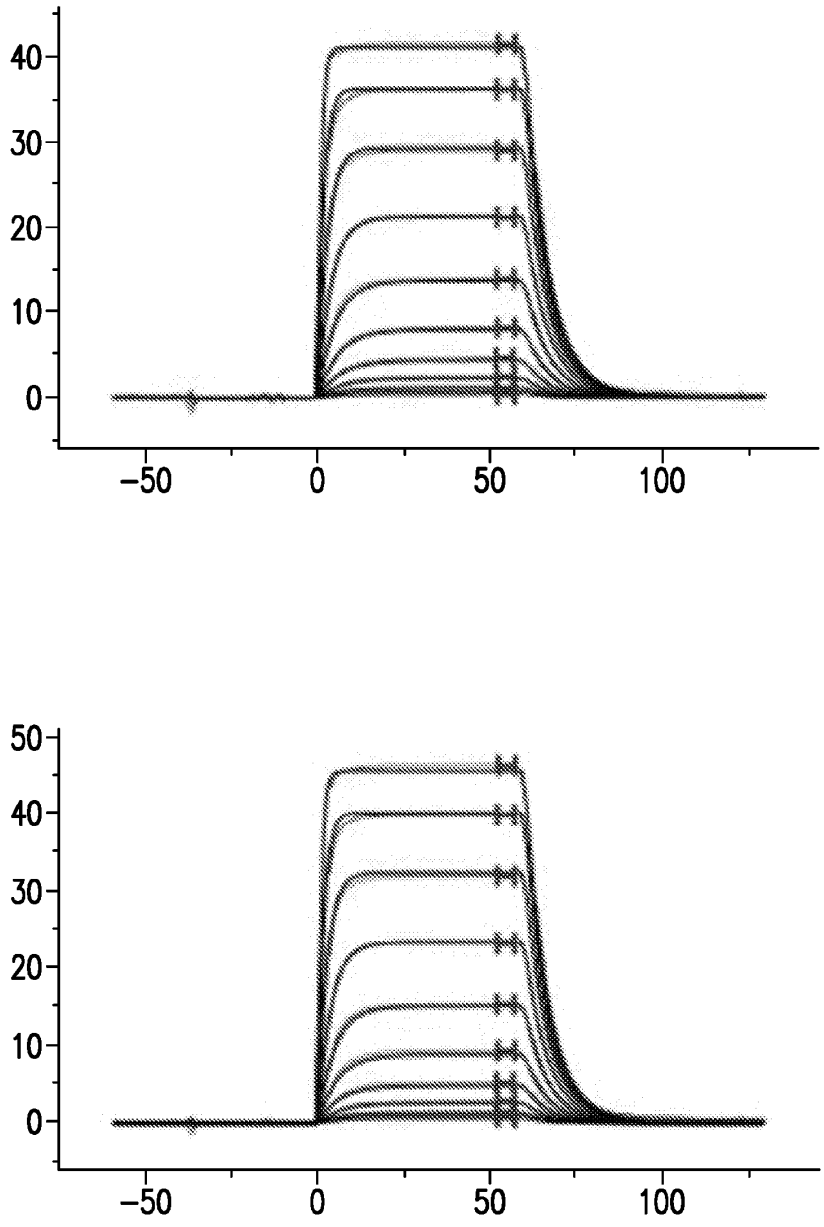
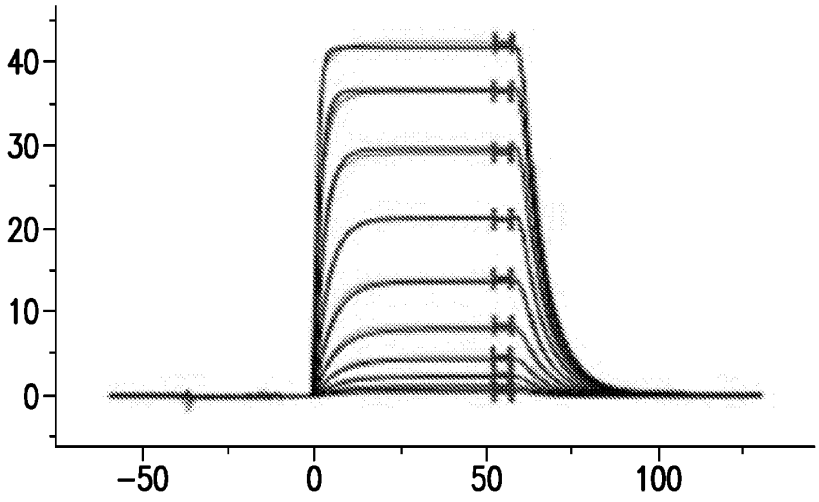
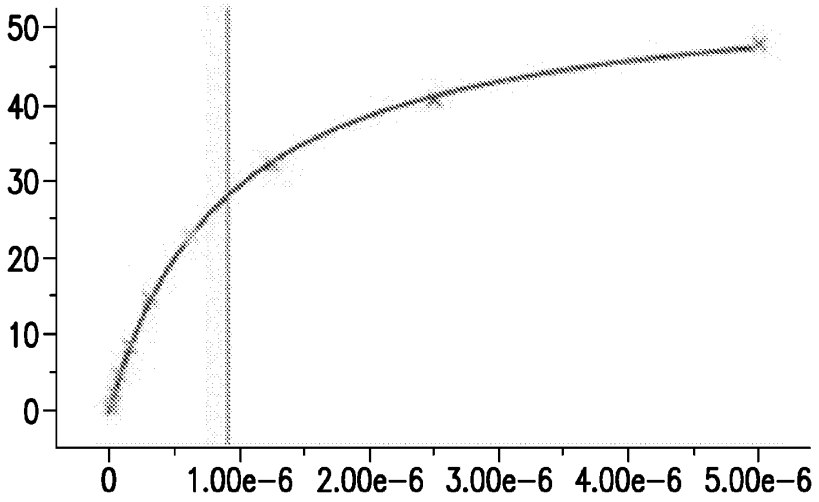


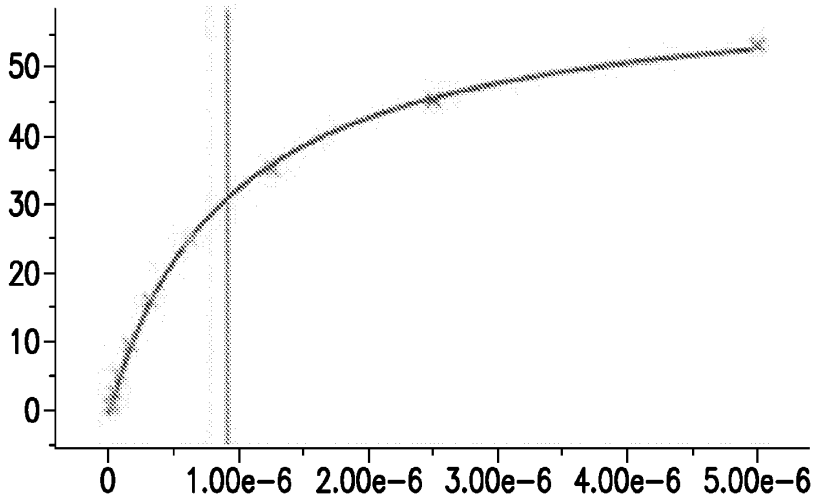
FIG. 233



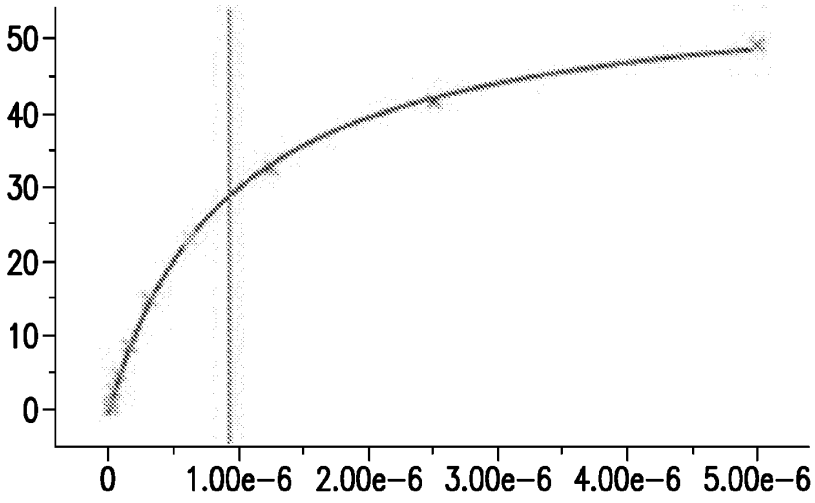


Group 7
AB0192−005; mFc−cNKG2D; Steady state affinity

FIG. 233 Continued

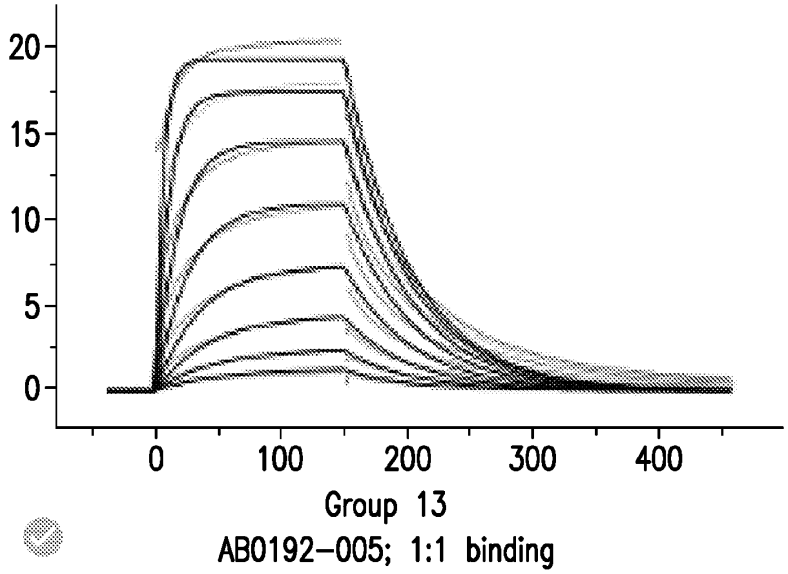


Group 8
AB0192-005; mFc-cNKG2D; Steady state affinity



Group 9
AB0192-005; mFc-cNKG2D; Steady state affinity

FIG. 233 Continued



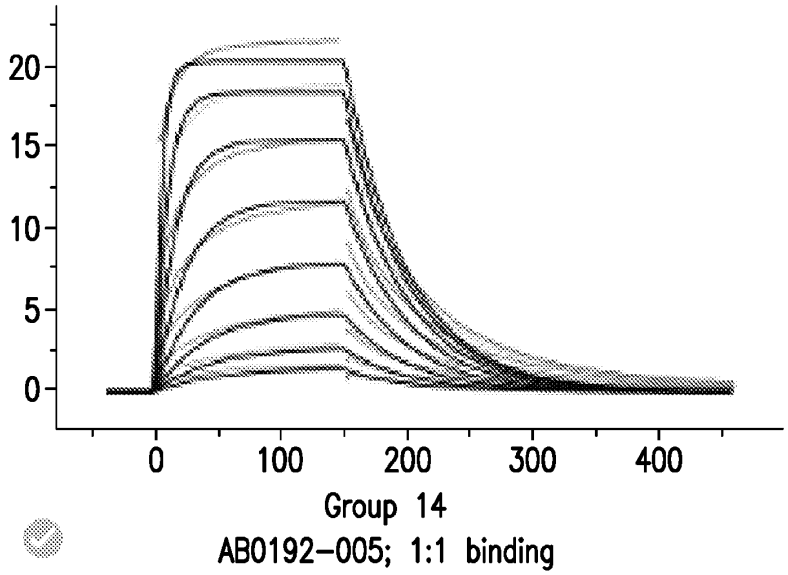
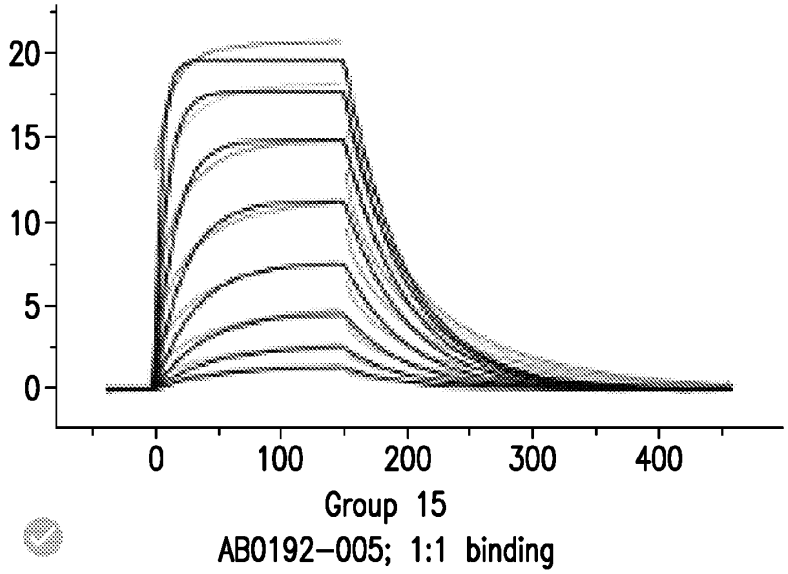


FIG. 234 Continued



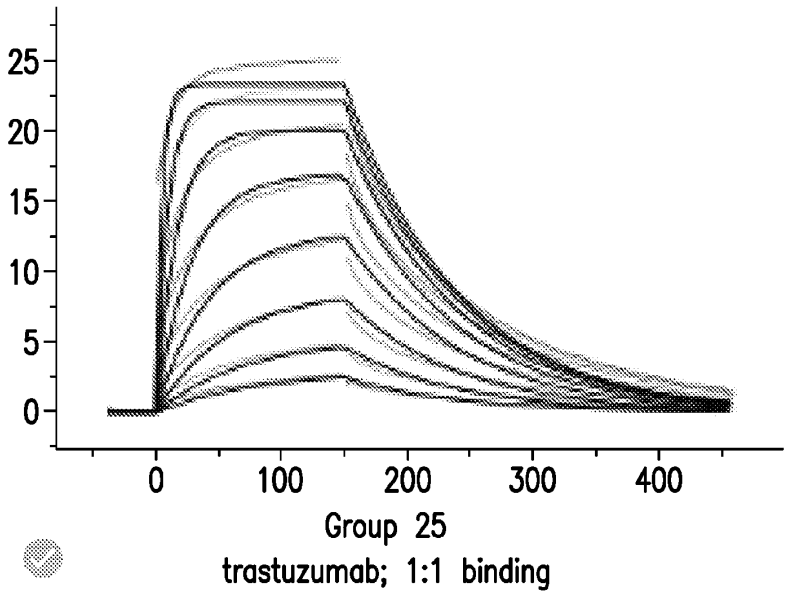
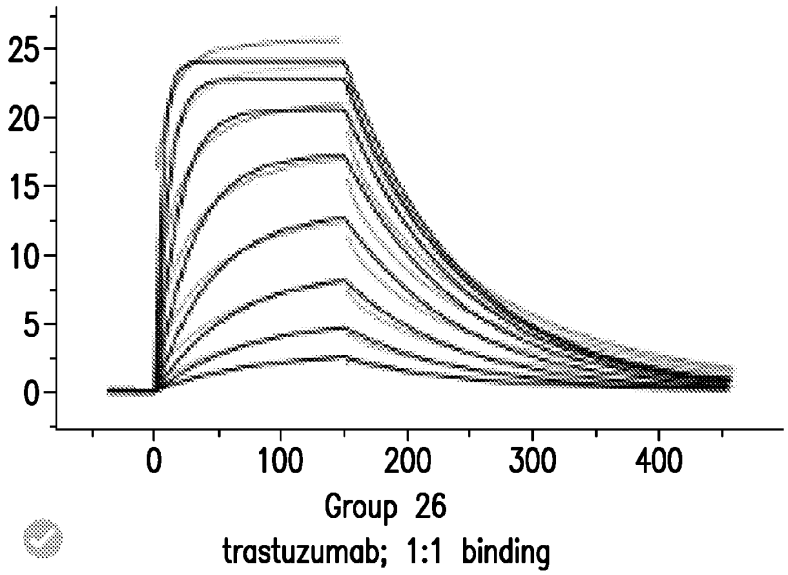


FIG. 234 Continued



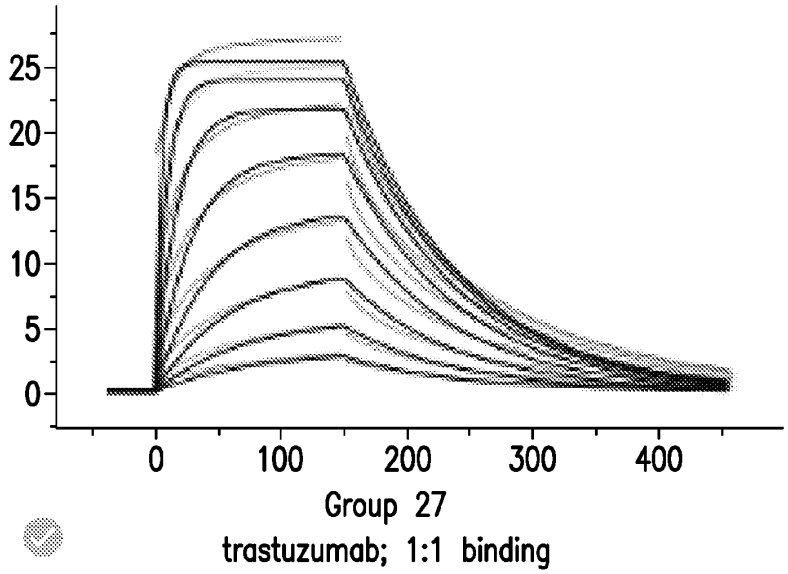


FIG. 234 Continued

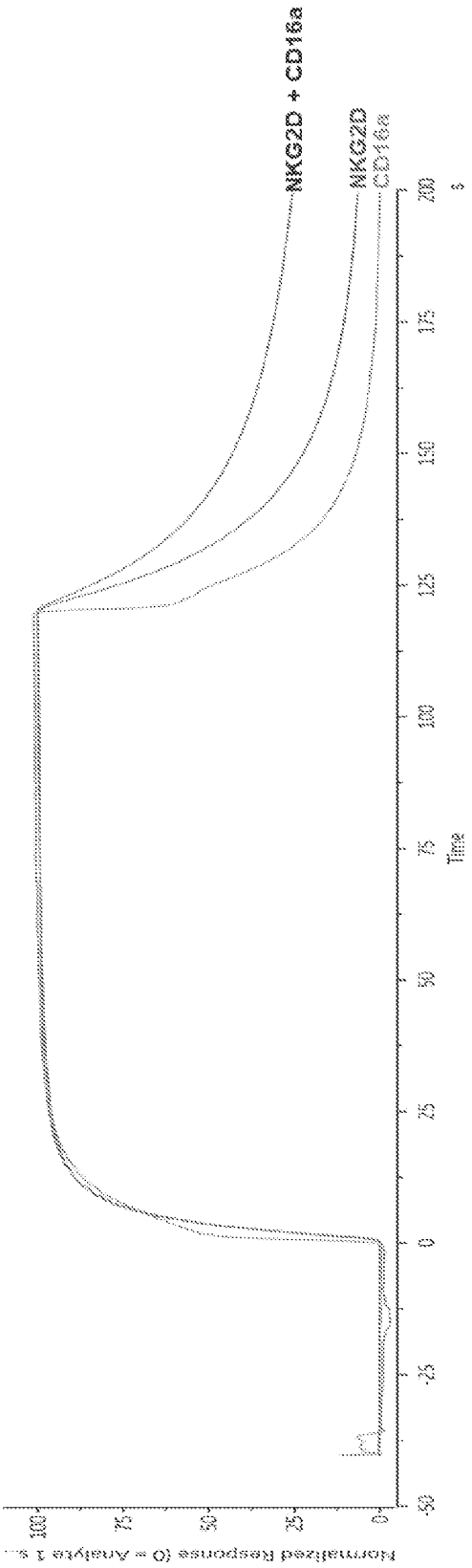

NKG2D + CD16a
NKG2D
CD16a
Normalized Response (0 = Analyte 1 s...
100
75
50
25
0
-50
-25
0
25
50
75
100
125
150
175
200
Time
s

FIG. 235

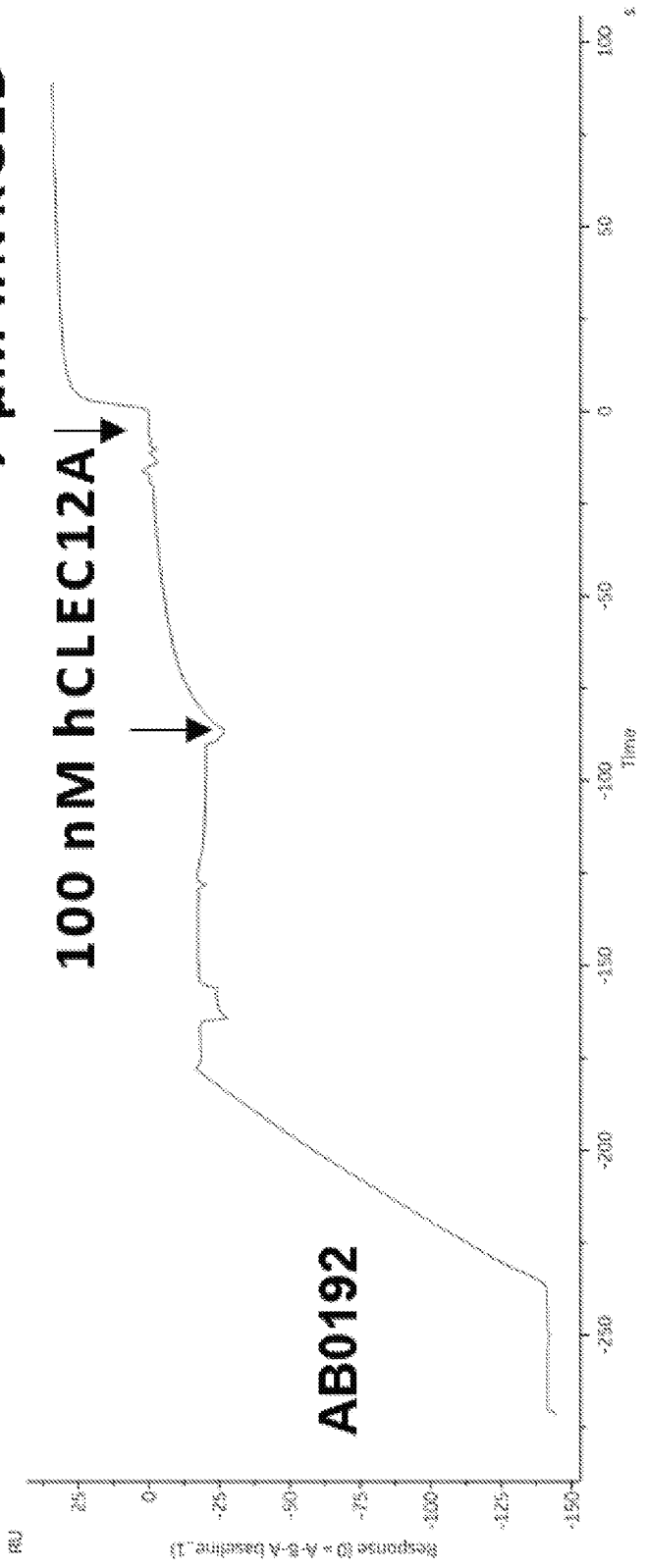

7 µM hNKG2D
100 nM hCLEC12A
AB0192
Response (D = A-B-A baseline_1)
nm
Time
s
25
0
-25
-50
-75
-100
-125
-150
-250
-200
-150
-100
-50
0
50
100

FIG. 236

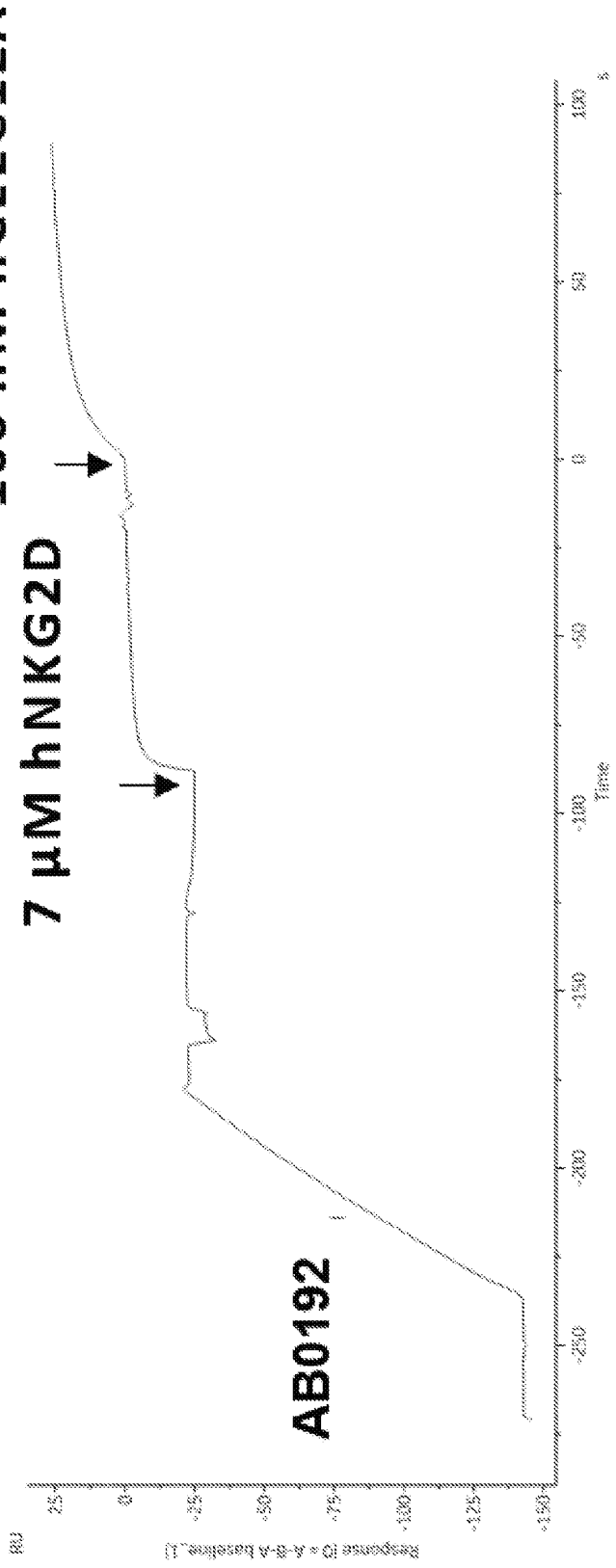
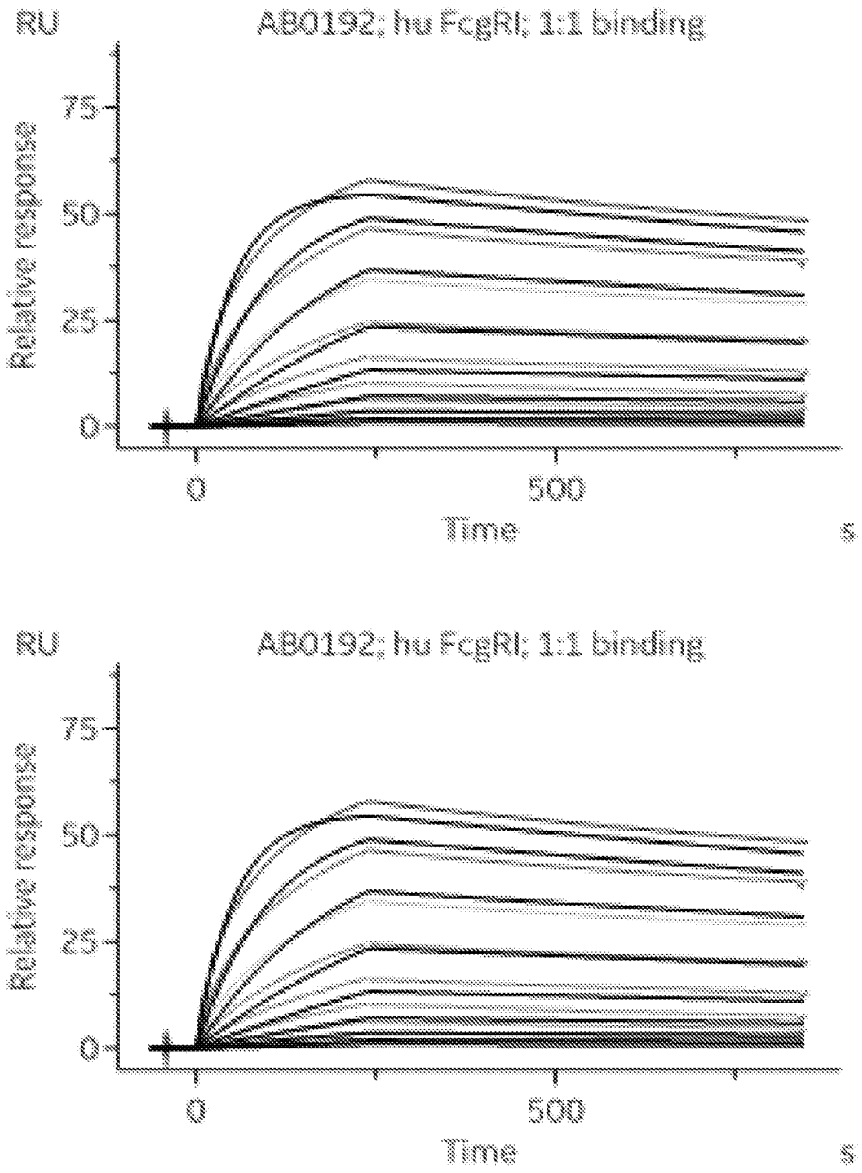


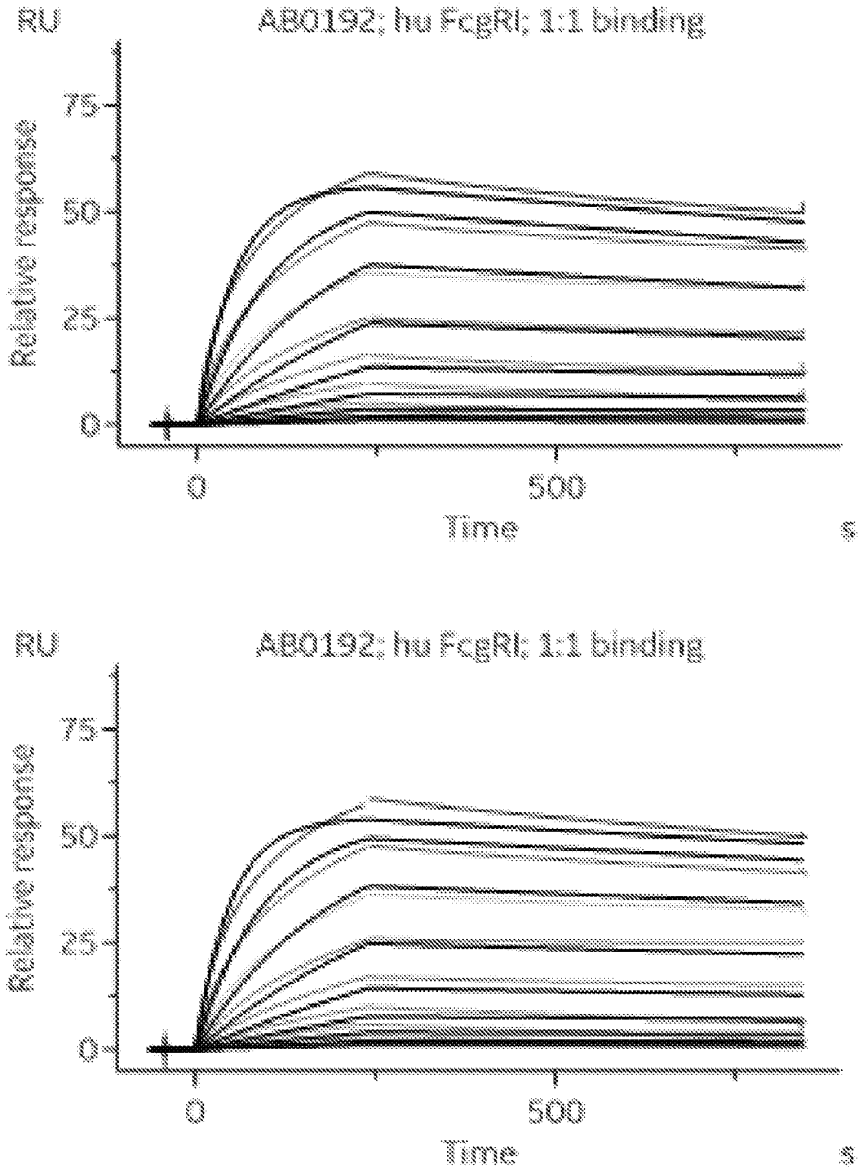
FIG. 236 Continued



AB0192

$K_D = 5.2 \pm 1.2$ nM

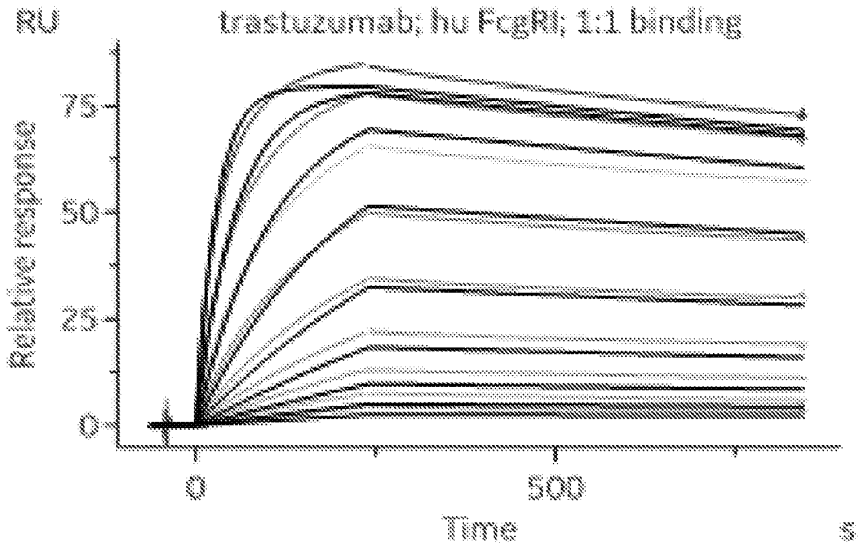
FIG. 237

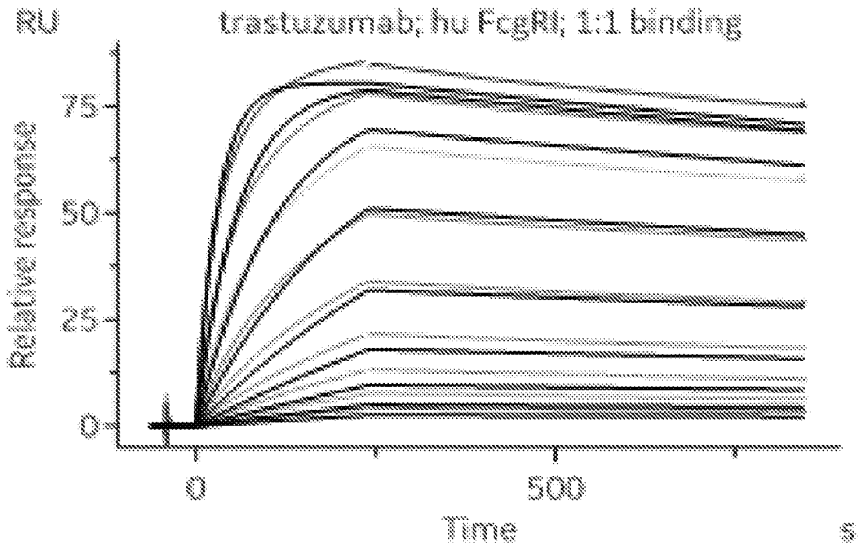


AB0192

$K_D = 5.2 \pm 1.2$ nM

FIG. 237 Continued

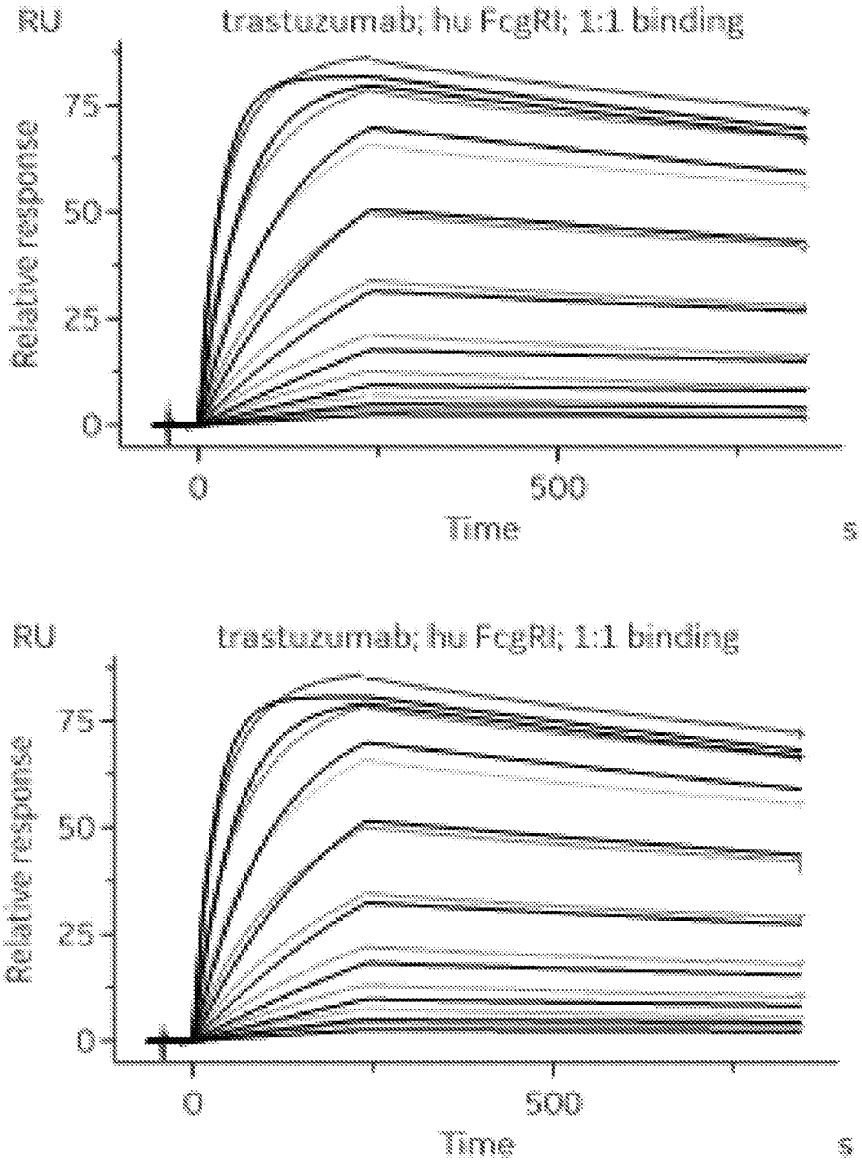




trastuzumab

$K_D = 2.9 \pm 0.4$ nM

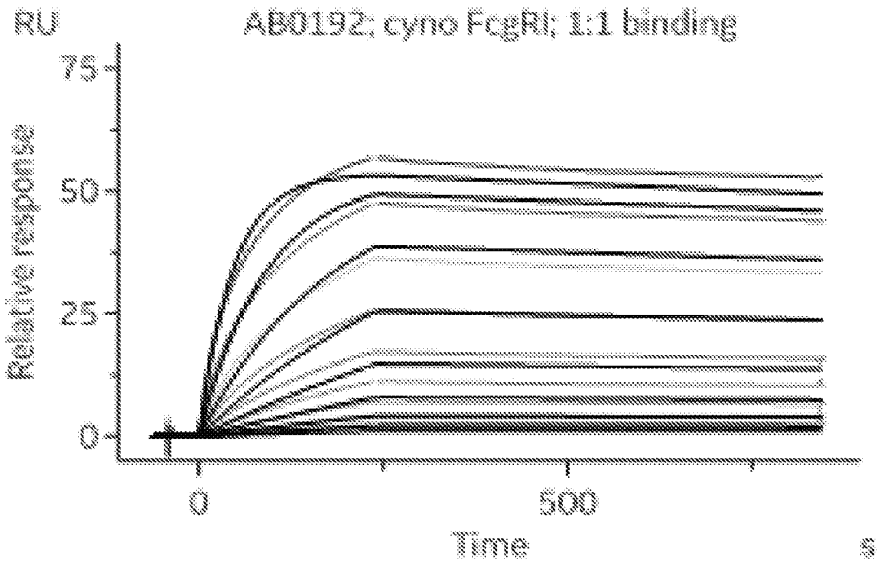
FIG. 237 Continued

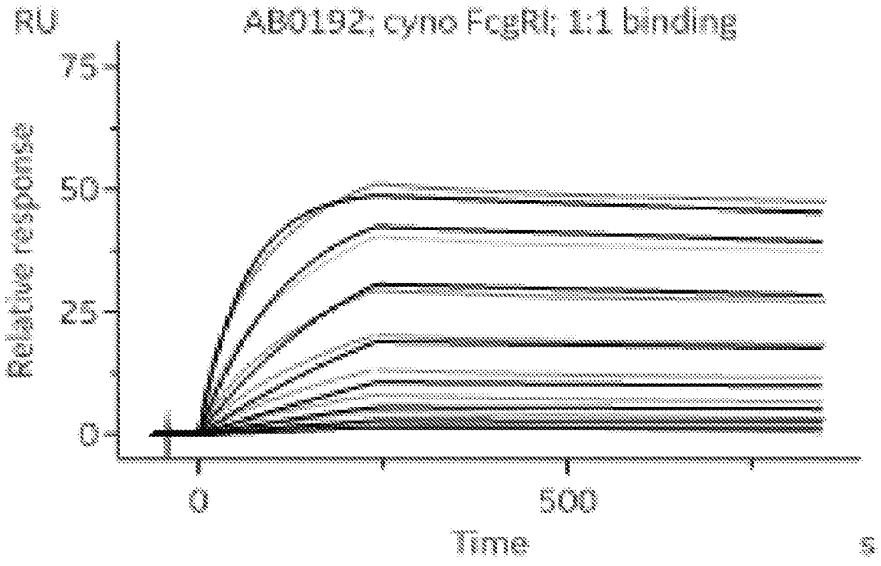


trastuzumab

$K_D = 2.9 \pm 0.4$ nM

FIG. 237 Continued

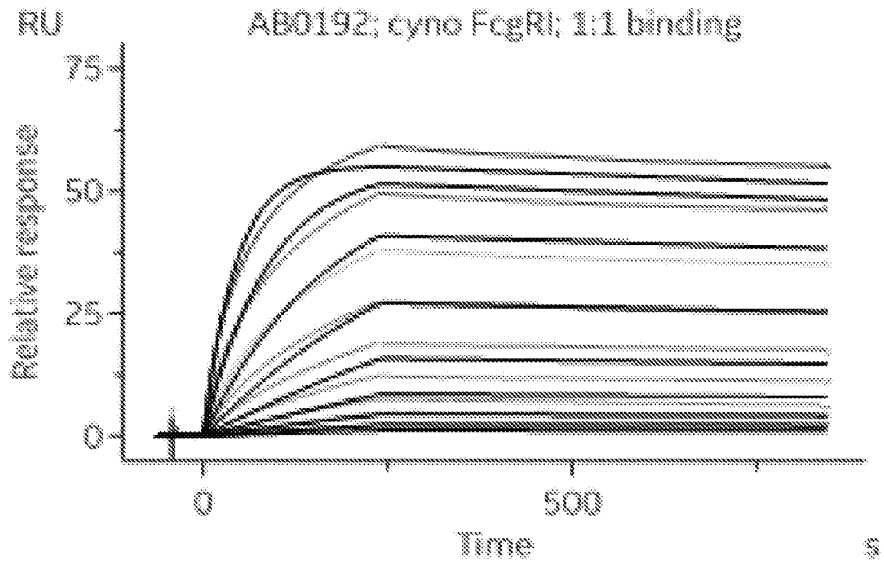


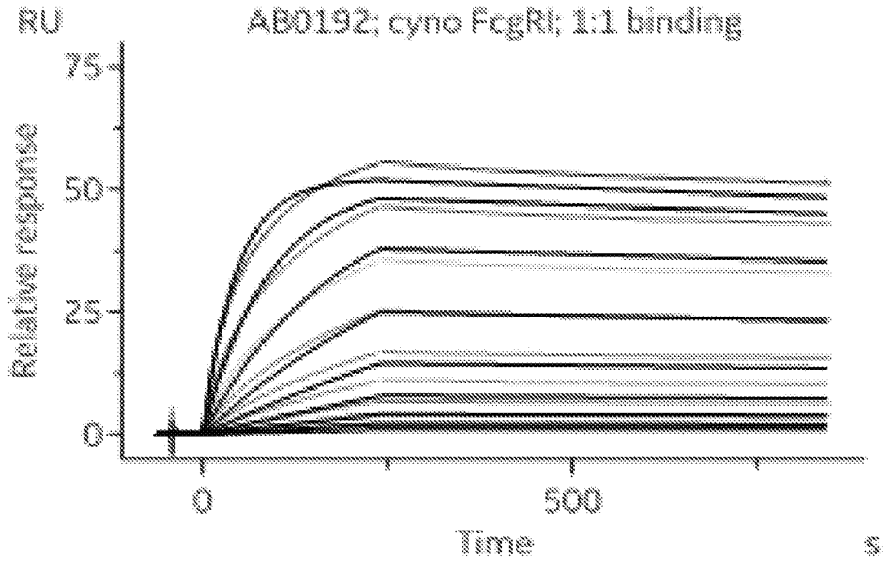


AB0192

$K_D = 1.9 \pm 0.3$ nM

FIG. 238

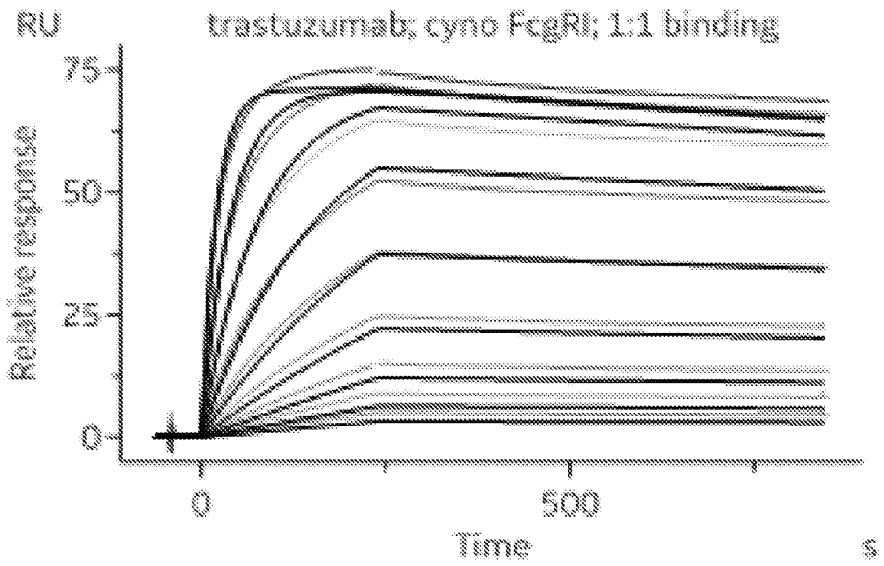


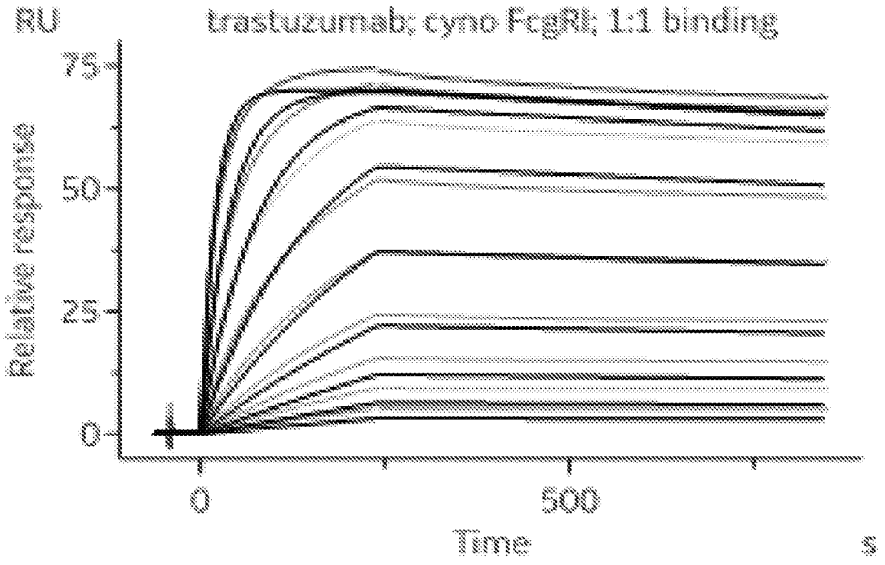


AB0192

$K_D = 1.9 \pm 0.3$ nM

FIG. 238 Continued

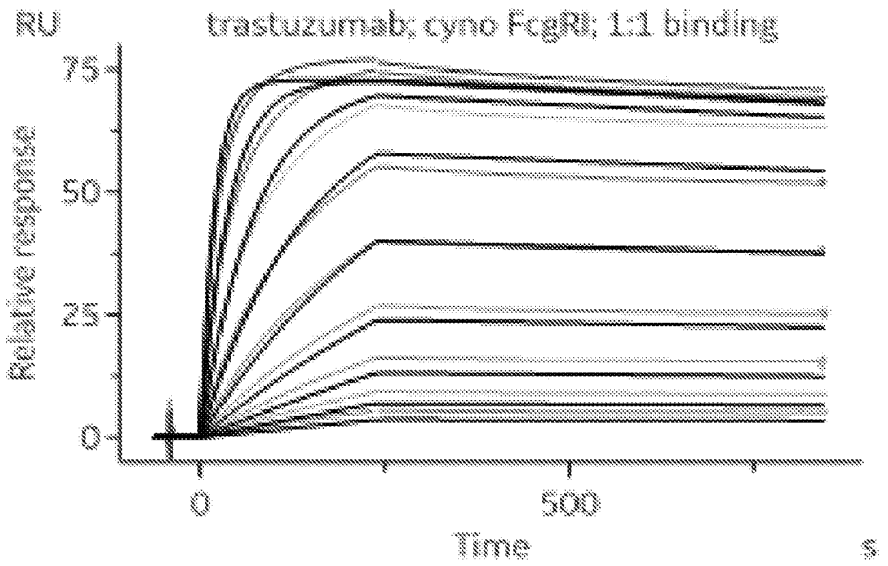


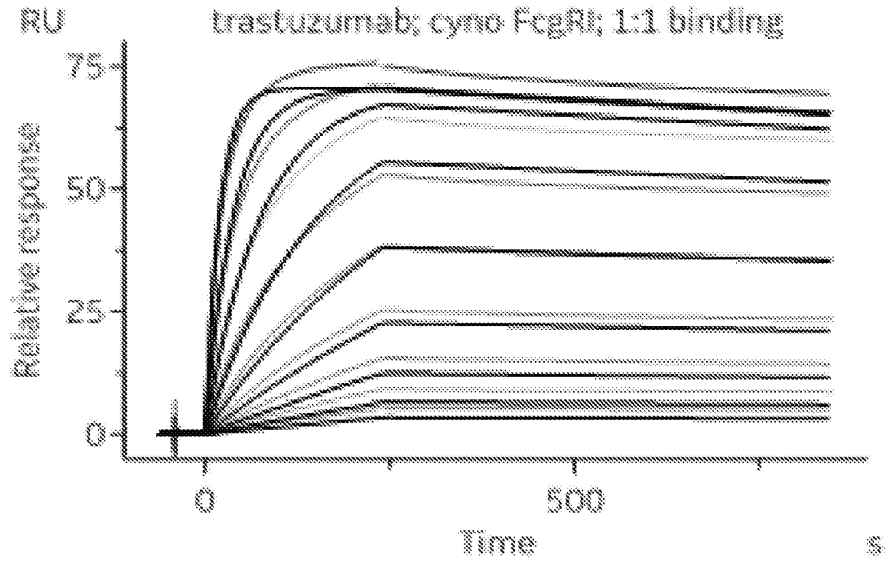


trastuzumab

K_D = 1.0 ± 0.1 nM

FIG. 238 Continued

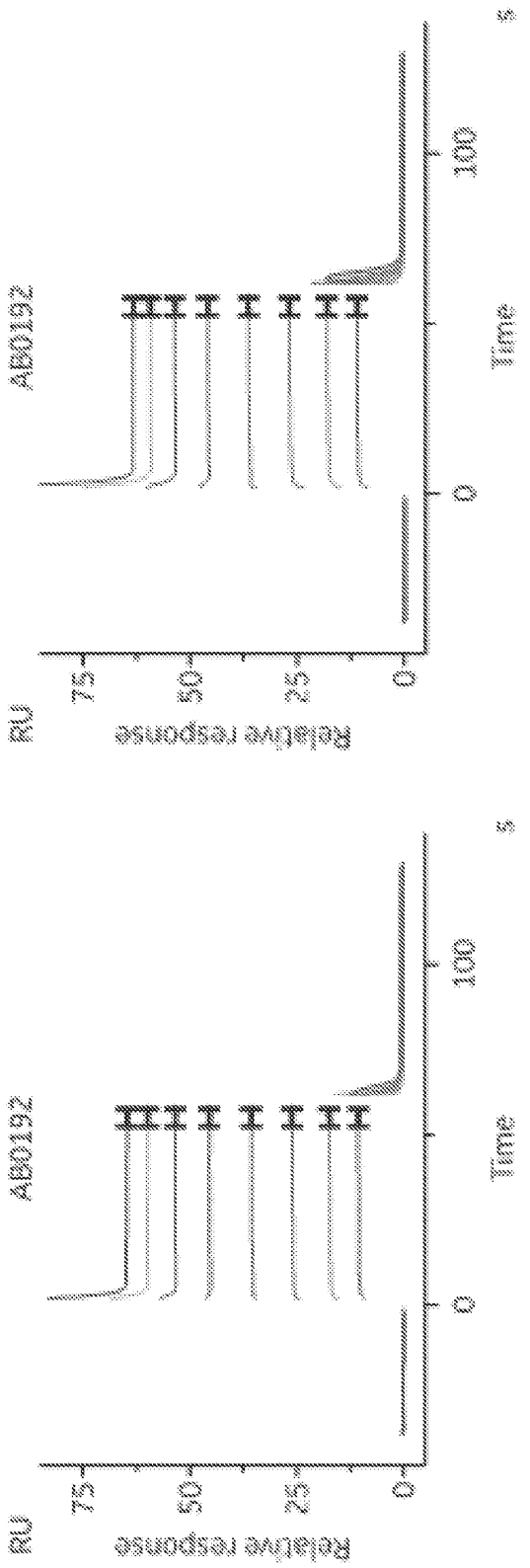




trastuzumab

K_D = 1.0 ± 0.1 nM

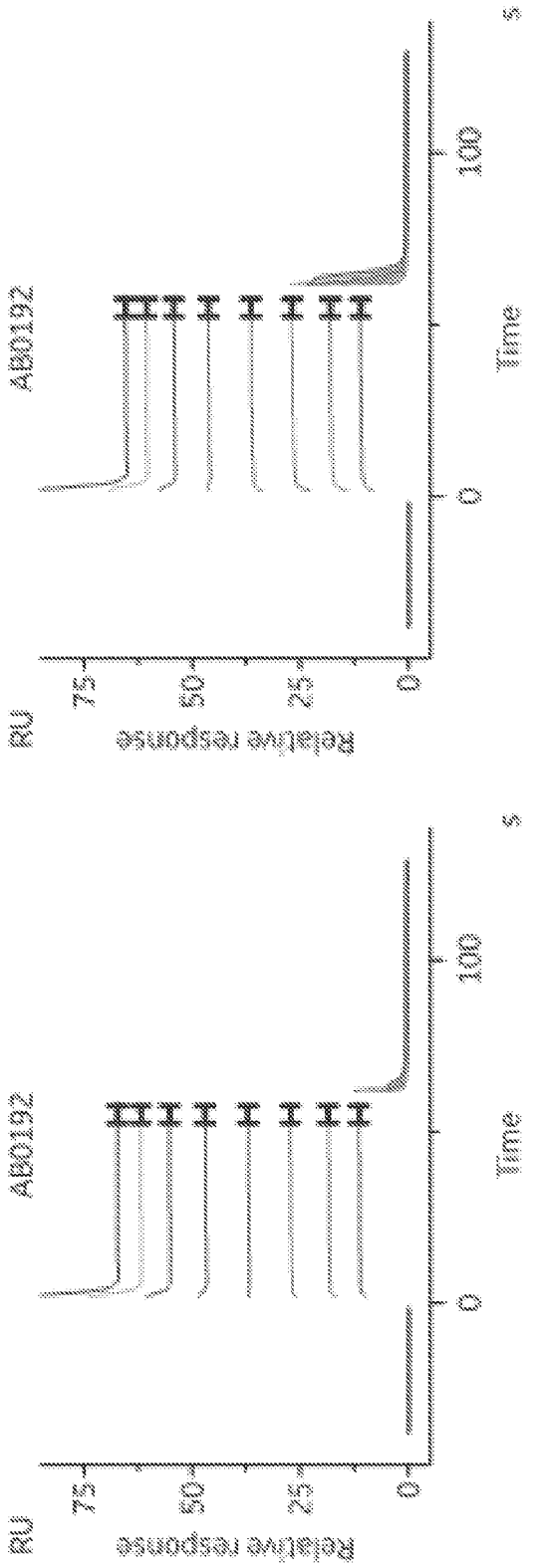
FIG. 238 Continued



AB0192

K_D = 1011.3 ± 63.3 nM

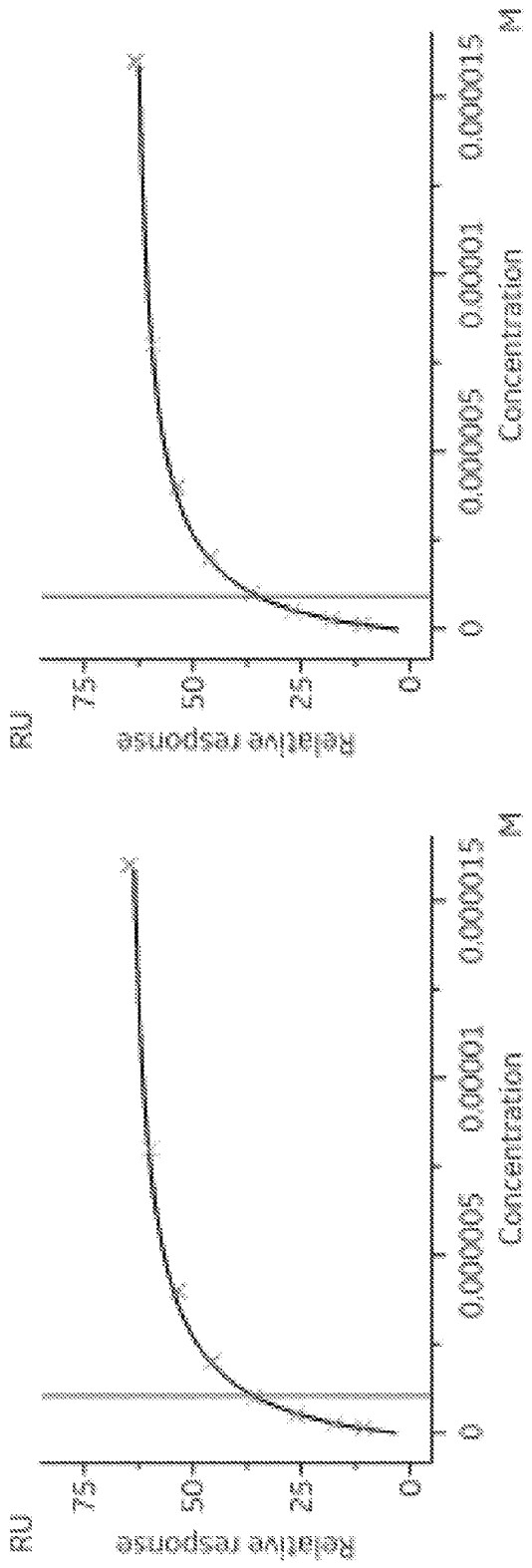
FIG. 239



AB0192

K_D = 1011.3 ± 63.3 nM

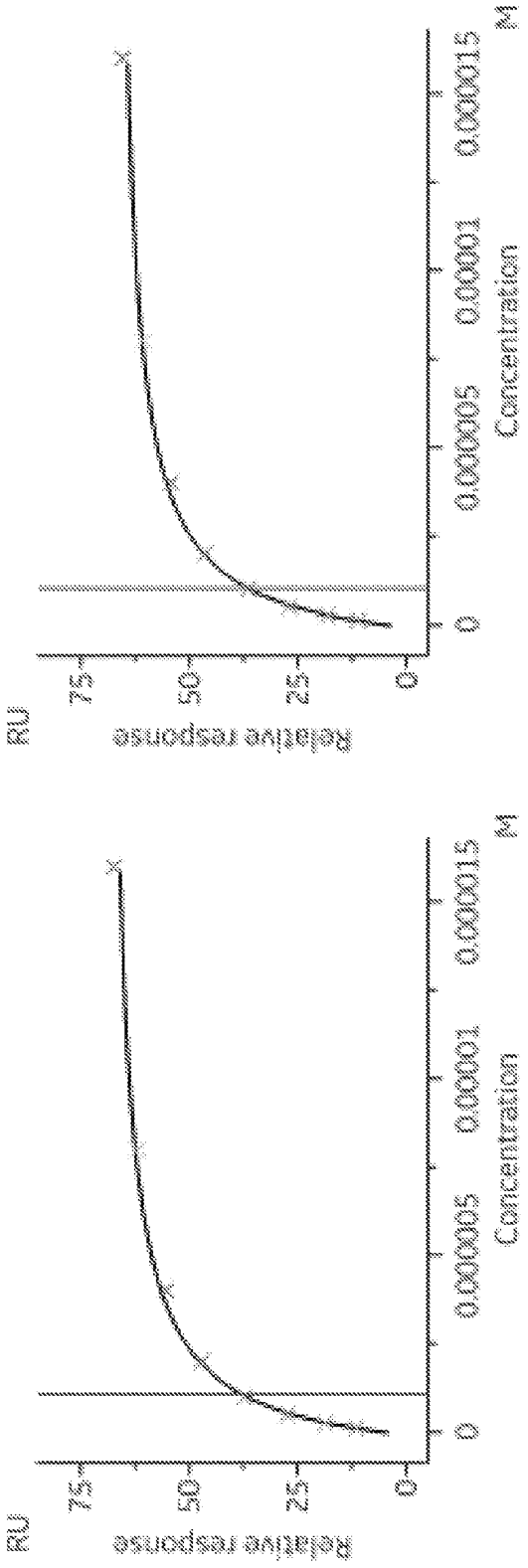
FIG. 239 Continued



AB0192

K_D = 1011.3 ± 63.3 nM

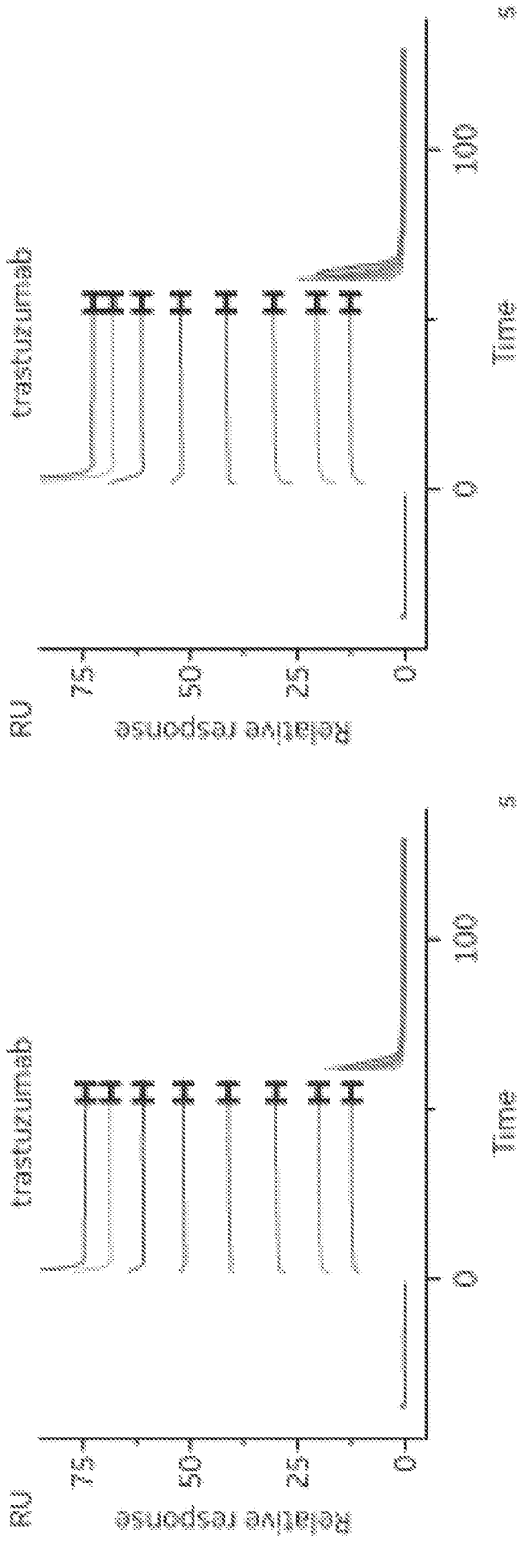
FIG. 239 Continued



AB0192

K_D = 1011.3 ± 63.3 nM

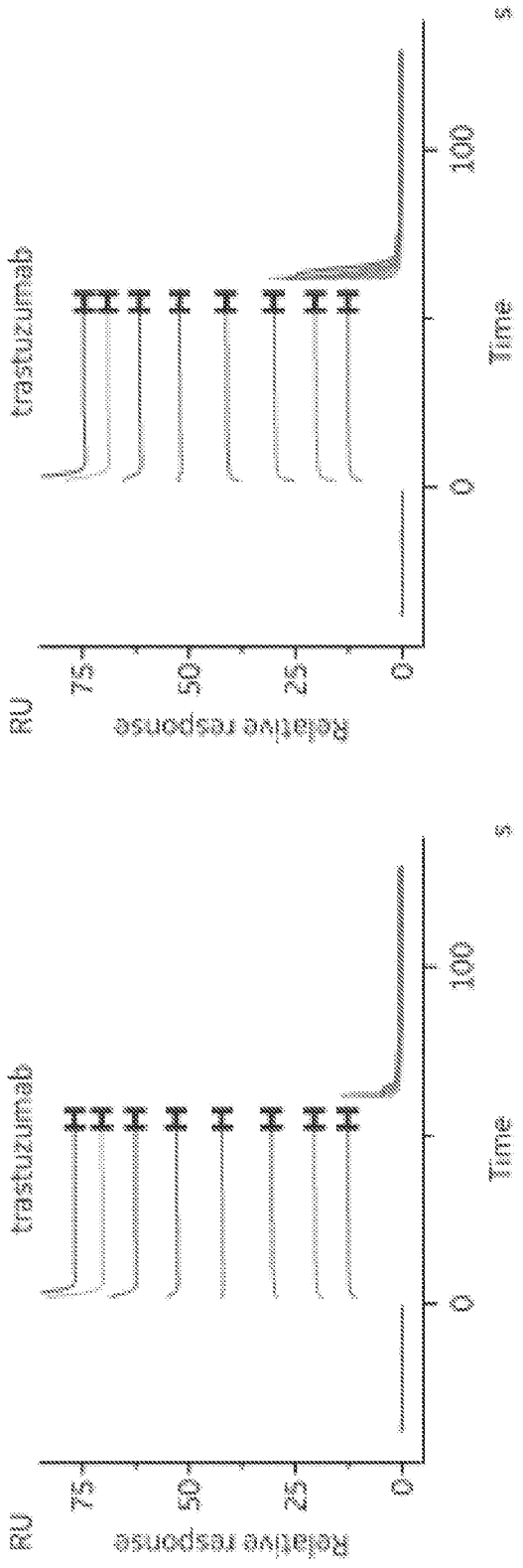
FIG. 239 Continued



trastuzumab

K_D = 1045.1 ± 62.2 nM

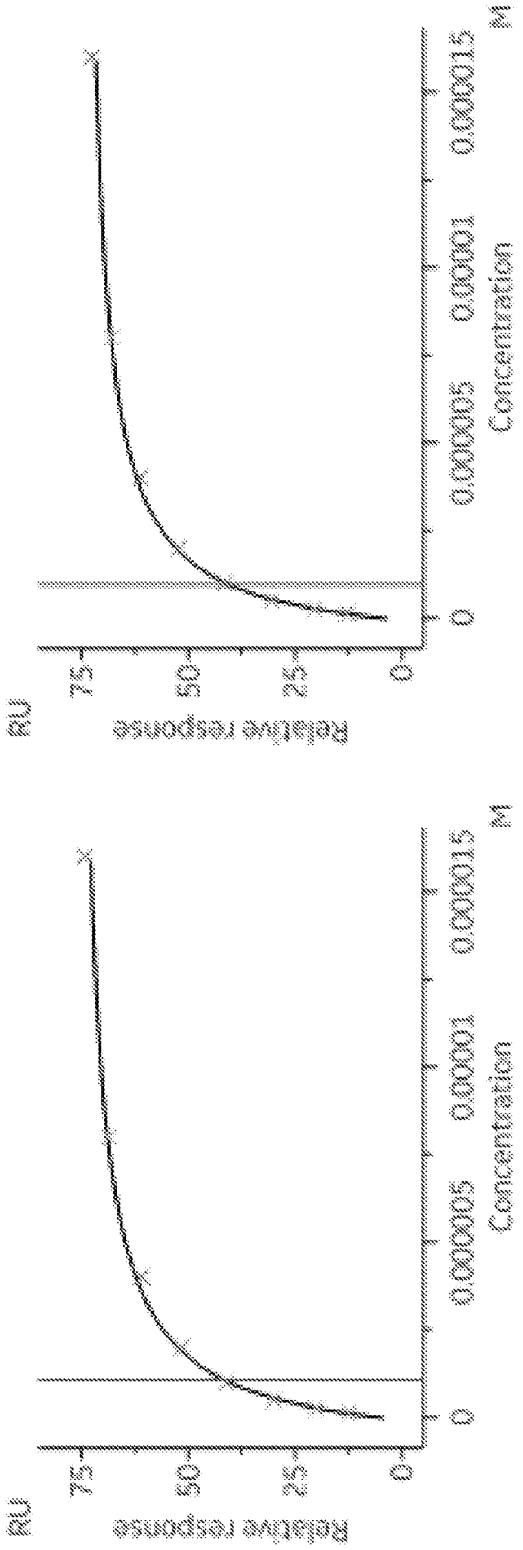
FIG. 239 Continued



trastuzumab

K_D = 1045.1 ± 62.2 nM

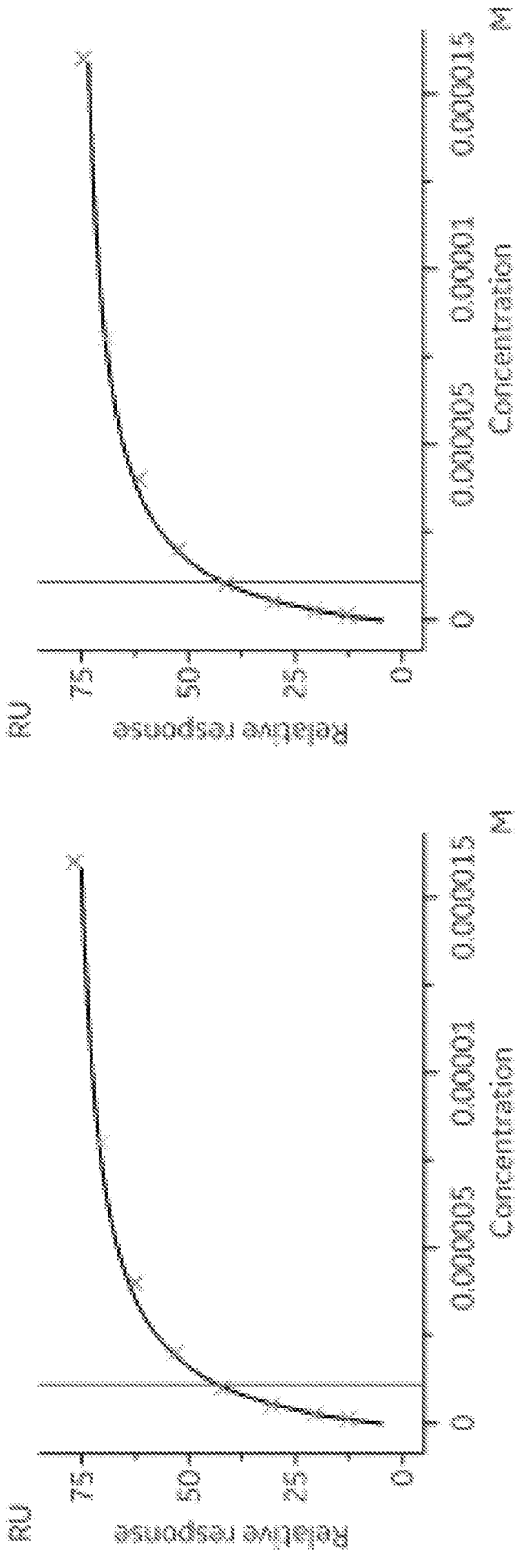
FIG. 239 Continued



trastuzumab

K_D = 1045.1 ± 62.2 nM

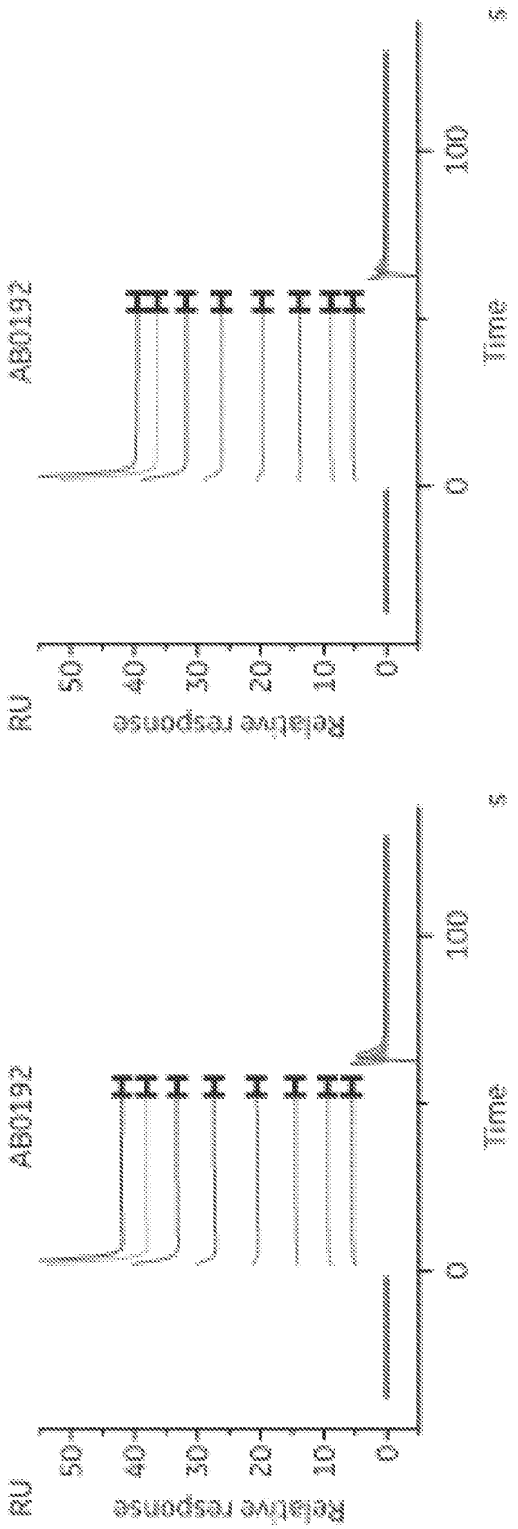
FIG. 239 Continued



trastuzumab

K_D = 1045.1 ± 62.2 nM

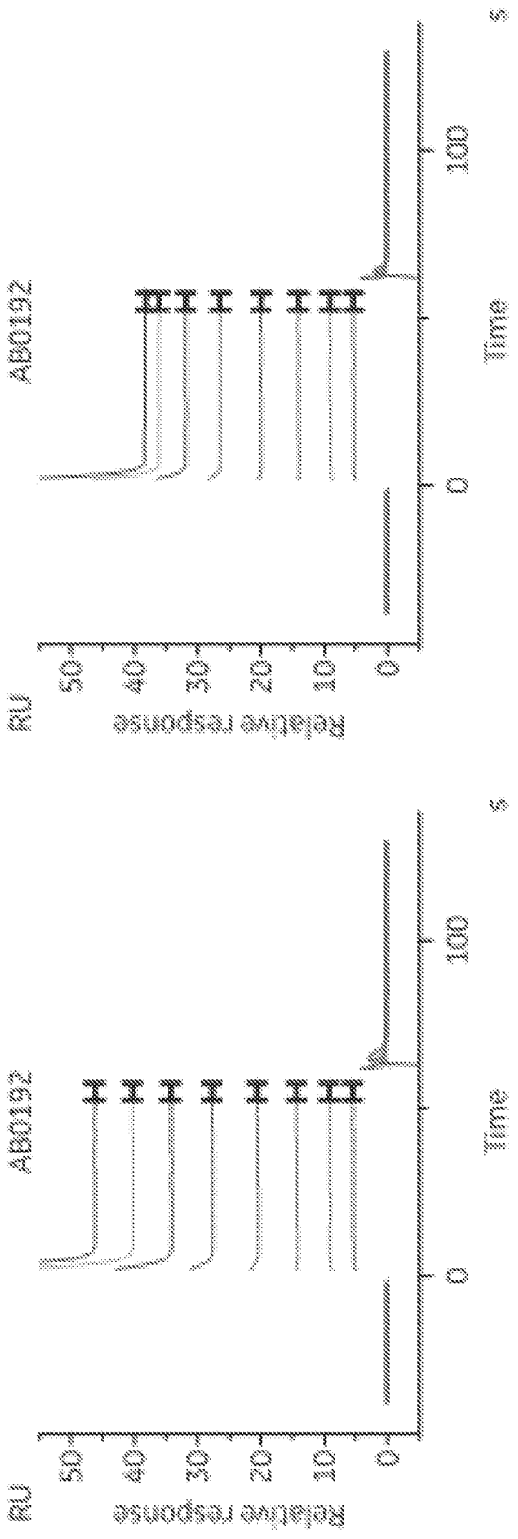
FIG. 239 Continued



AB0192

K_D = 1398.8 ± 286.1 nM

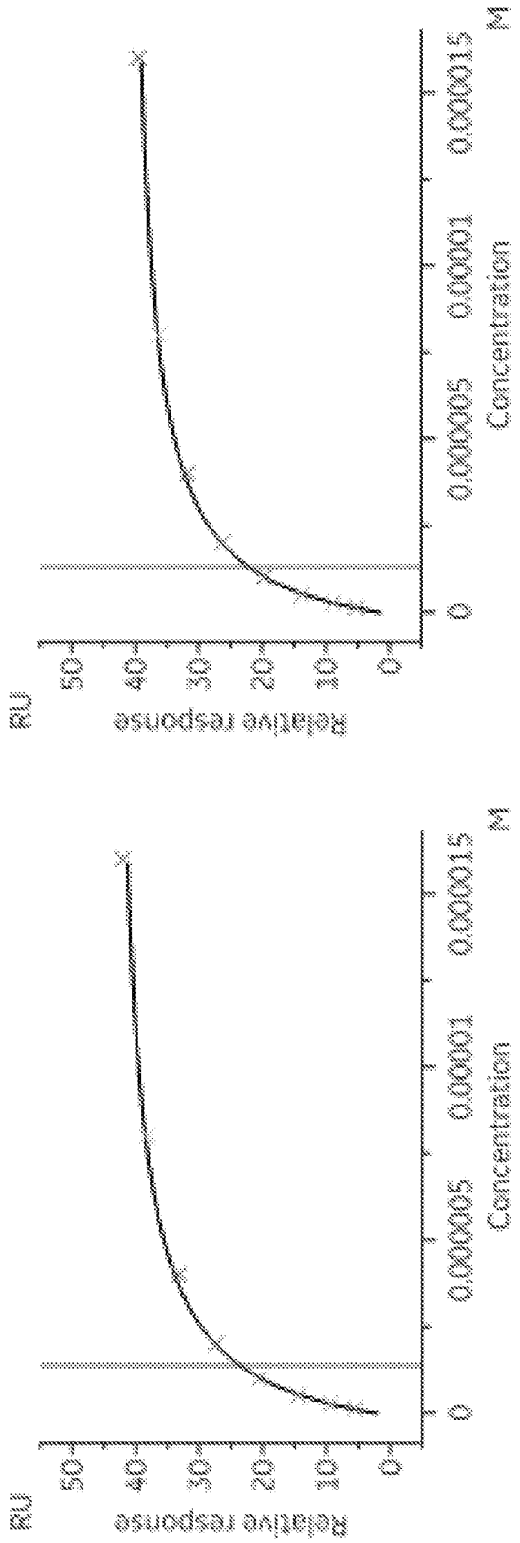
FIG. 240



AB0192

K_D = 1398.8 ± 286.1 nM

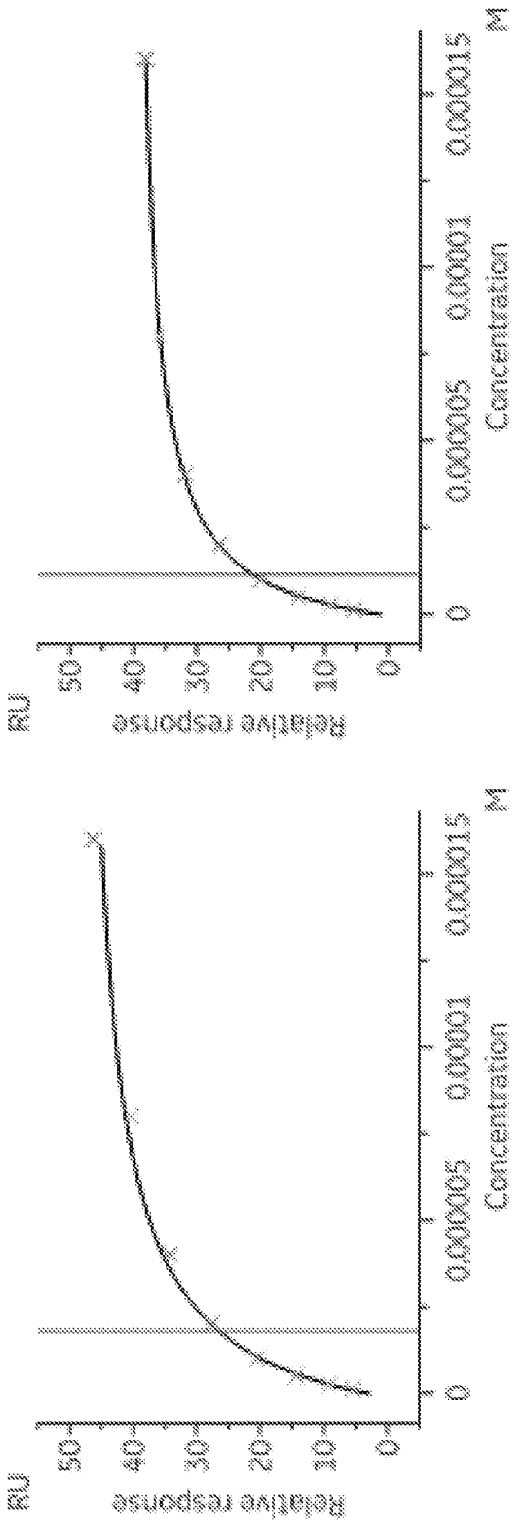
FIG. 240 Continued



AB0192

K_D = 1398.8 ± 286.1 nM

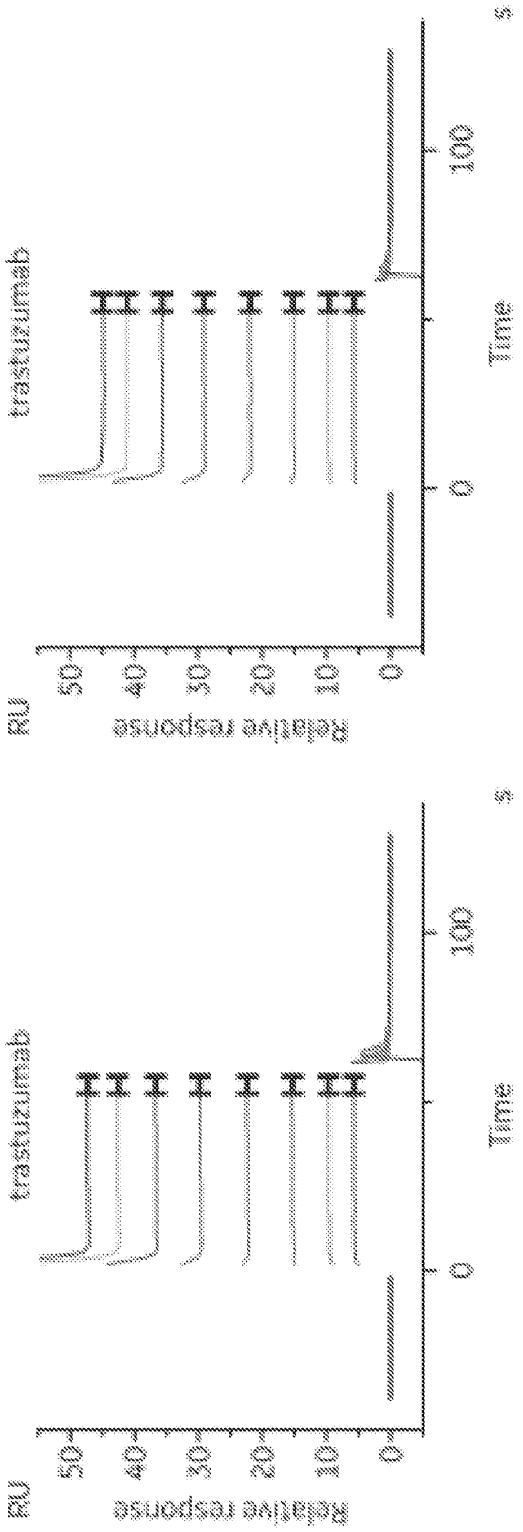
FIG. 240 Continued



AB0192

K_D = 1398.8 ± 286.1 nM

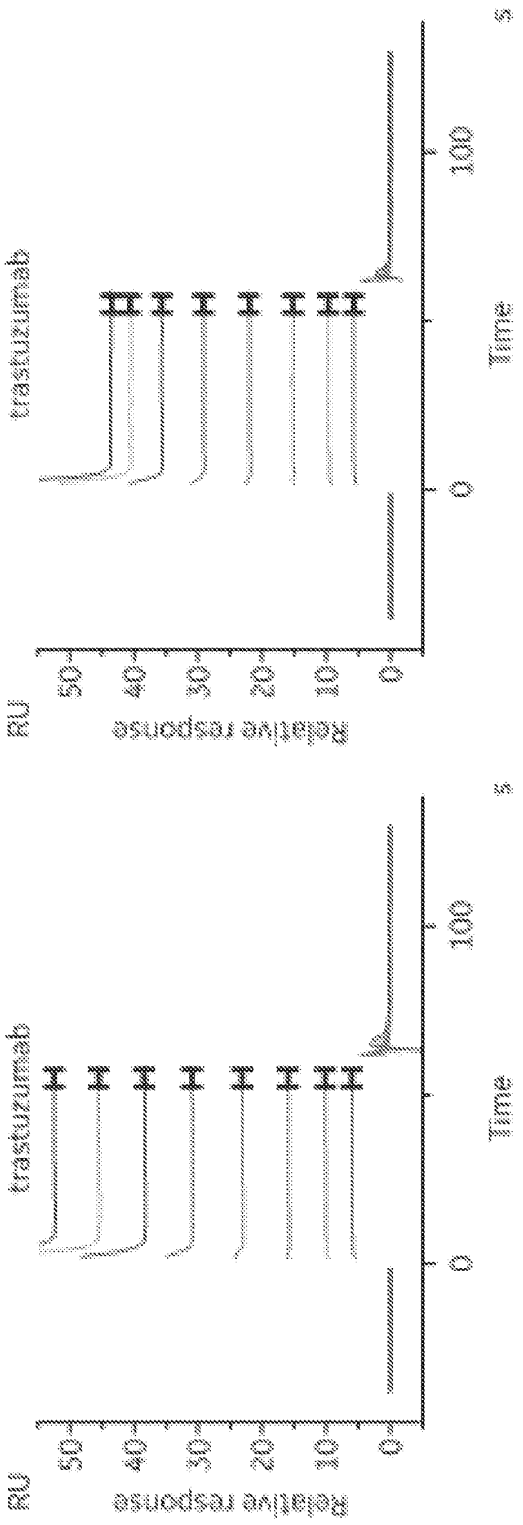
FIG. 240 Continued



trastuzumab

K_D = 1493.2 ± 265.9 nM

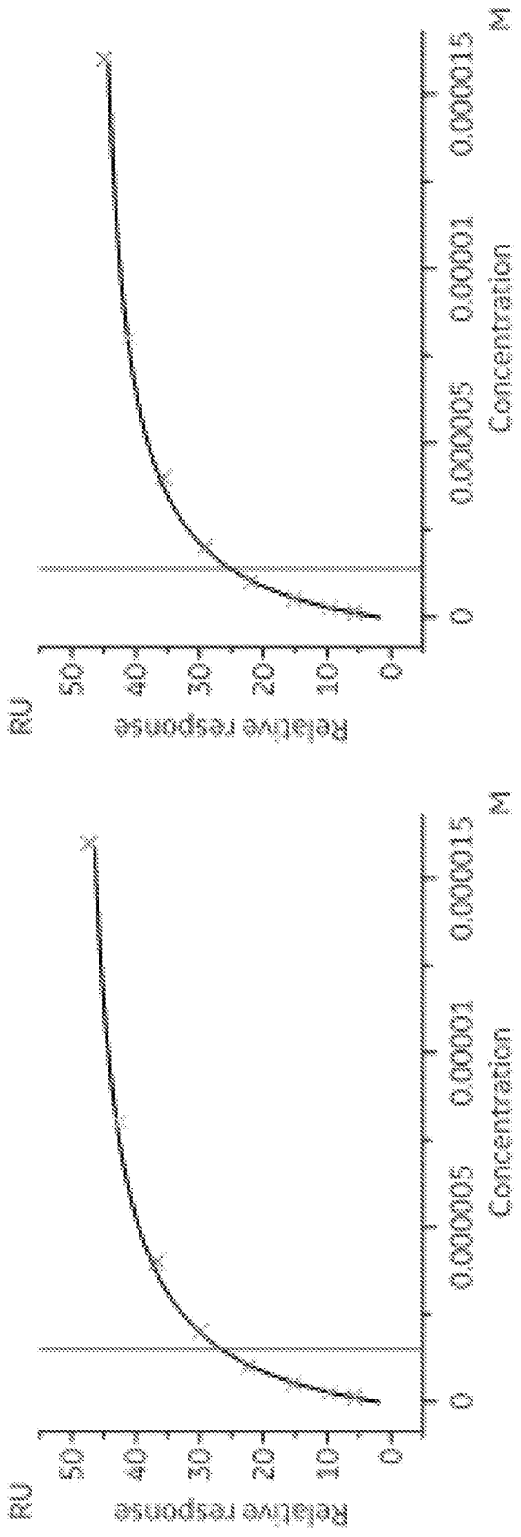
FIG. 240 Continued



trastuzumab

K_D = 1493.2 ± 265.9 nM

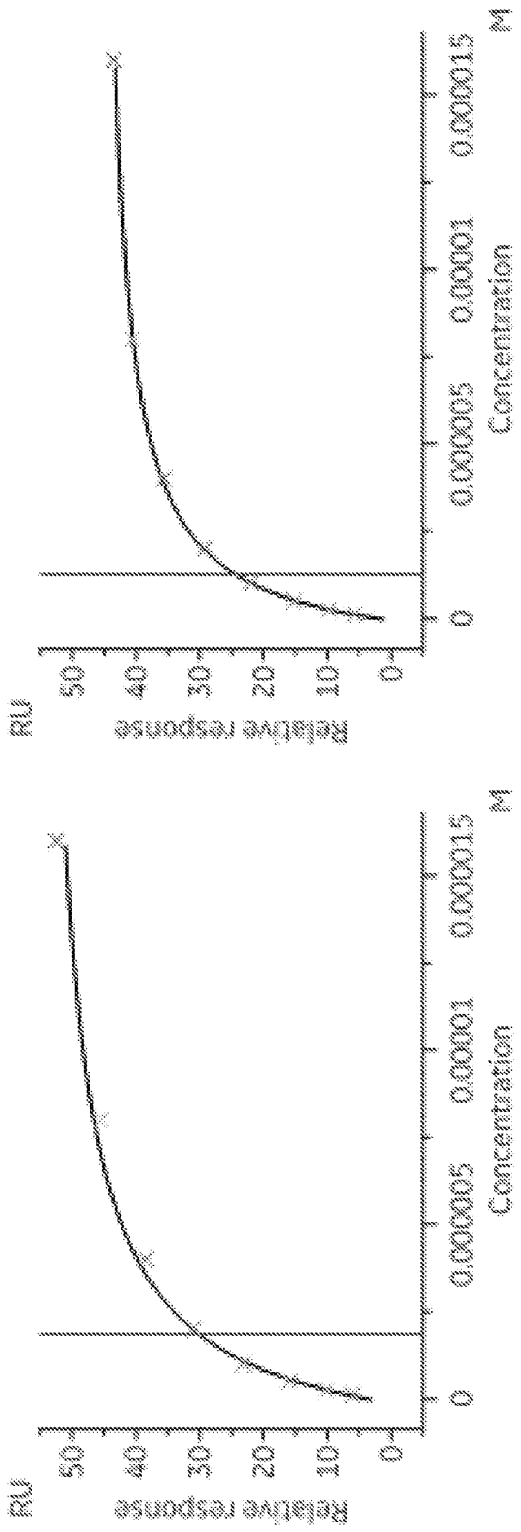
FIG. 240 Continued



trastuzumab

K_D = 1493.2 ± 265.9 nM

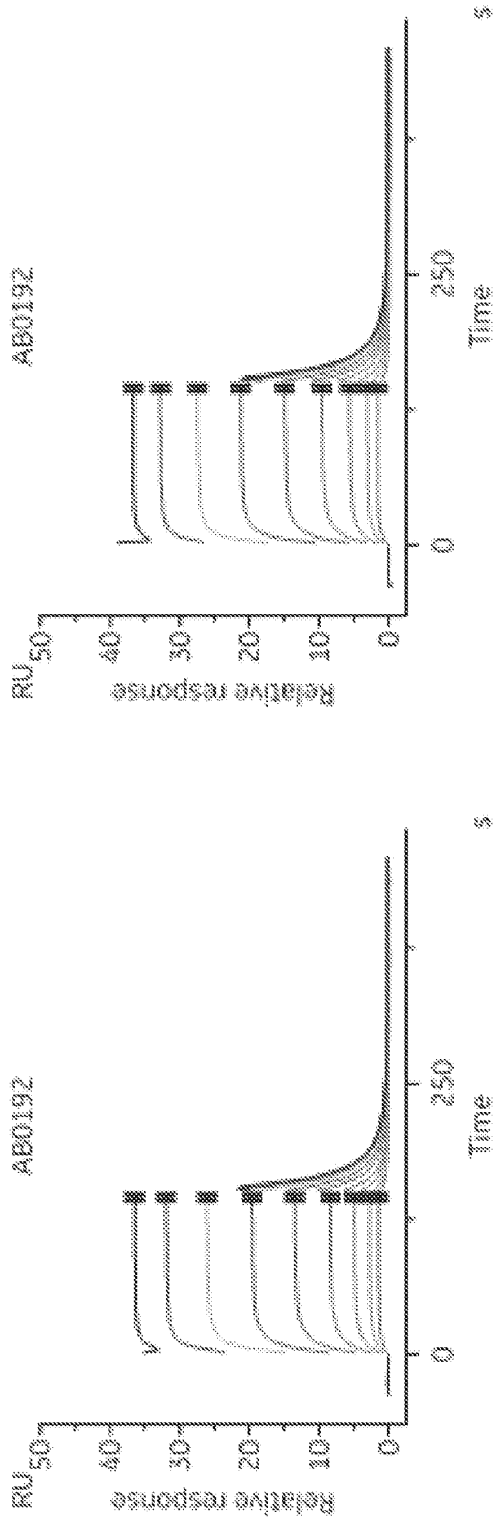
FIG. 240 Continued



trastuzumab

K_D = 1493.2 ± 265.9 nM

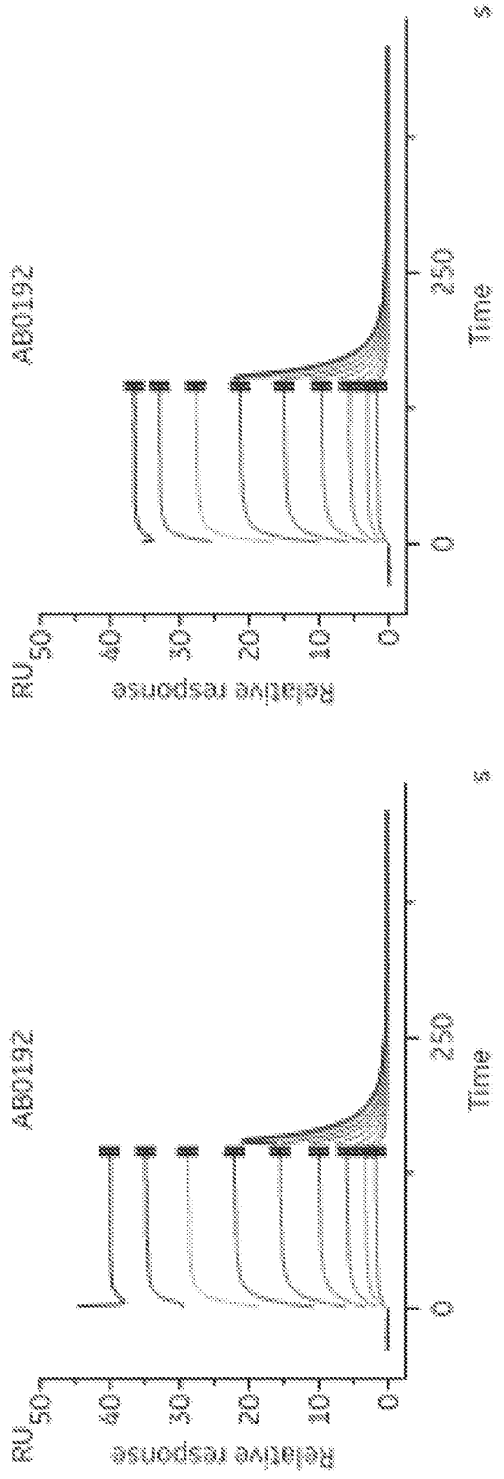
FIG. 240 Continued



AB0192

K_D = 680.0 ± 69.6 nM

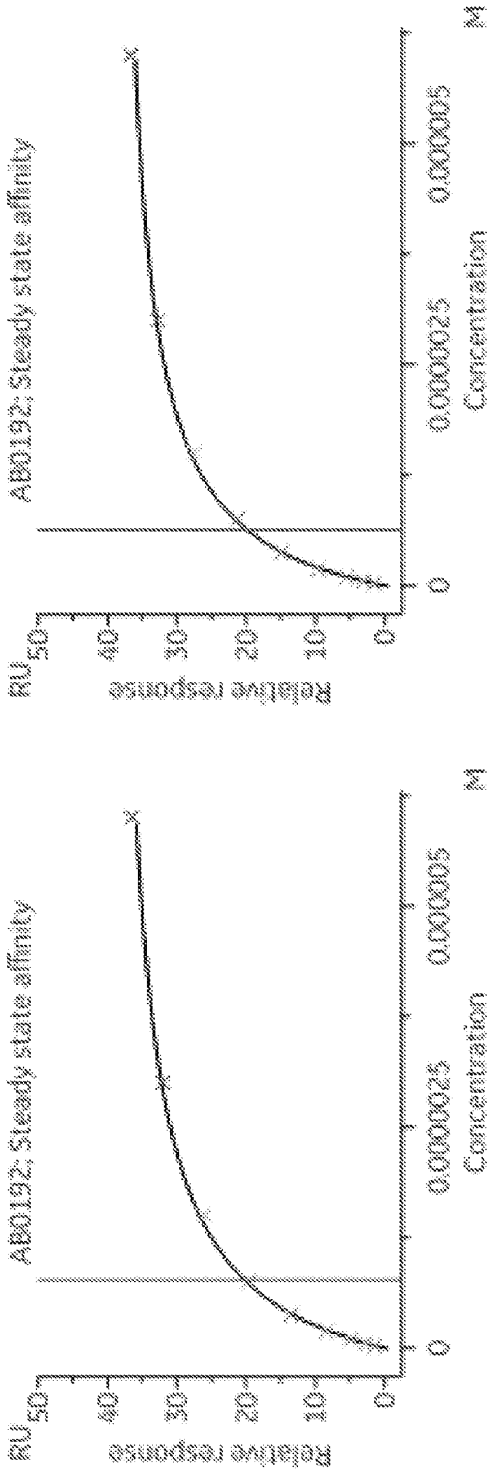
FIG. 241



AB0192

K_D = 680.0 ± 69.6 nM

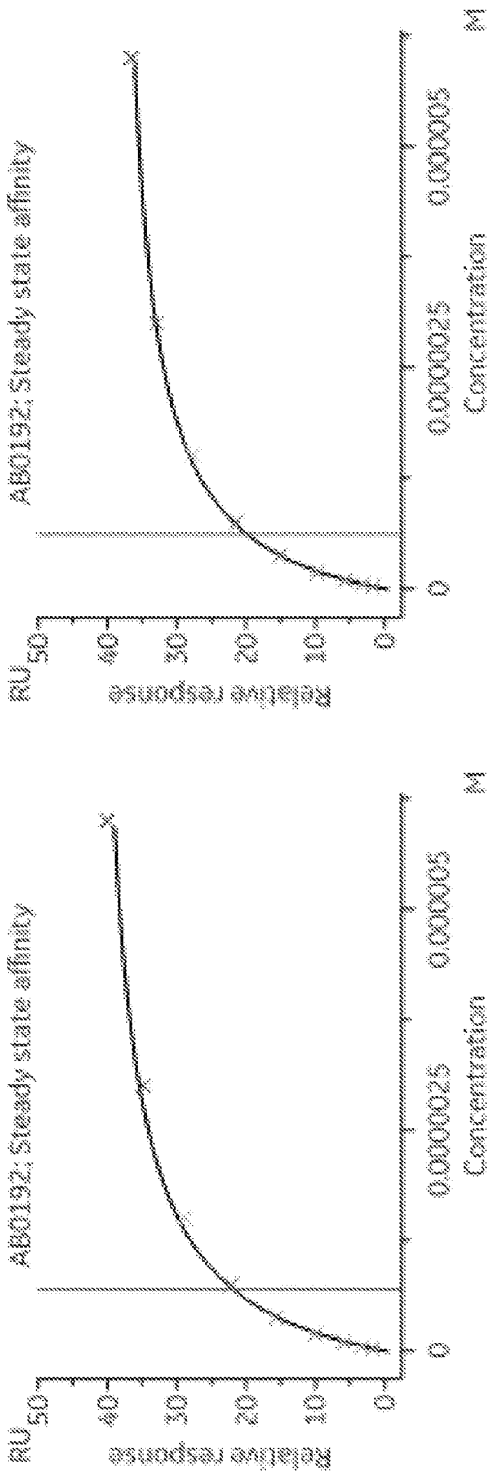
FIG. 241 Continued



AB0192

K_D = 680.0 ± 69.6 nM

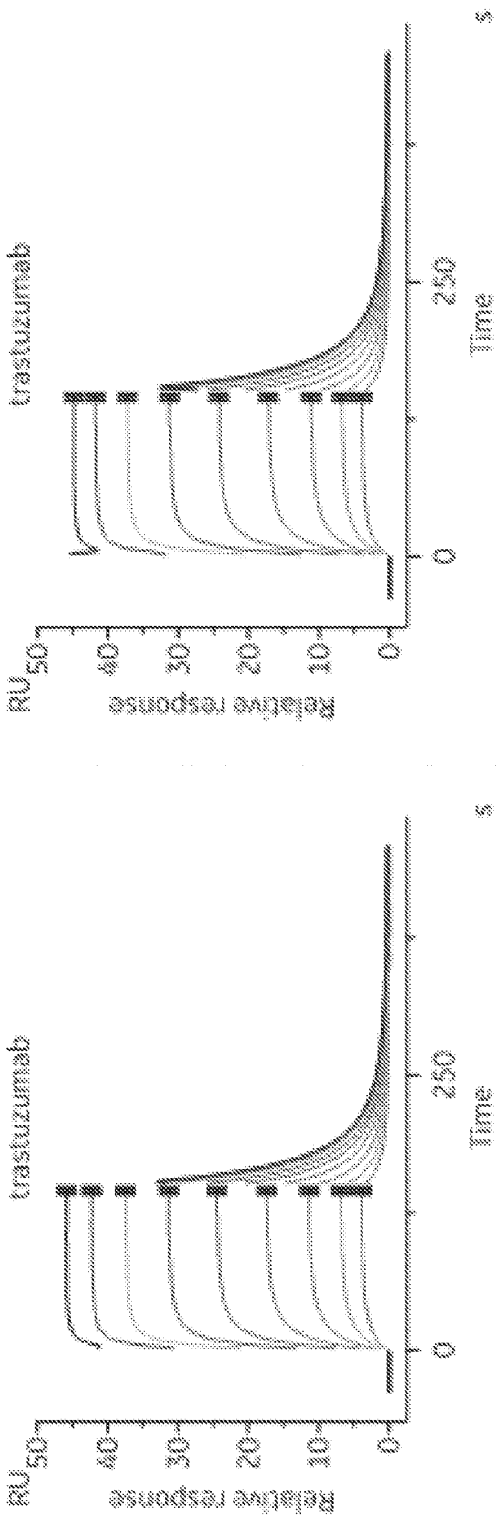
FIG. 241 Continued



AB0192

K_D = 680.0 ± 69.6 nM

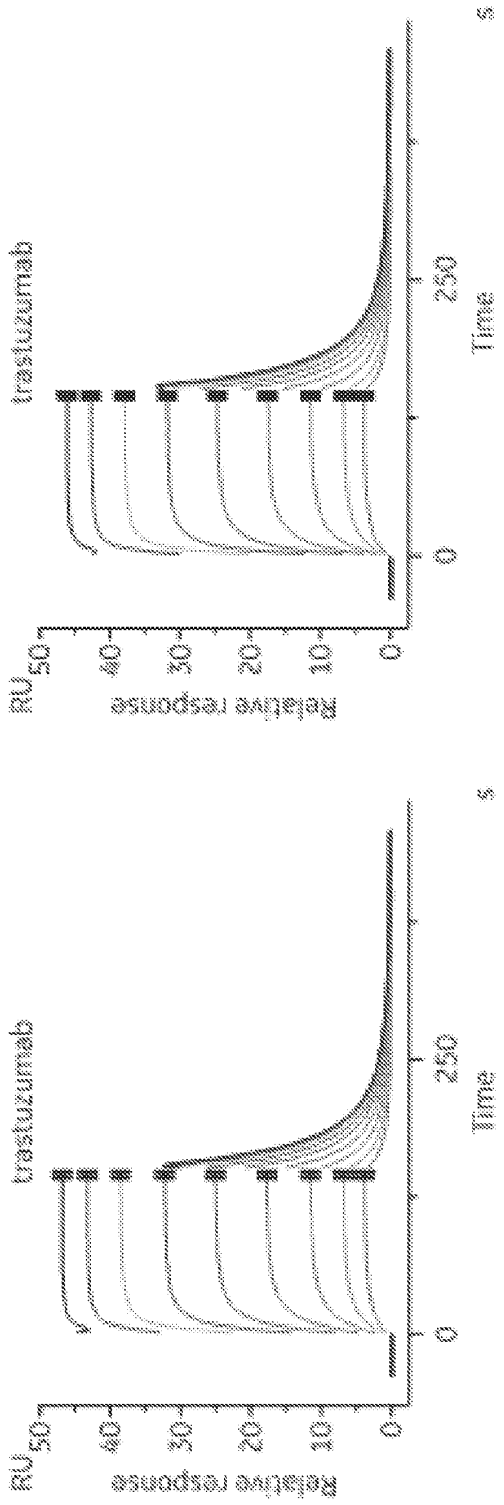
FIG. 241 Continued



trastuzumab

K_D = 331.4 ± 3.3 nM

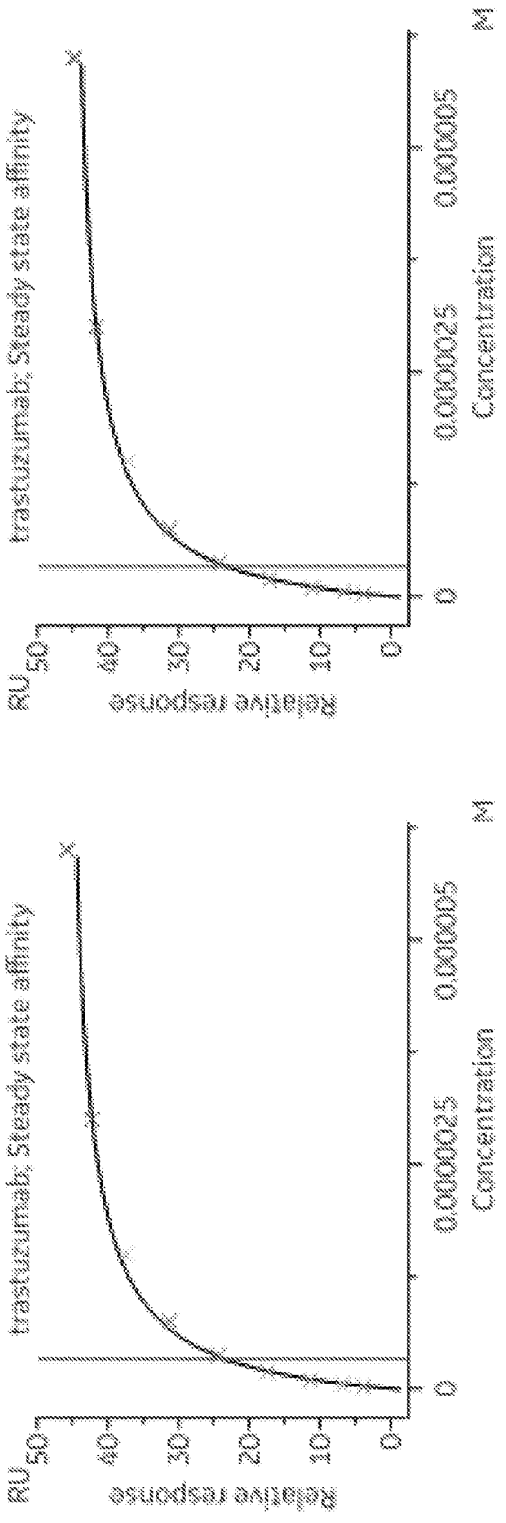
FIG. 241 Continued



trastuzumab

K_D = 331.4 ± 3.3 nM

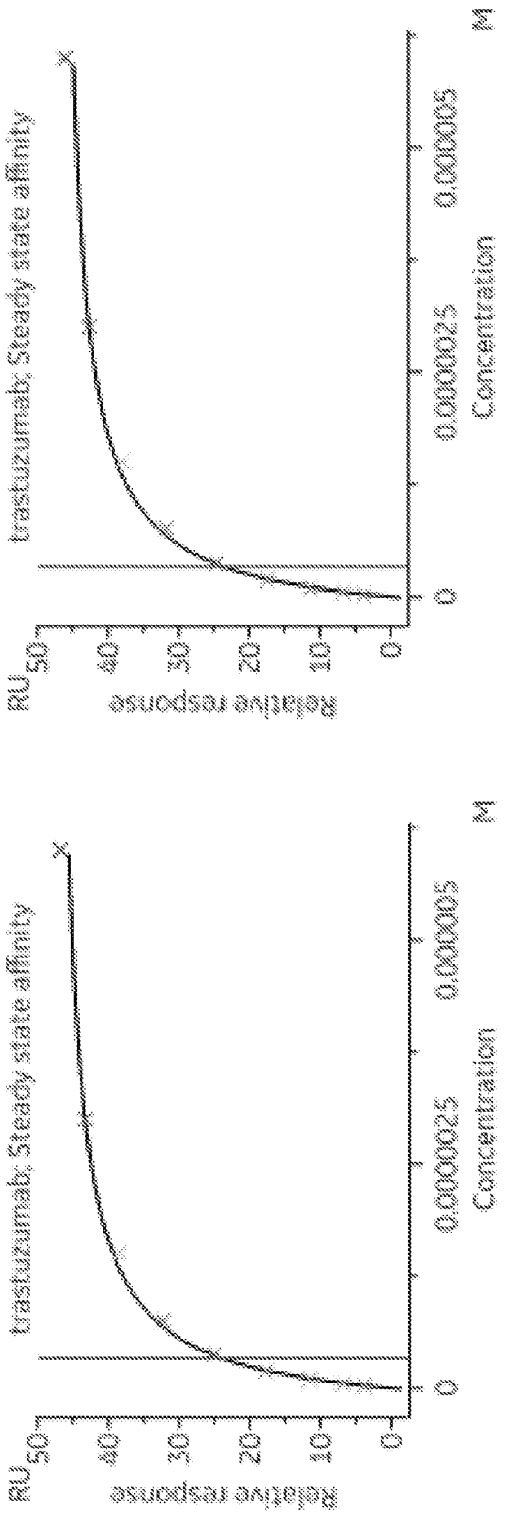
FIG. 241 Continued



trastuzumab

K_D = 331.4 ± 3.3 nM

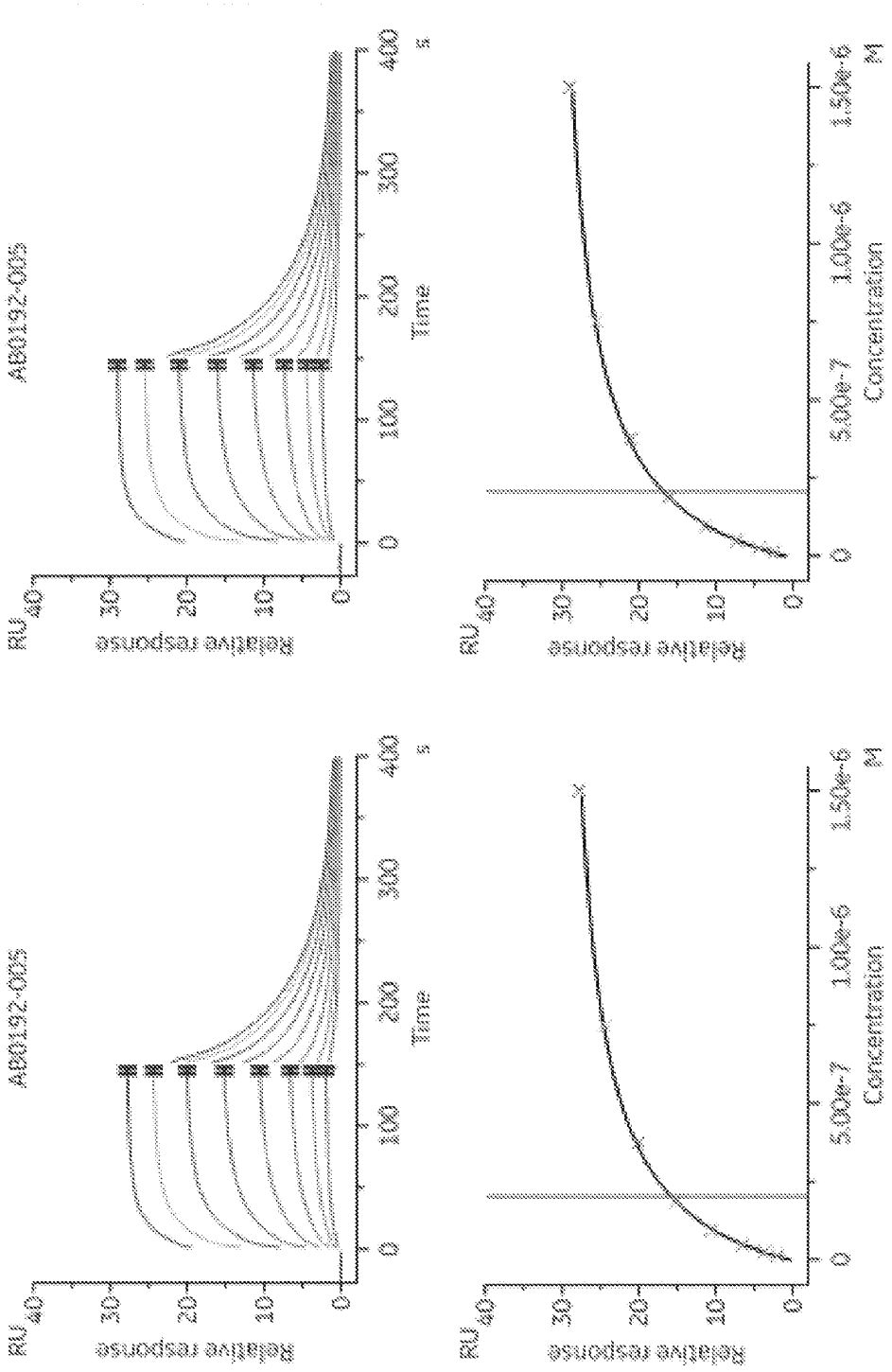
FIG. 241 Continued



trastuzumab

K_D = 331.4 ± 3.3 nM

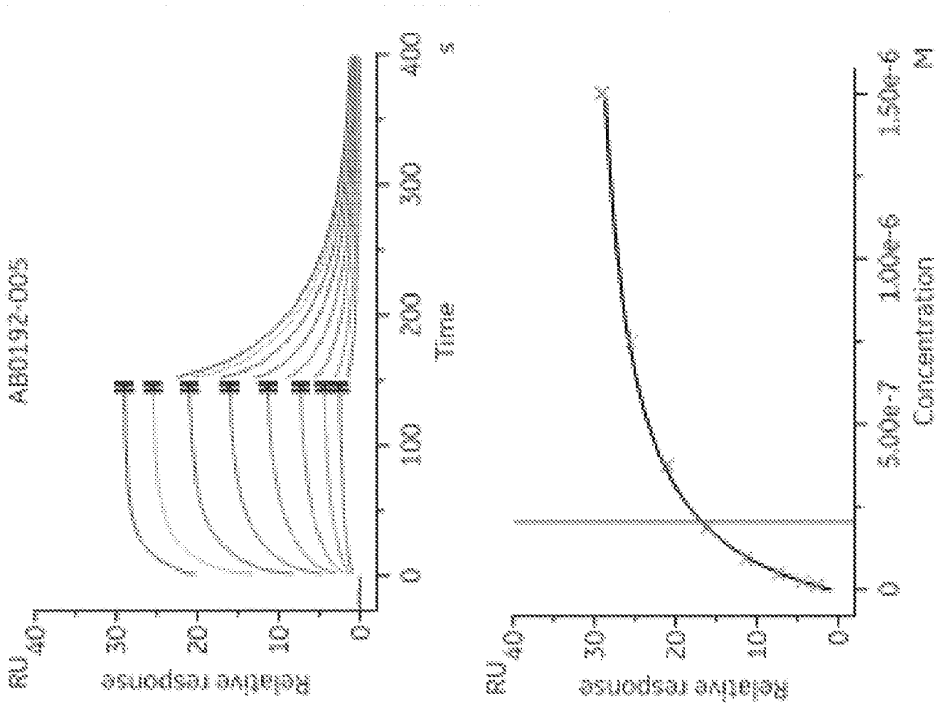
FIG. 241 Continued



AB0192

K_D = 203.8 ± 1.0 nM

FIG. 242



AB0192

K_D = 203.8 ± 1.0 nM

FIG. 242 Continued

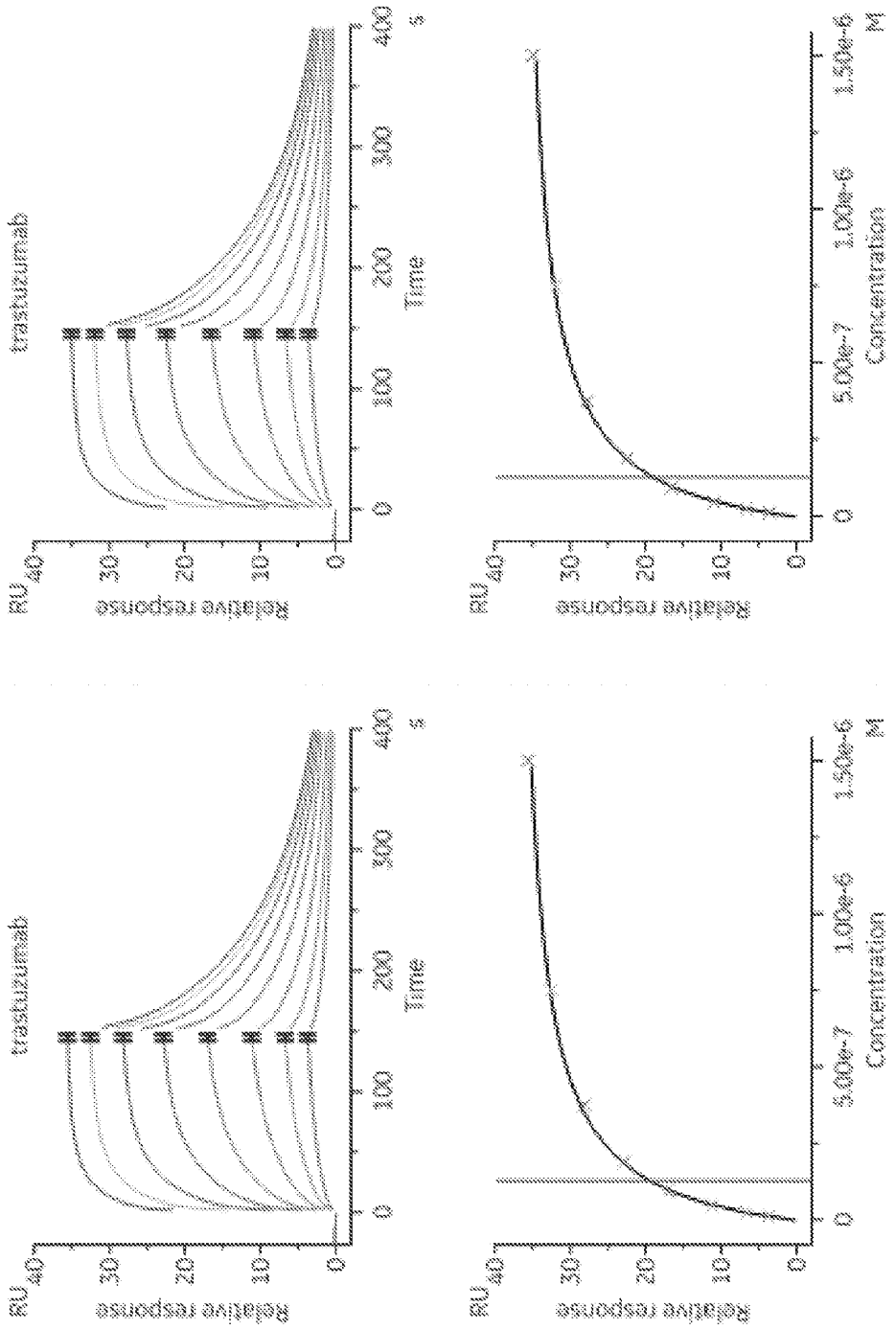
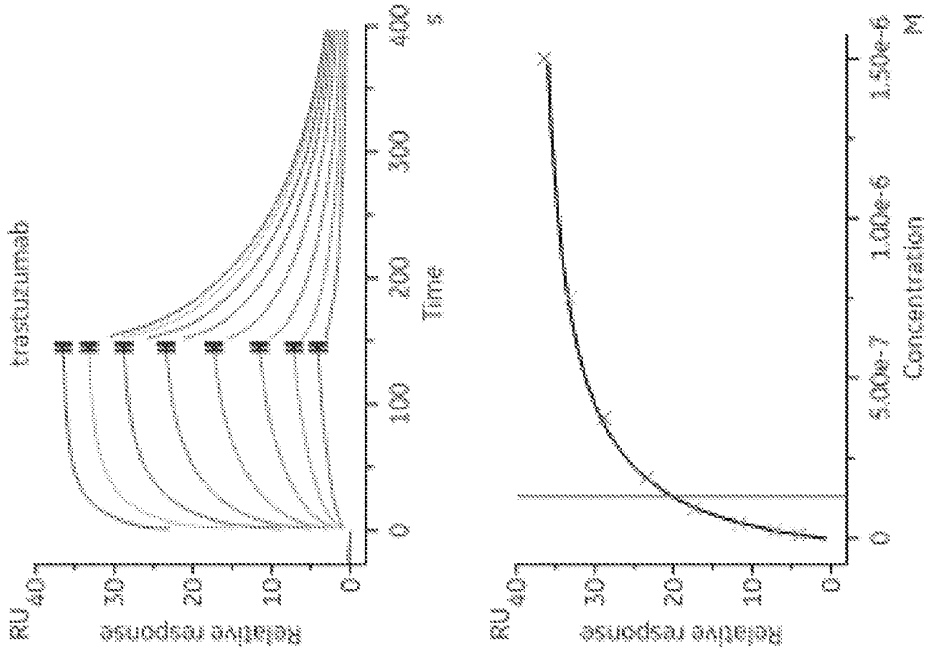


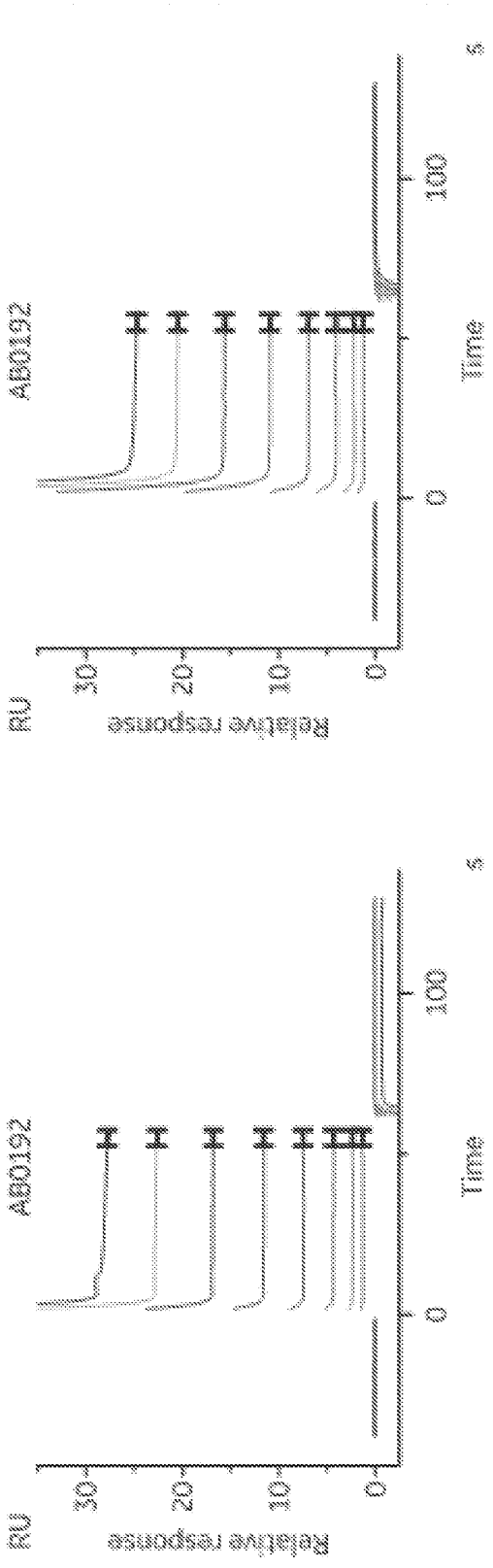
FIG. 242 Continued



trastuzumab

K_D = 128.8 ± 2.1 nM

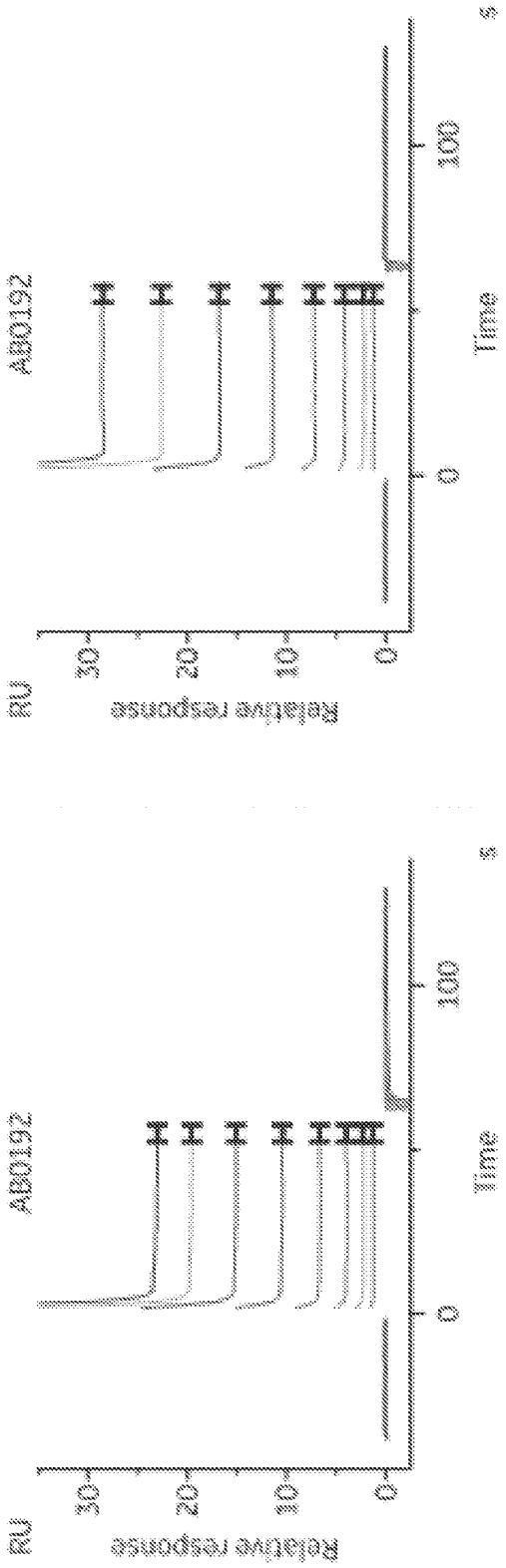
FIG. 242 Continued



AB0192

$K_D = 6.1 \pm 0.8\ \mu M$

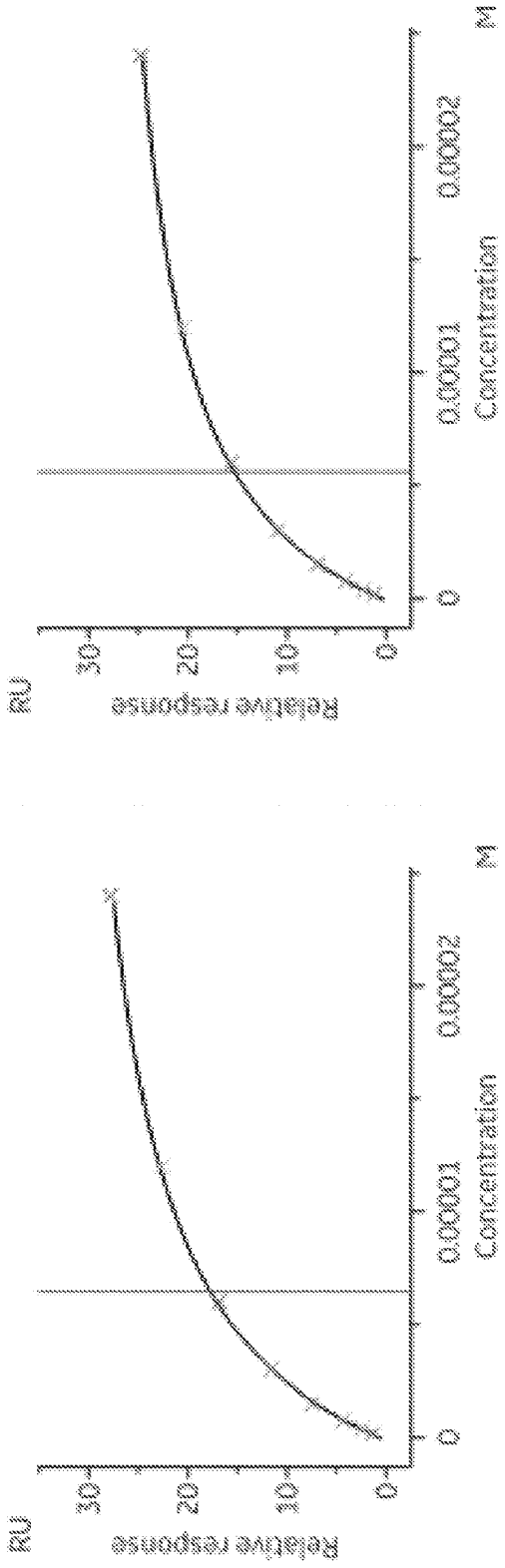
FIG. 243



AB0192

$K_D = 6.1 \pm 0.8\ \mu M$

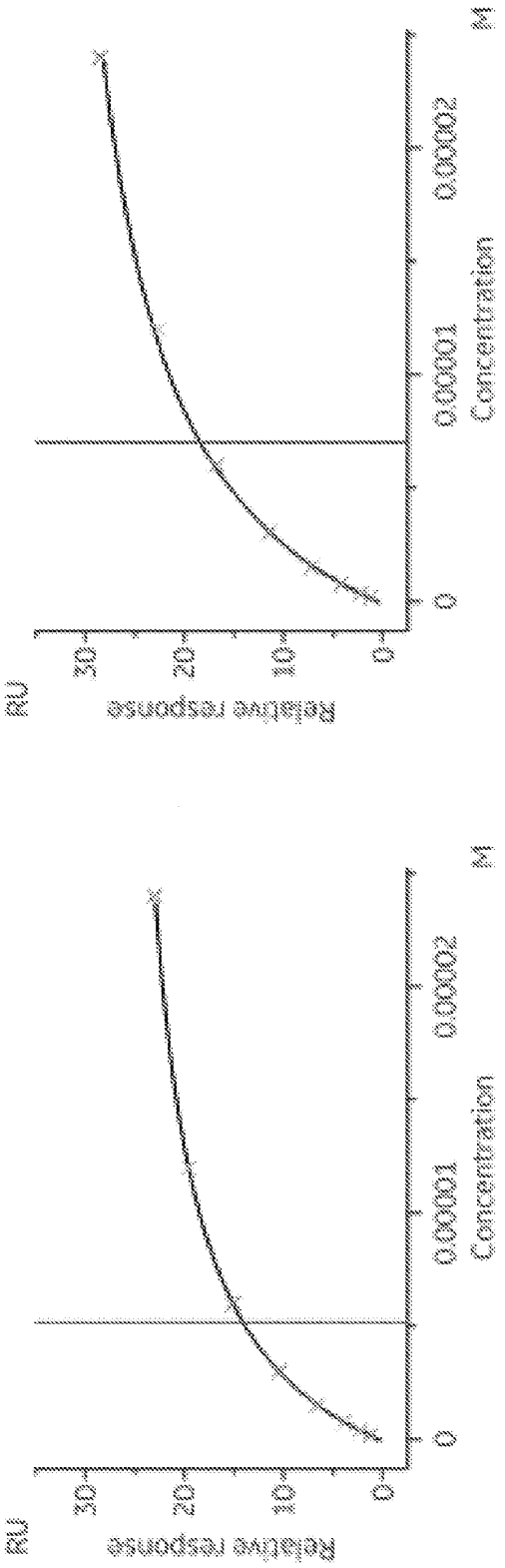
FIG. 243 Continued



AB0192

K_D = 6.1 ± 0.8 µM

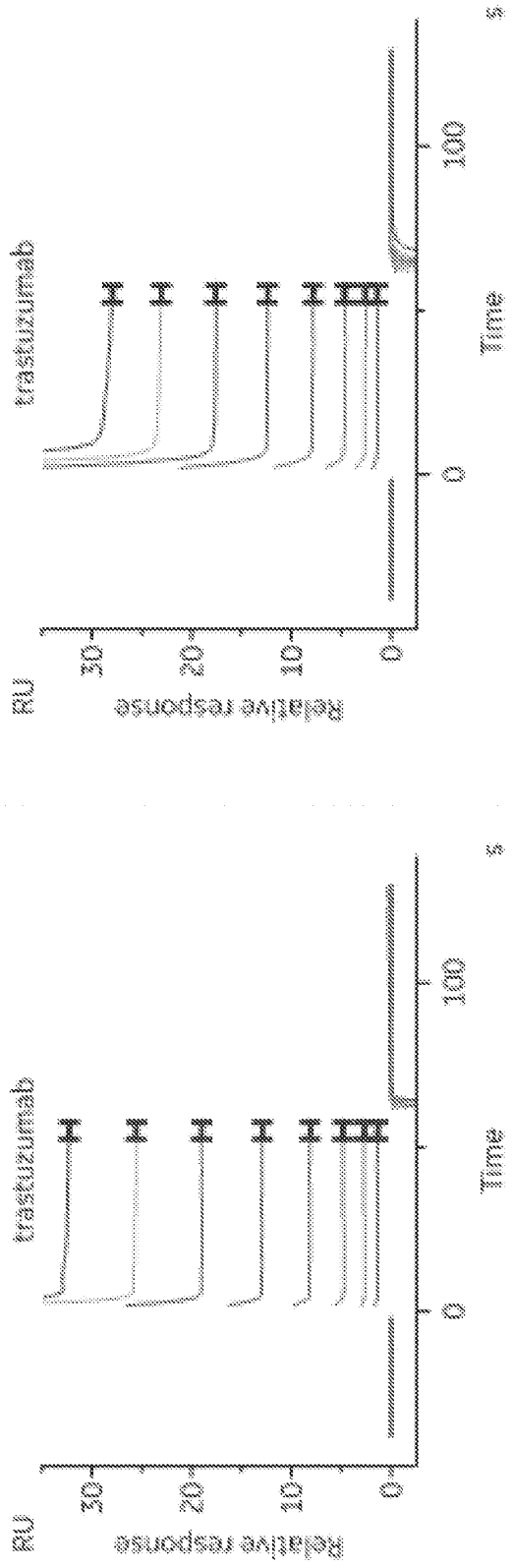
FIG. 243 Continued



AB0192

$K_D = 6.1 \pm 0.8\ \mu M$

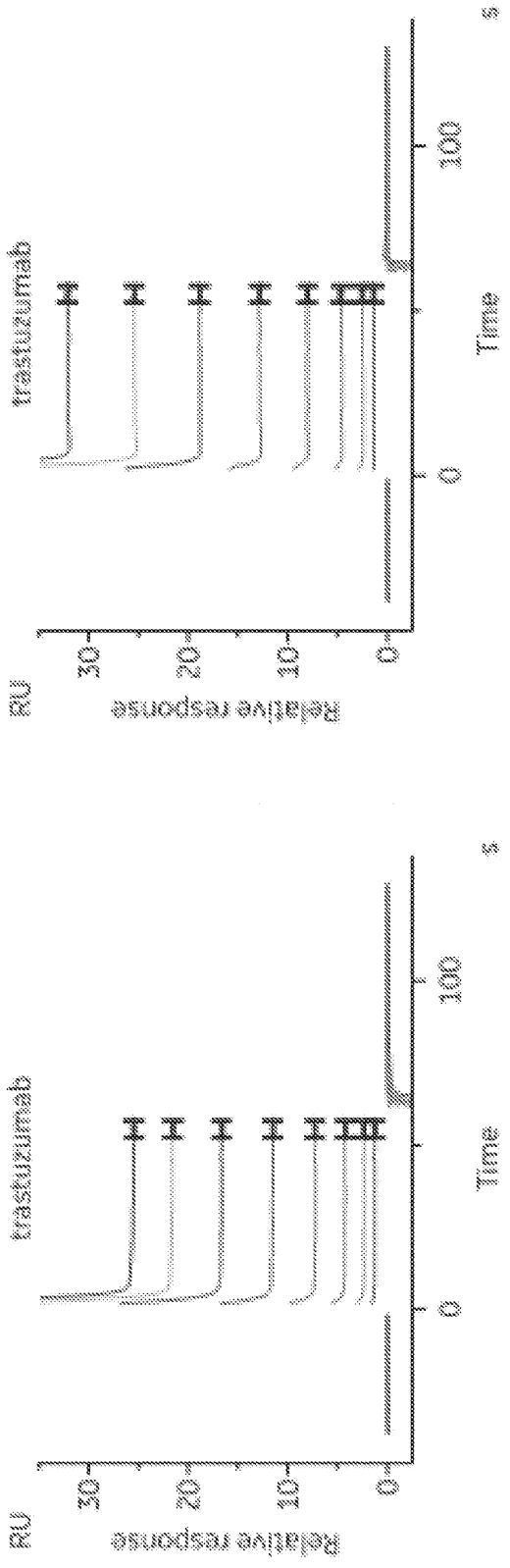
FIG. 243 Continued



trastuzumab

K_D = 6.3 ± 1.0 µM

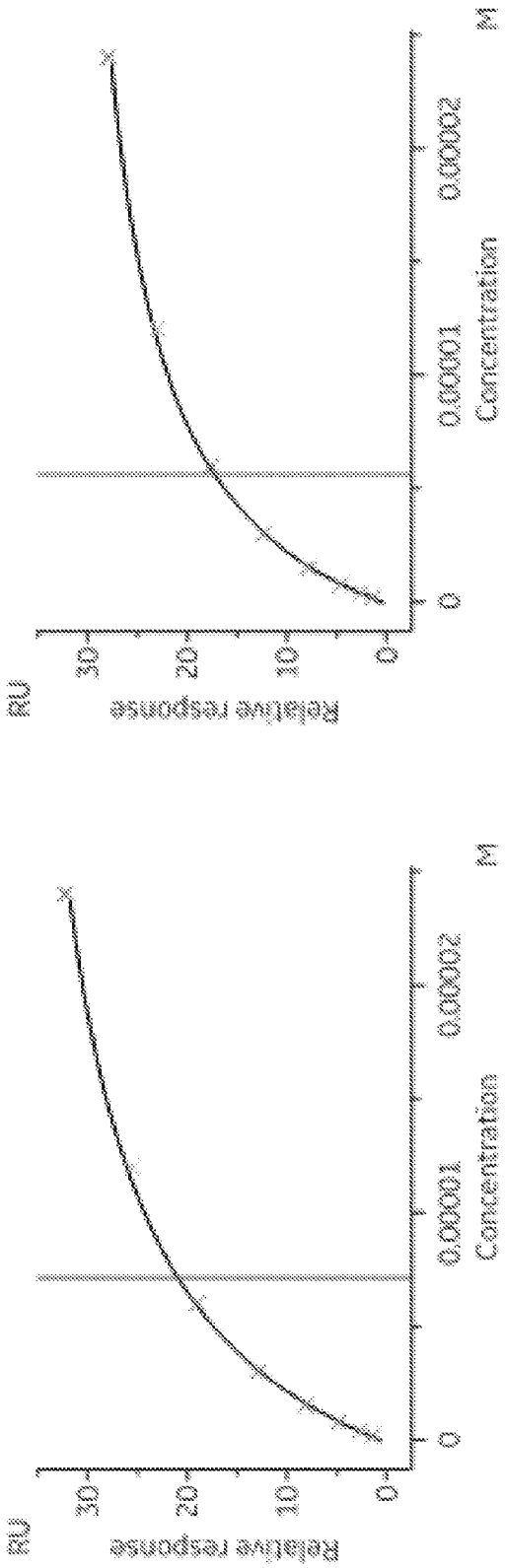
FIG. 243 Continued



trastuzumab

K_D = 6.3 ± 1.0 µM

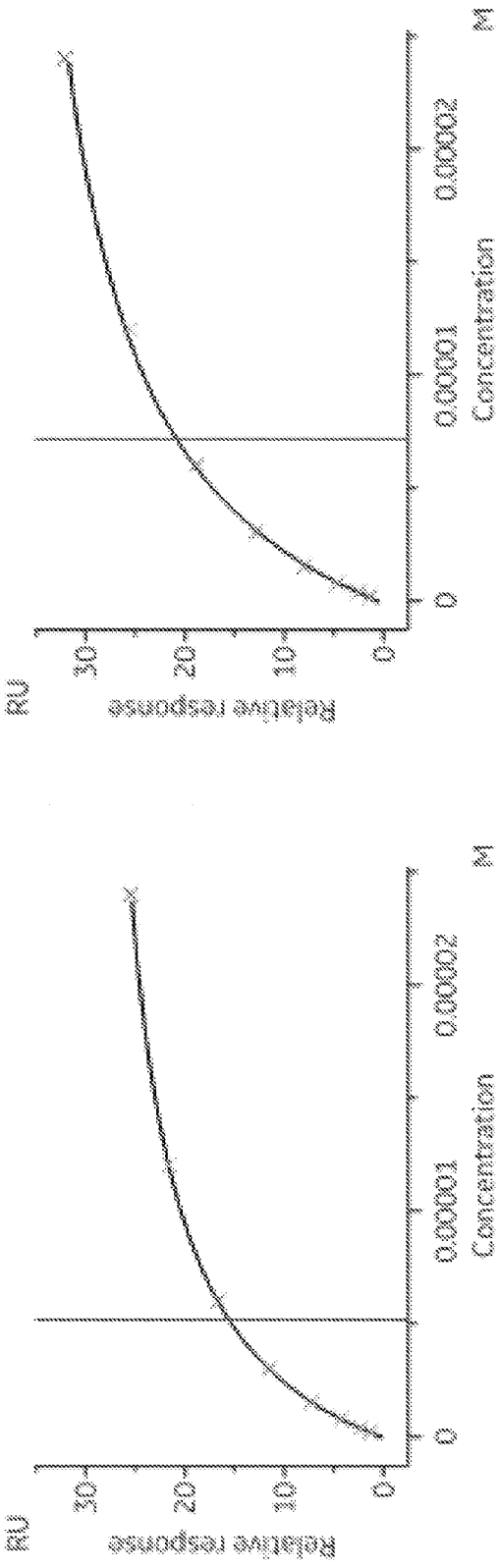
FIG. 243 Continued



trastuzumab

$K_D = 6.3 \pm 1.0\ \mu M$

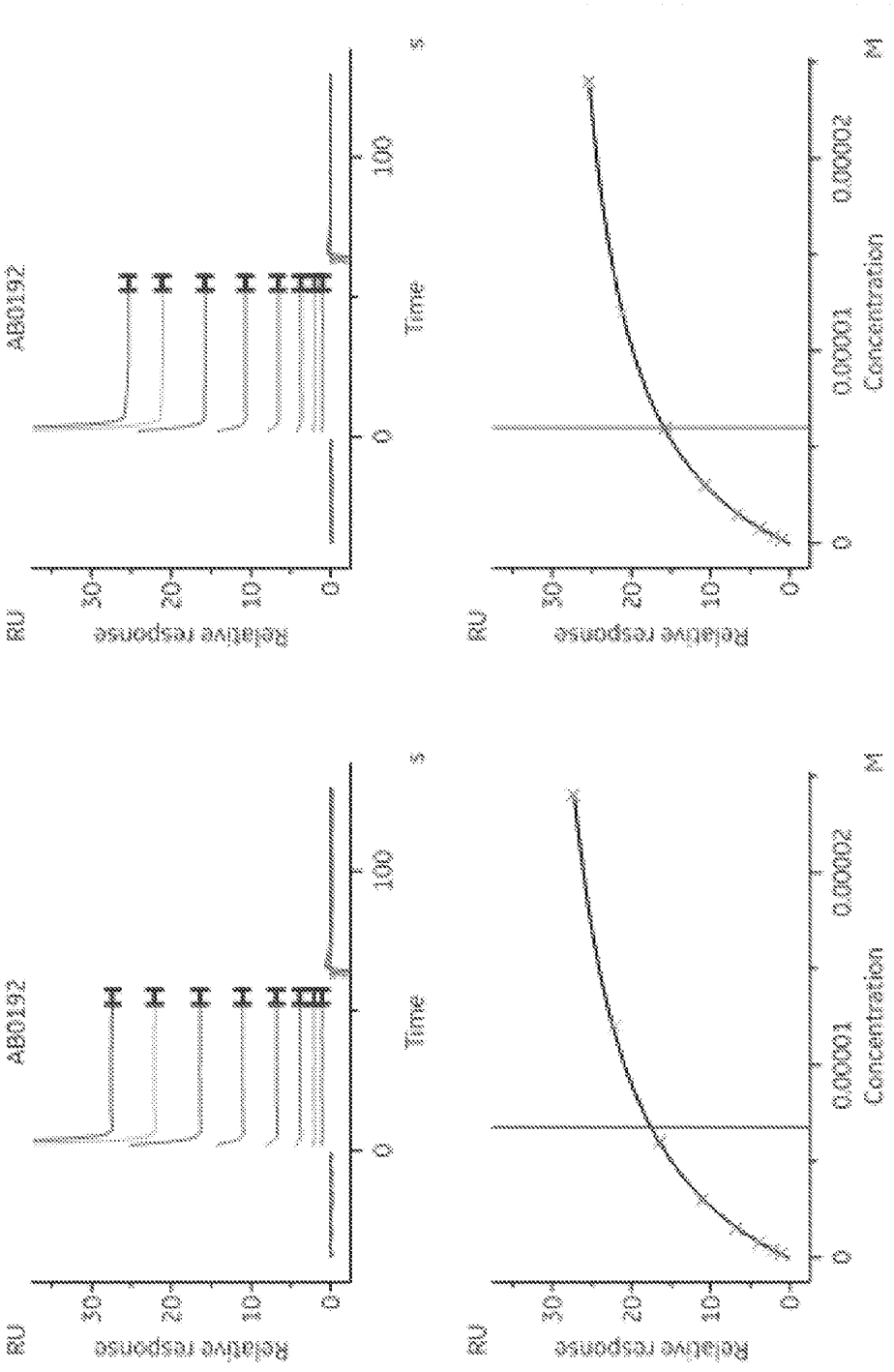
FIG. 243 Continued



trastuzumab

$K_D = 6.3 \pm 1.0\ \mu M$

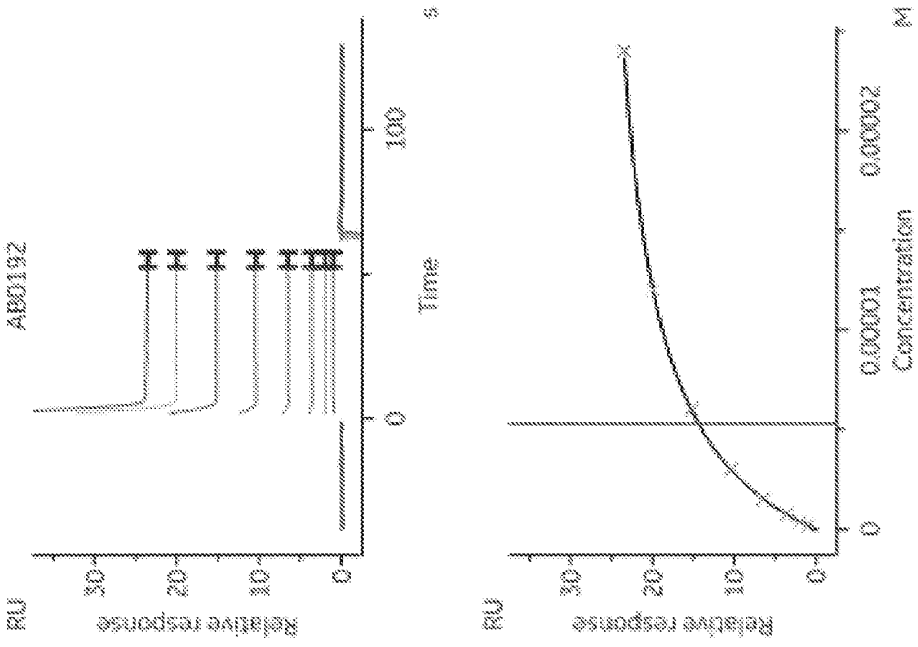
FIG. 243 Continued



AB0192

$K_D = 6.0 \pm 0.7$ µM

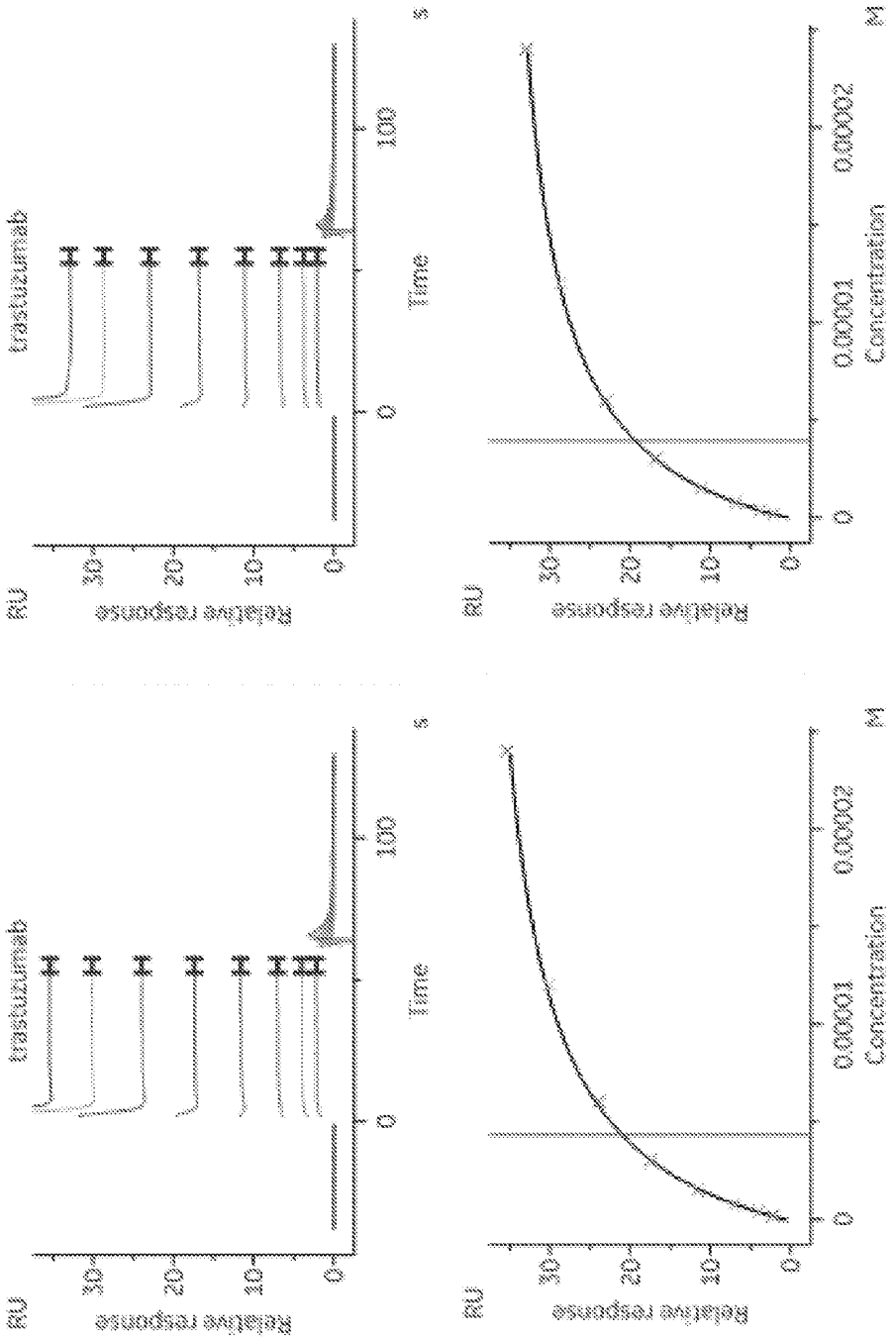
FIG. 244



AB0192

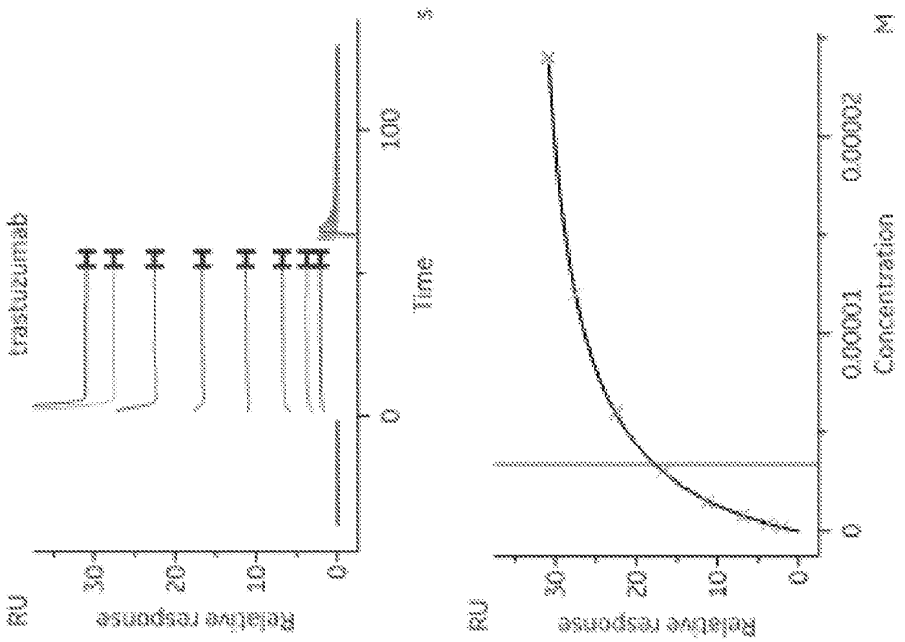
$K_D = 6.0 \pm 0.7$ µM

FIG. 244 Continued



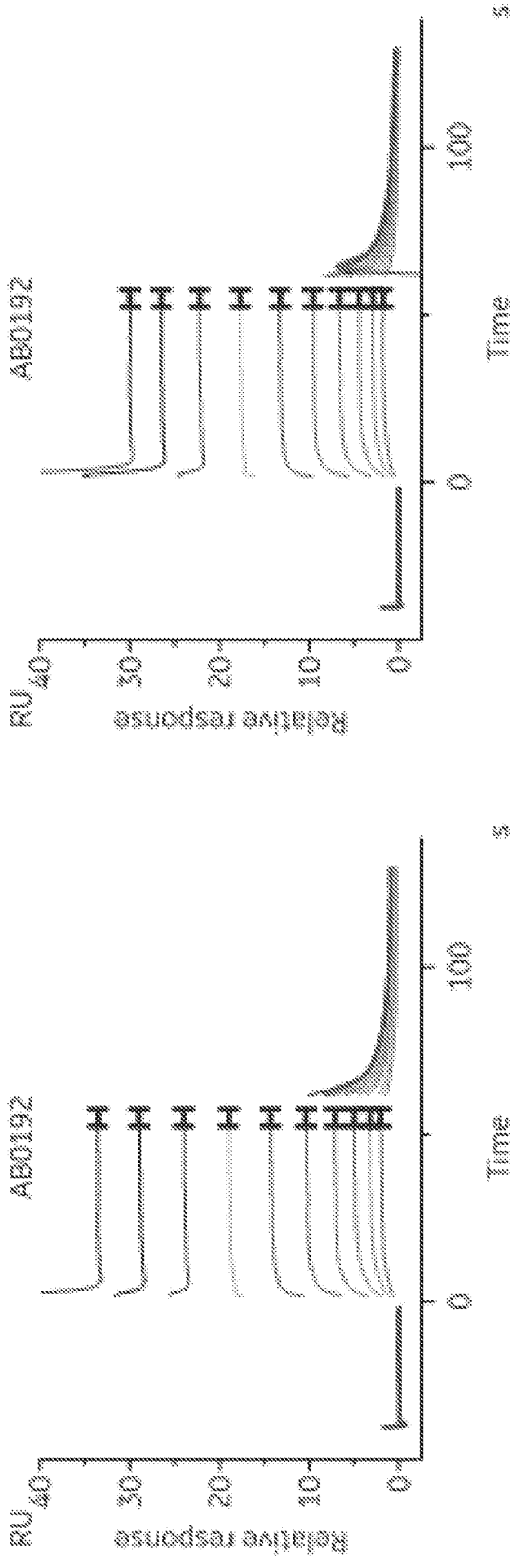
trastuzumab

K_D = 3.9 ± 0.5 µM FIG. 244 Continued



trastuzumab

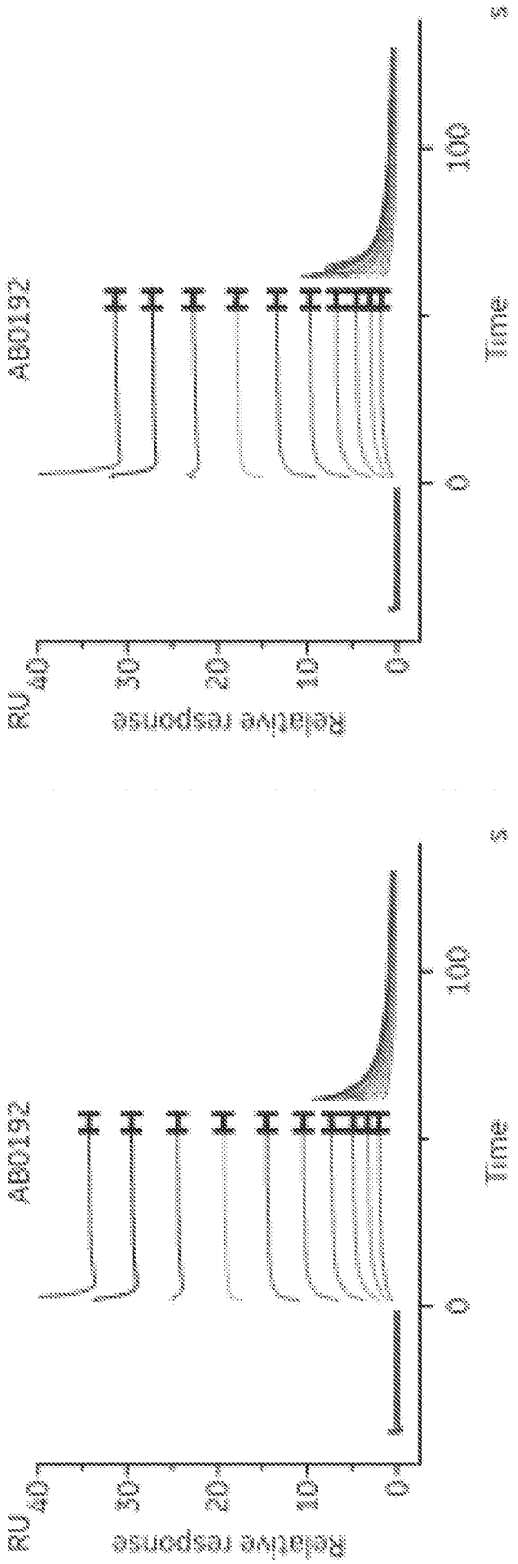
K_D = 3.9 ± 0.5 µM FIG. 244 Continued



AB0192

K_D = 1.5 ± 0.1 μM

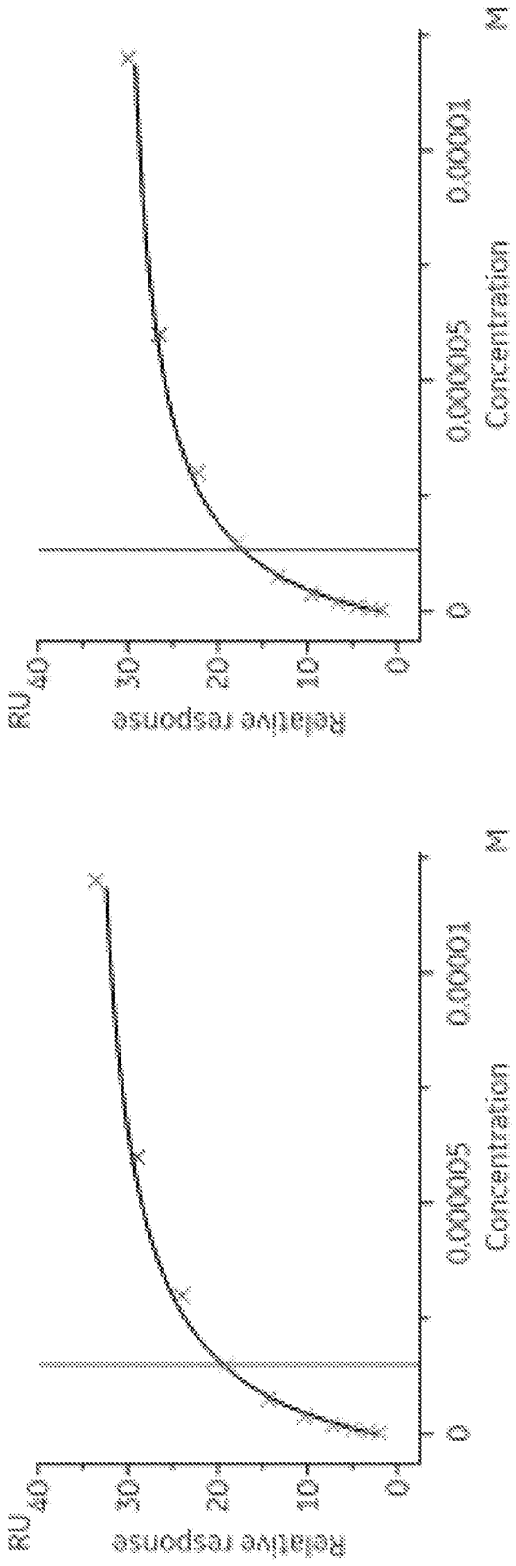
FIG. 245



AB0192

K_D = 1.5 ± 0.1 µM

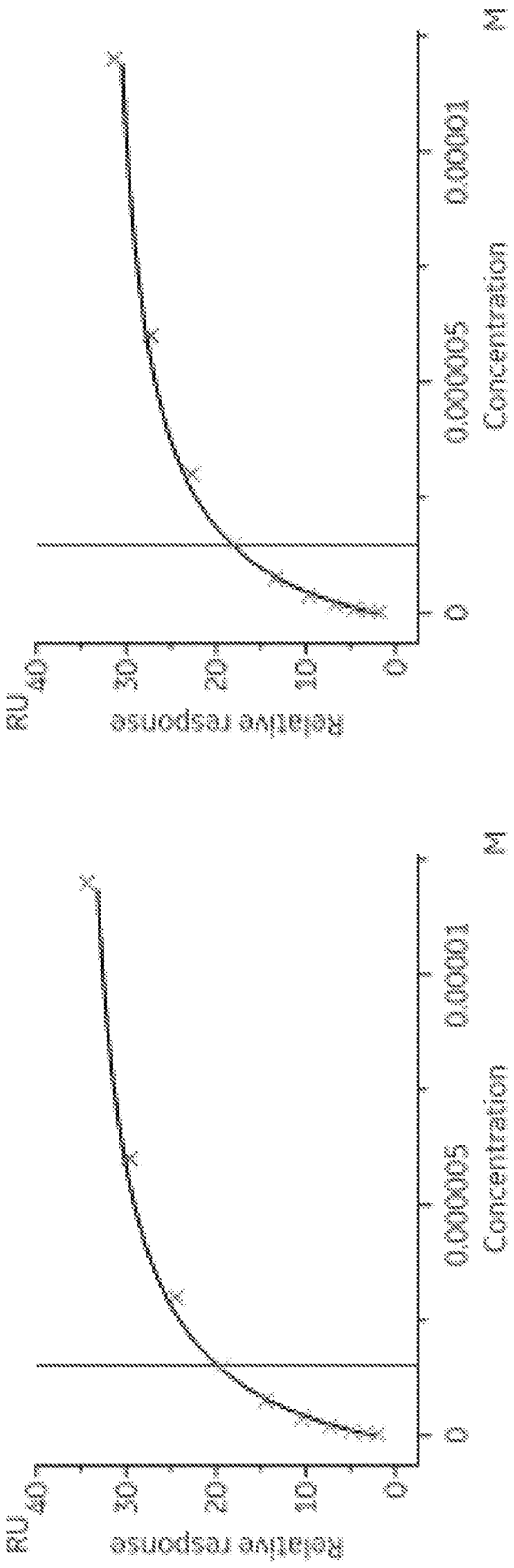
FIG. 245 Continued



AB0192

K_D = 1.5 ± 0.1 μM

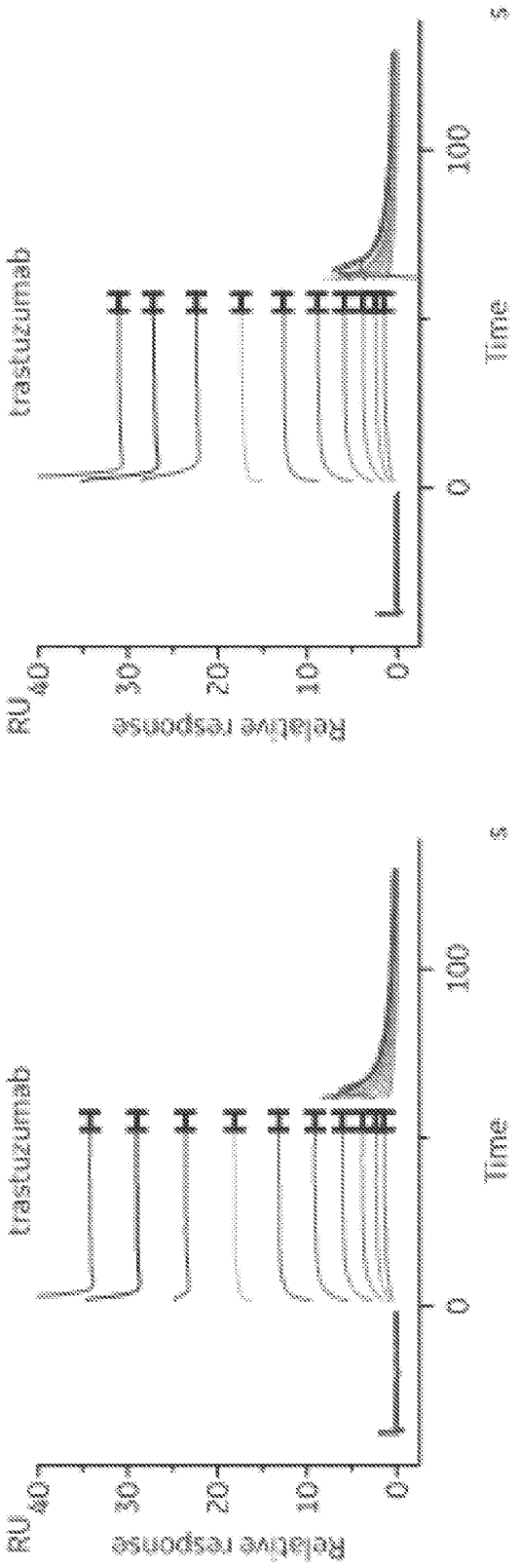
FIG. 245 Continued



AB0192

K_D = 1.5 ± 0.1 µM

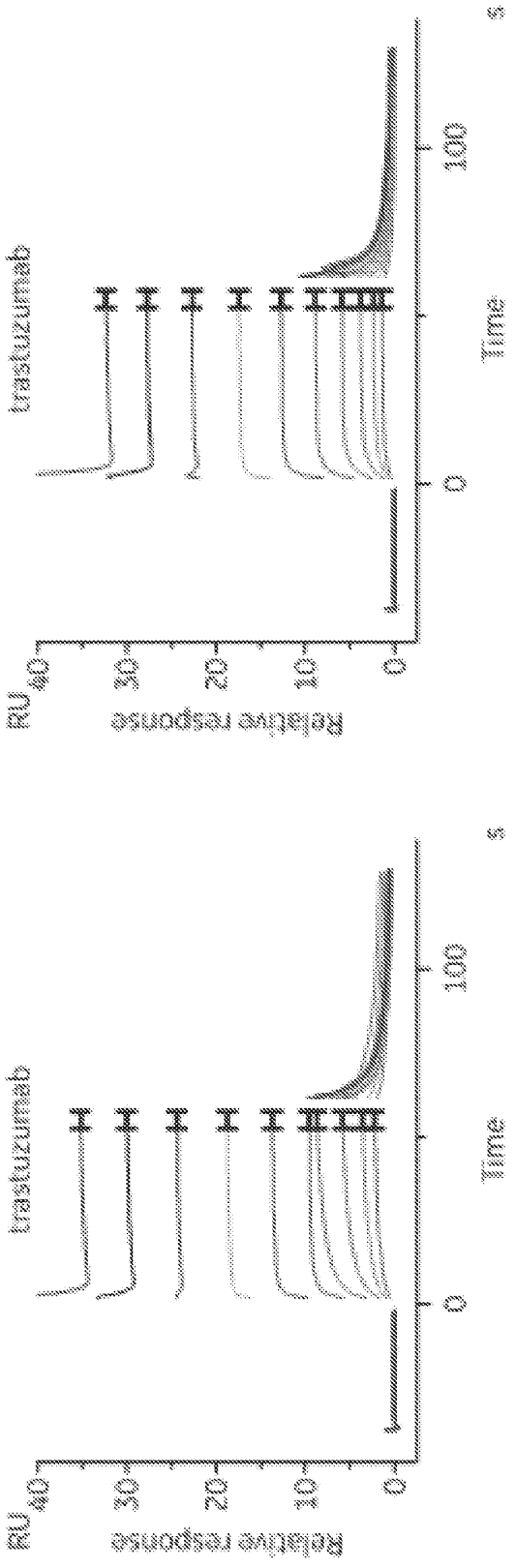
FIG. 245 Continued



trastuzumab

K_D = 1.7 ± 0.2 µM

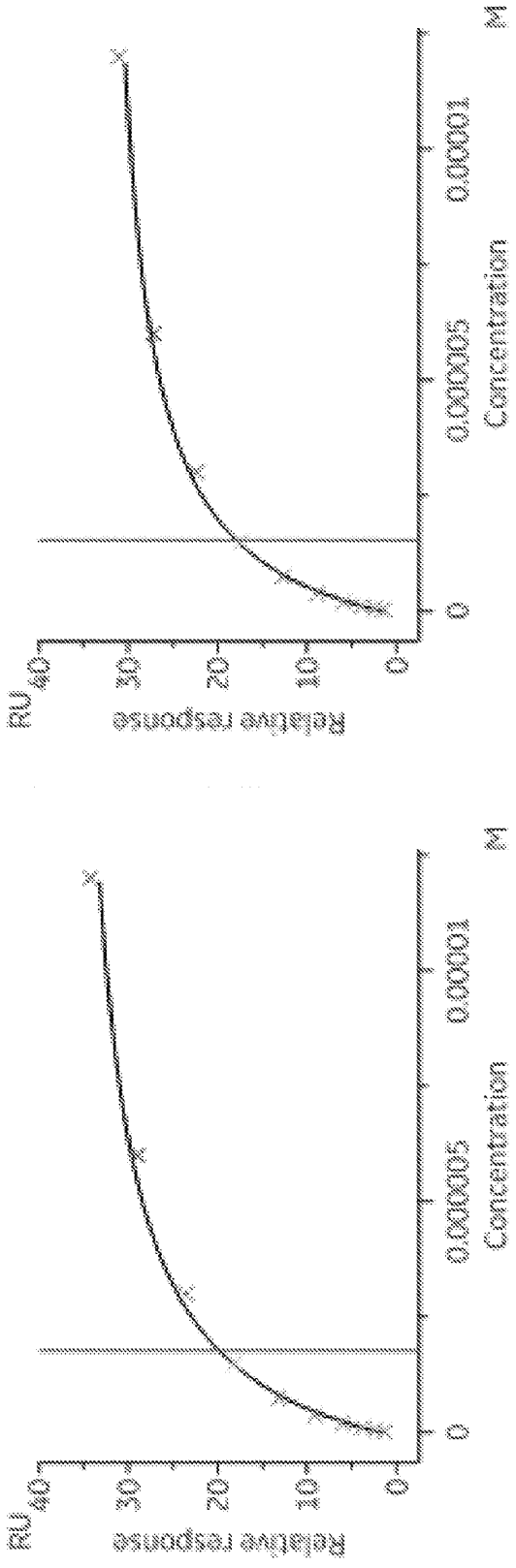
FIG. 245 Continued



trastuzumab

K_D = 1.7 ± 0.2 μM

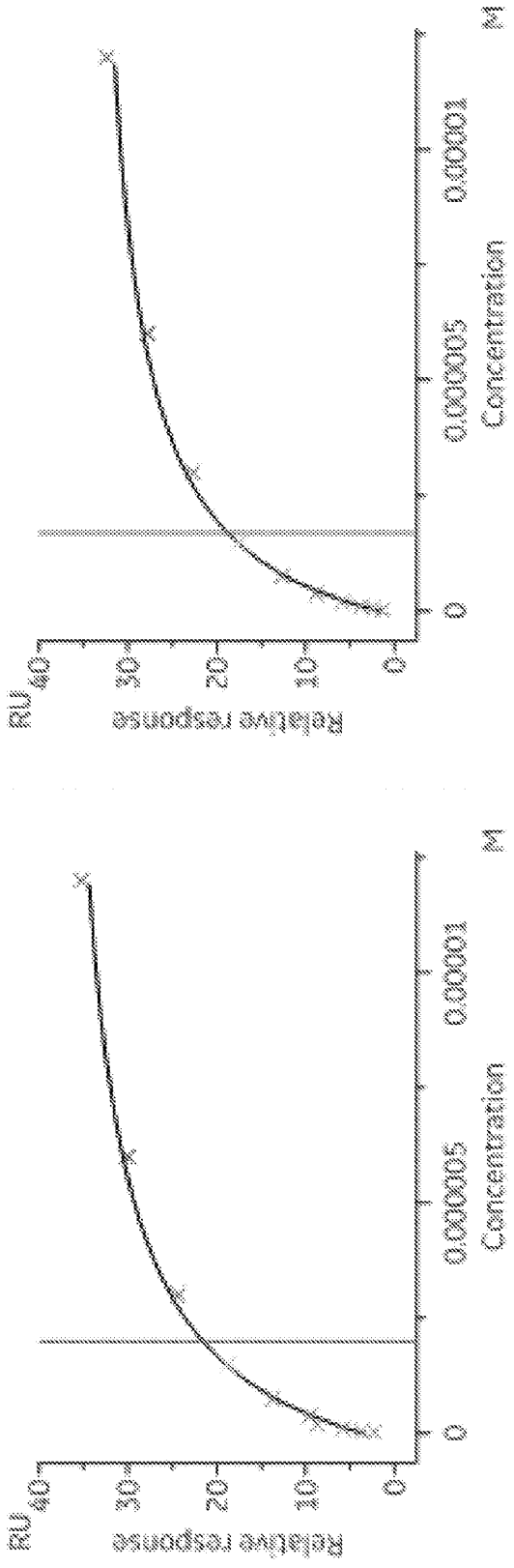
FIG. 245 Continued



trastuzumab

K_D = 1.7 ± 0.2 μM

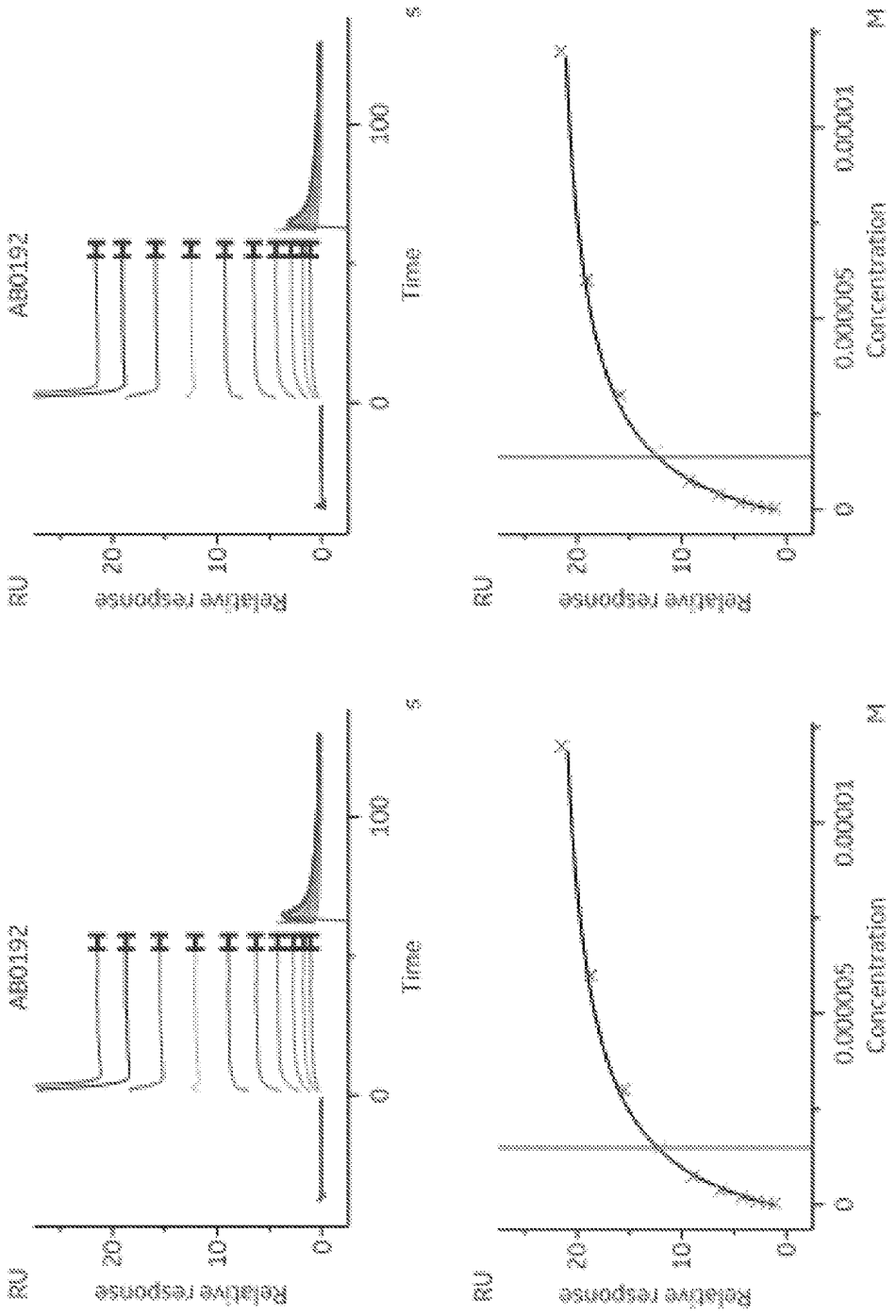
FIG. 245 Continued



trastuzumab

$K_D = 1.7 \pm 0.2$ μM

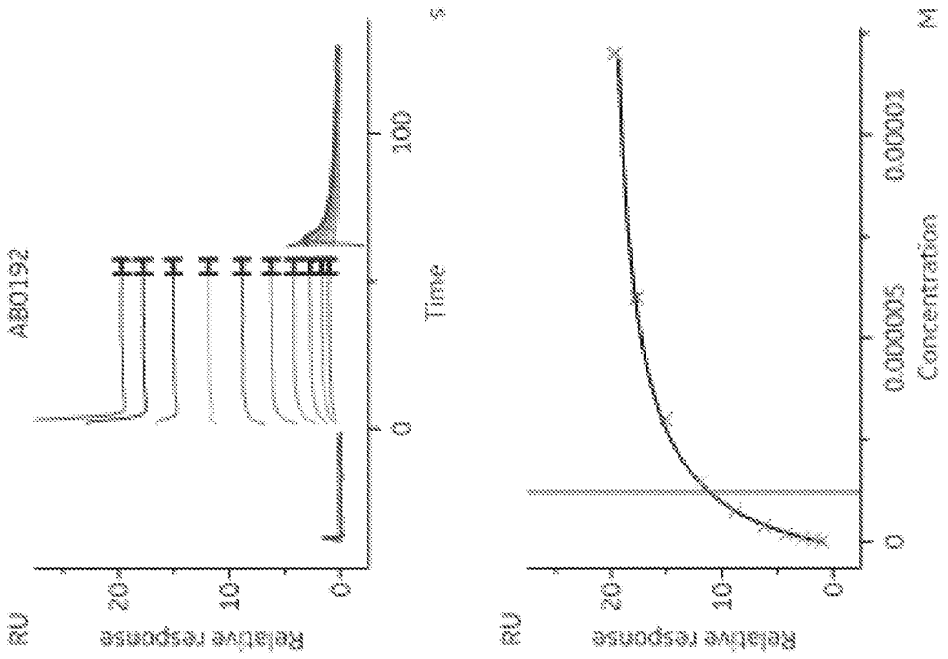
FIG. 245 Continued



AB0192

K_D = 1.4 ± 0.1 µM

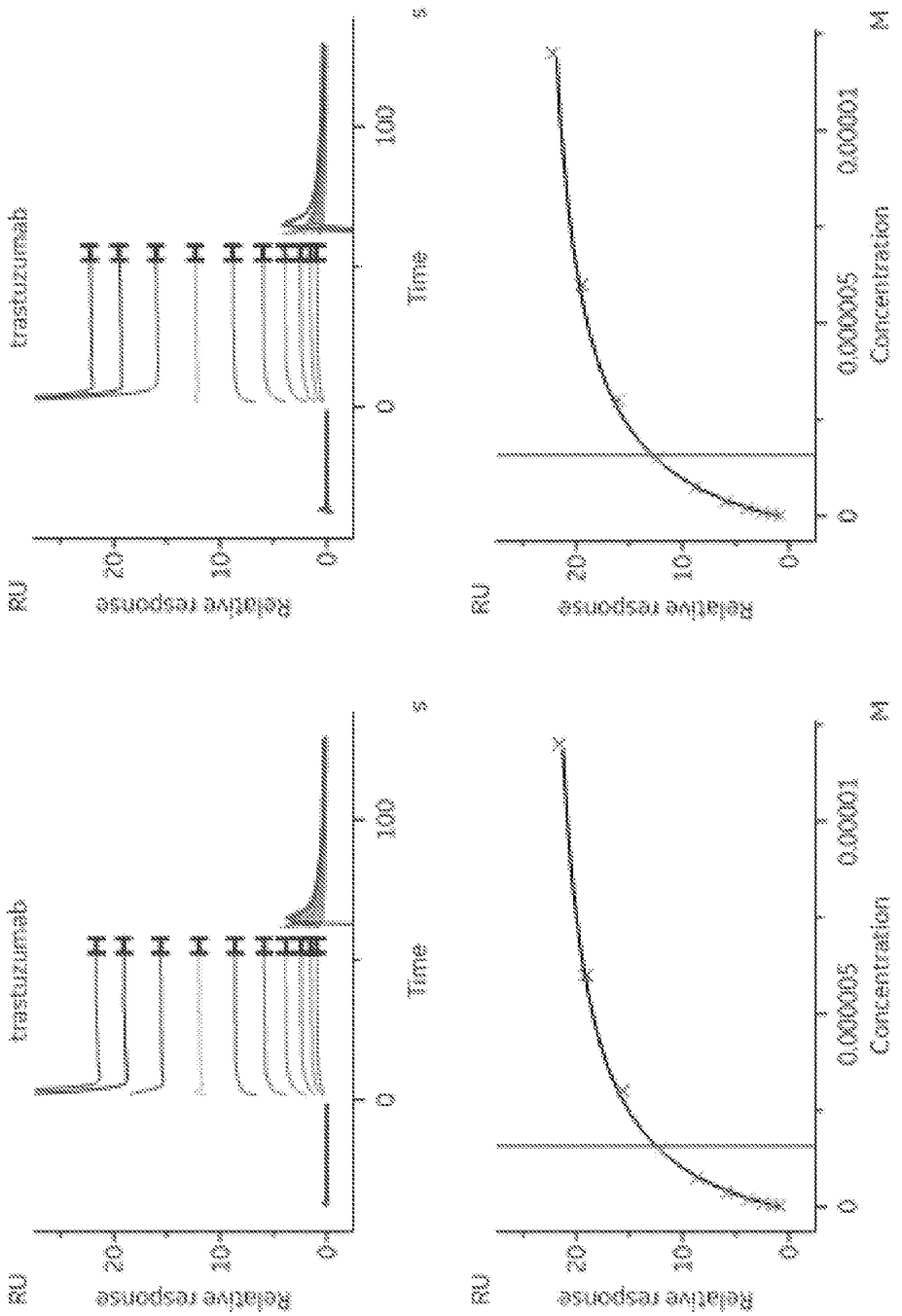
FIG. 246



AB0192

K_D = 1.4 ± 0.1 µM

FIG. 246 Continued



trastuzumab

K_D = 1.5 ± 0.1 µM

FIG. 246 Continued

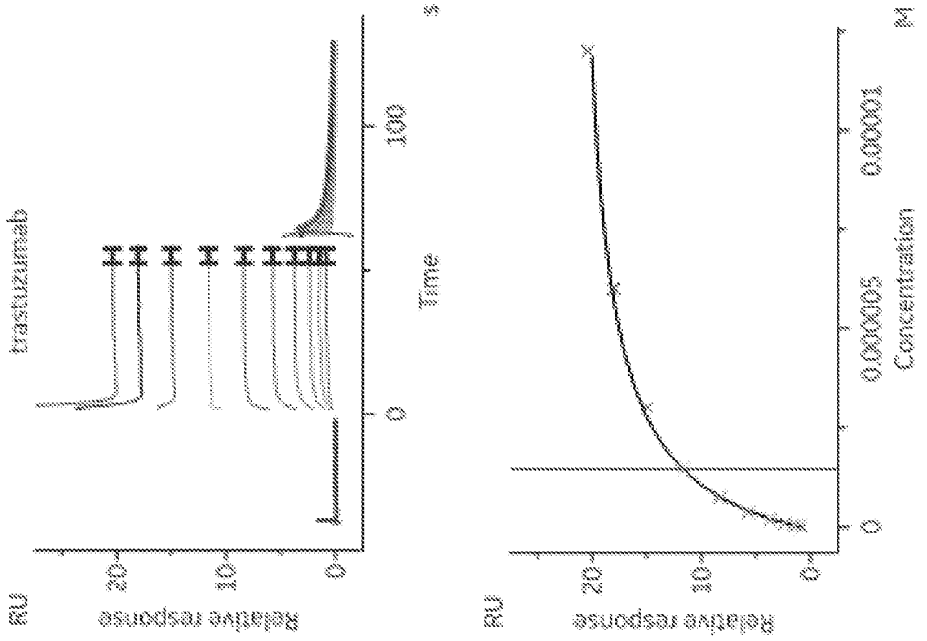


FIG. 246 Continued

FIG. 247

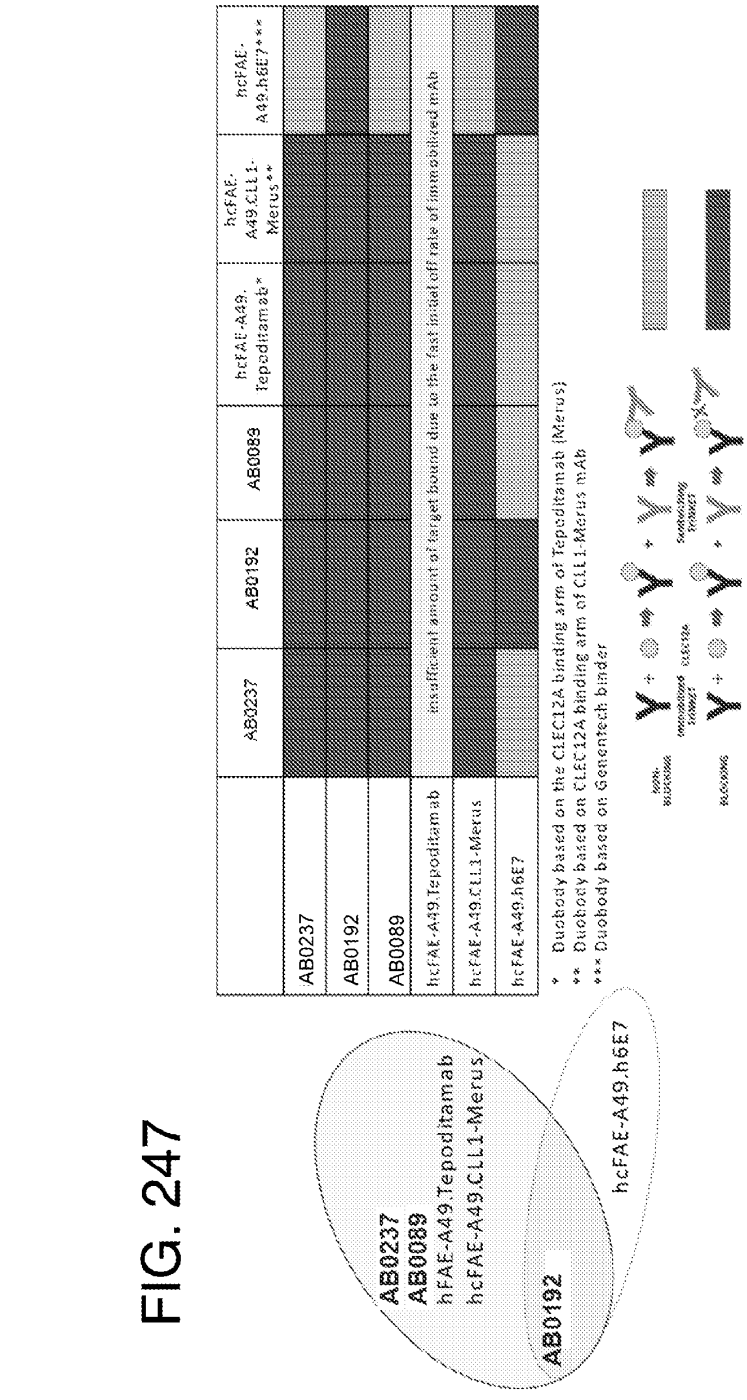

AB0237
AB0192
AB0089
hcFAE-A49.Tepoditamab*
hcFAE-A49.CLL1-Merus**
hcFAE-A49.h6E7***
AB0237
AB0192
AB0089
hcFAE-A49.Tepoditamab
hcFAE-A49.CLL1-Merus
hcFAE-A49.h6E7
insufficient amount of target bound due to the fast initial off rate of immobilized mAb
* Duobody based on the CLEC12A binding arm of Tepoditamab (Merus)
** Duobody based on CLEC12A binding arm of CLL1-Merus mAb
*** Duobody based on Genentech binder
AB0237
AB0089
hFAE-A49.Tepoditamab
hcFAE-A49.CLL1-Merus
AB0192
hcFAE-A49.h6E7

FIG. 248

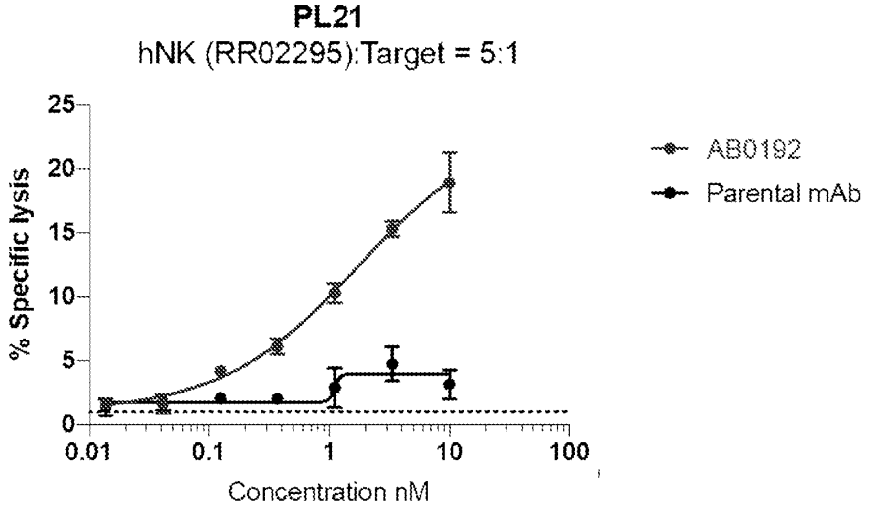
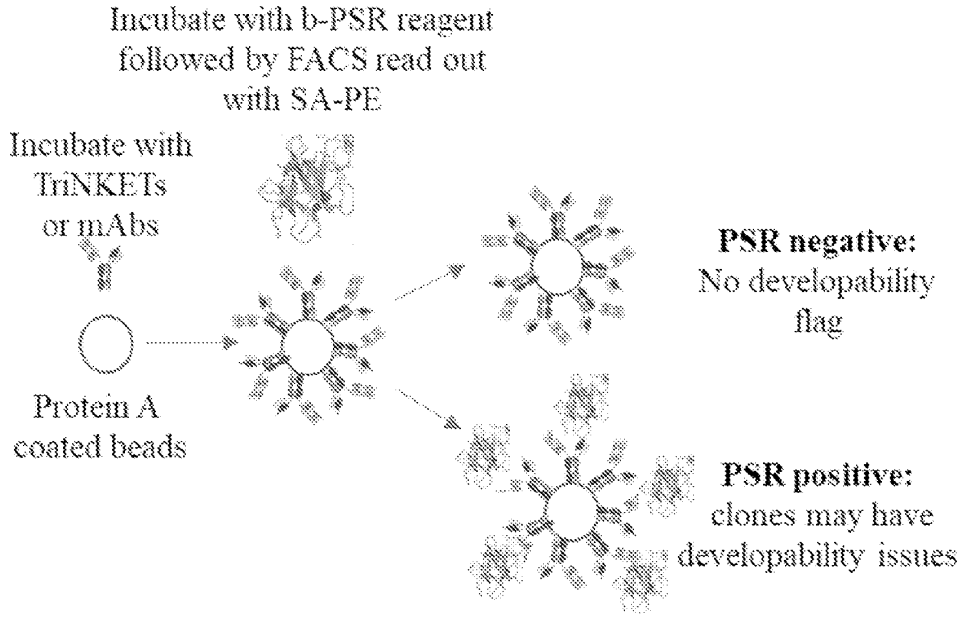


FIG. 249A



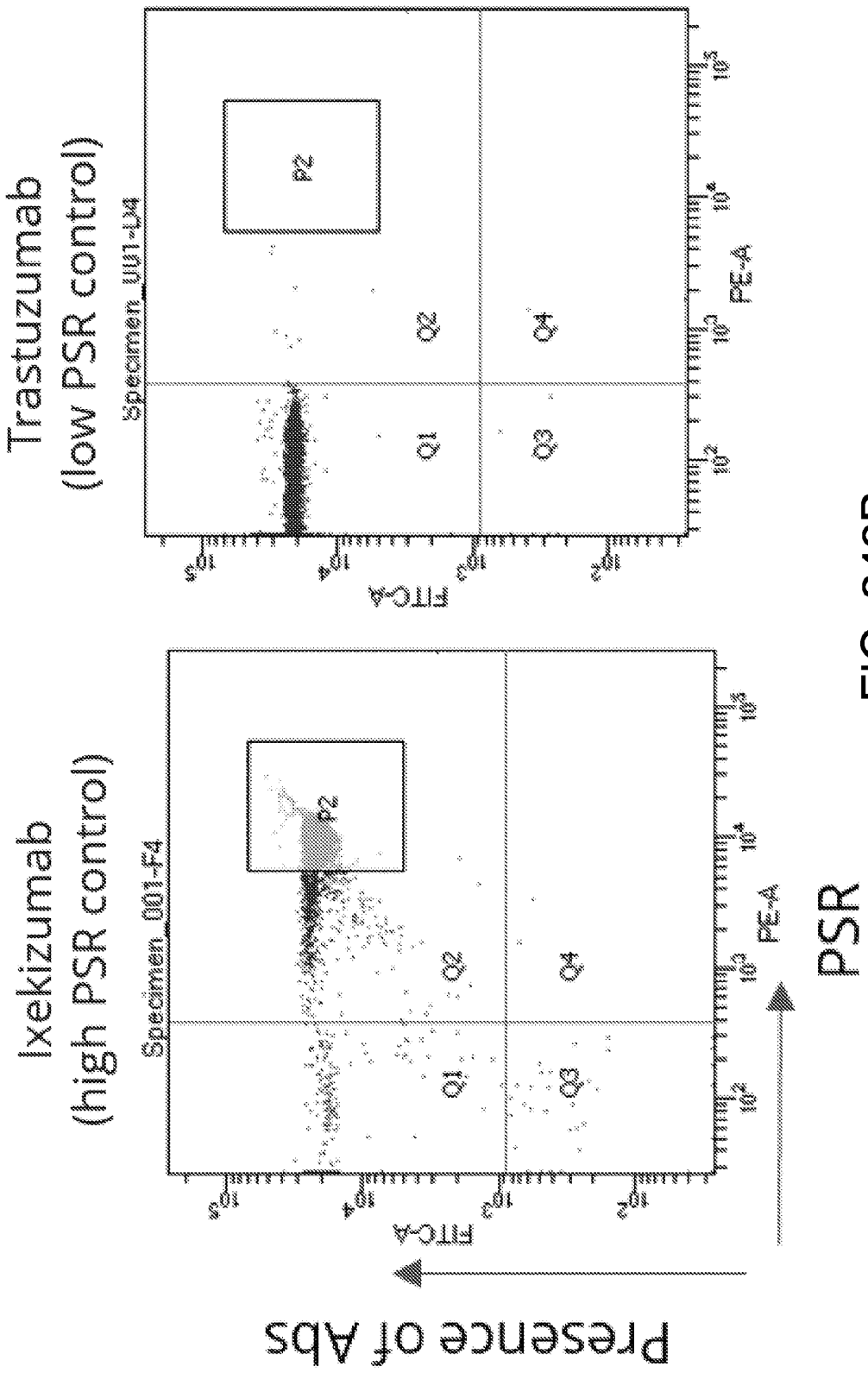

Trastuzumab
(low PSR control)
Specimen_UU1-LX4
P2
Q1
Q2
Q3
Q4
FITC-A
PE-A
Ixekizumab
(high PSR control)
Specimen_001-F4
P2
Q1
Q2
Q3
Q4
FITC-A
PE-A
Presence of Abs
PSR

FIG. 249B

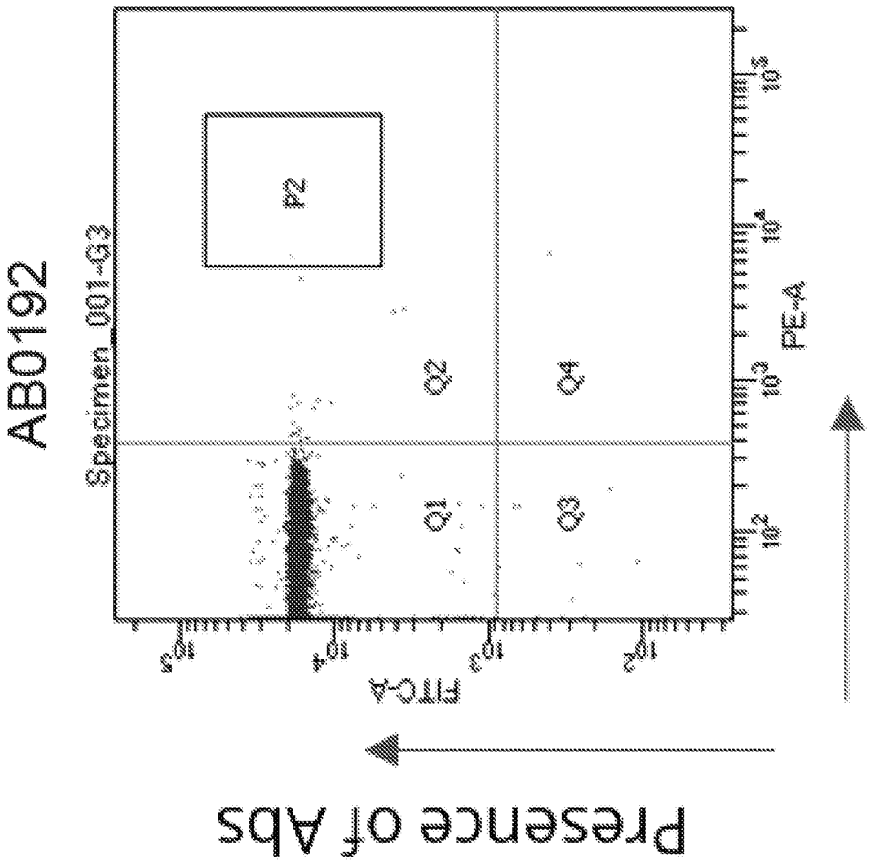
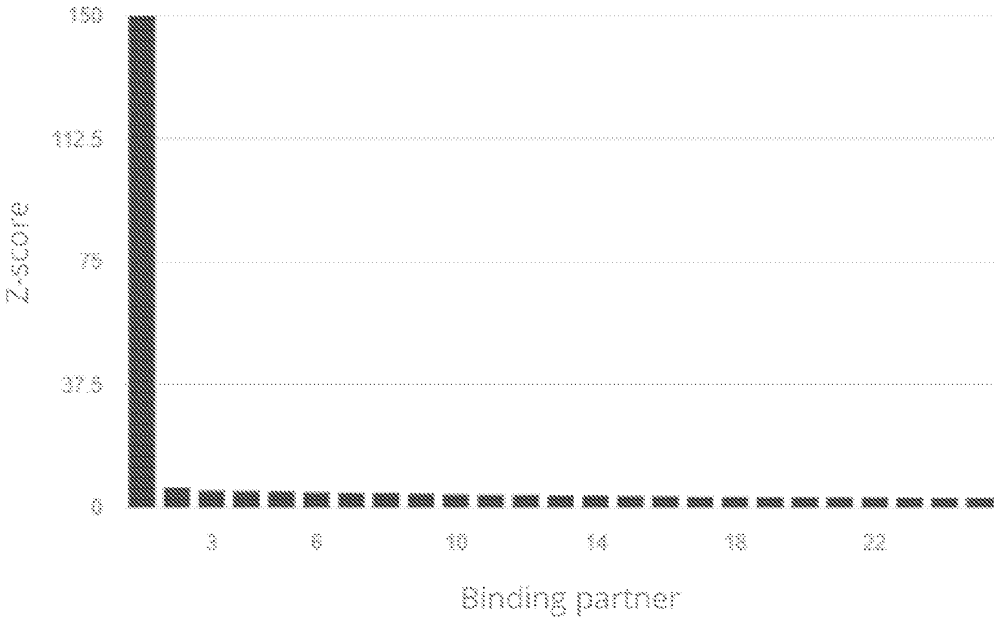


FIG. 249B Continued

FIG. 250



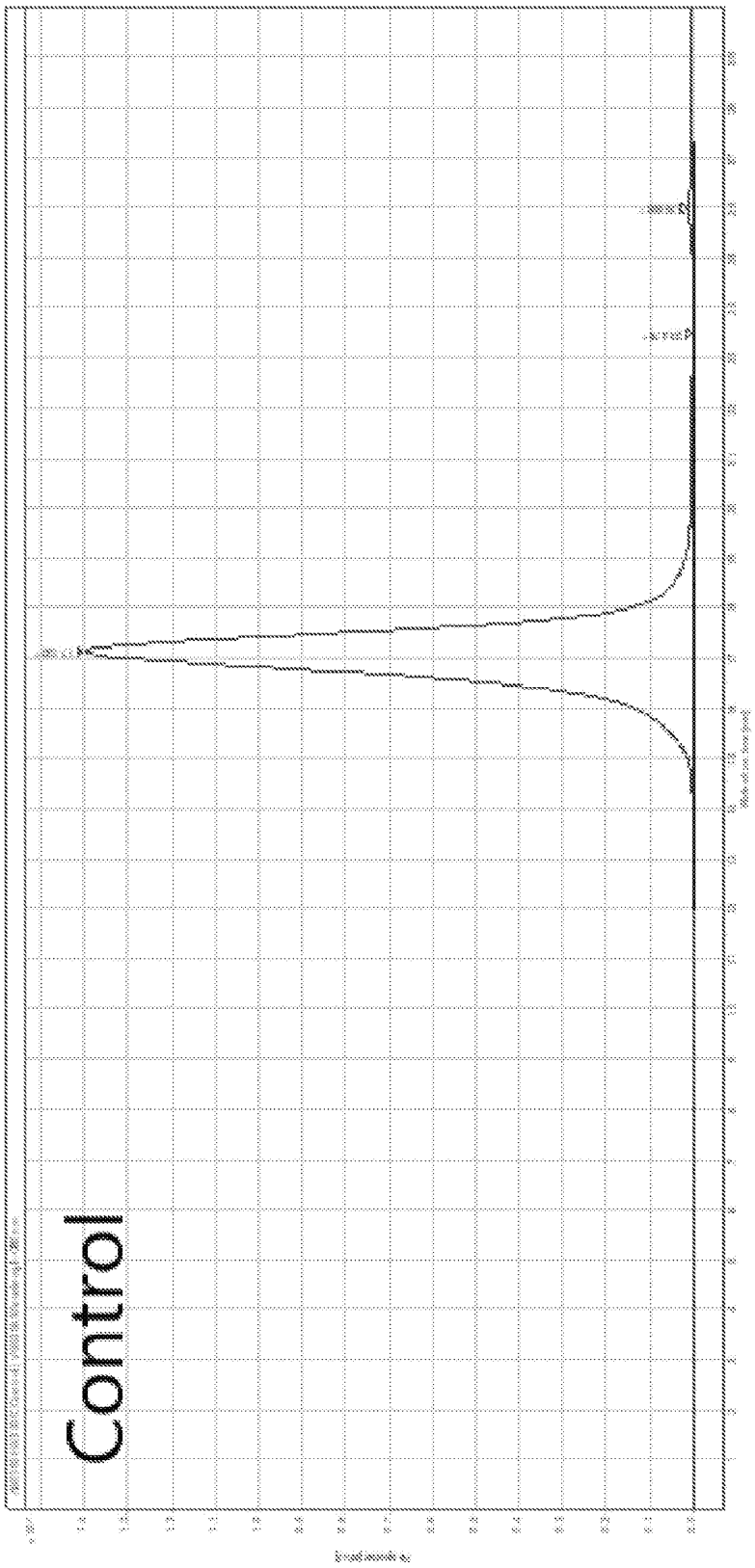


FIG. 251

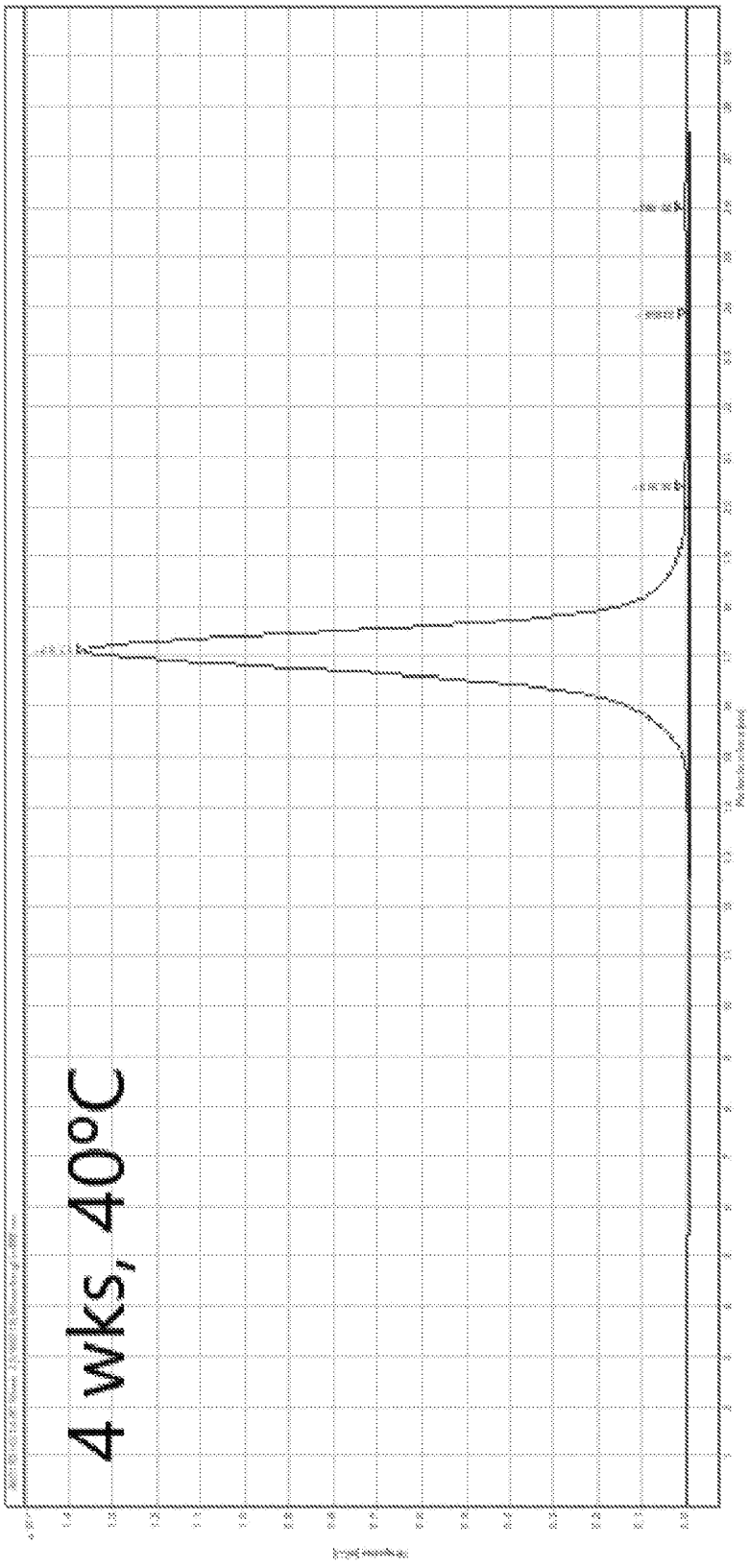


FIG. 251 Continued

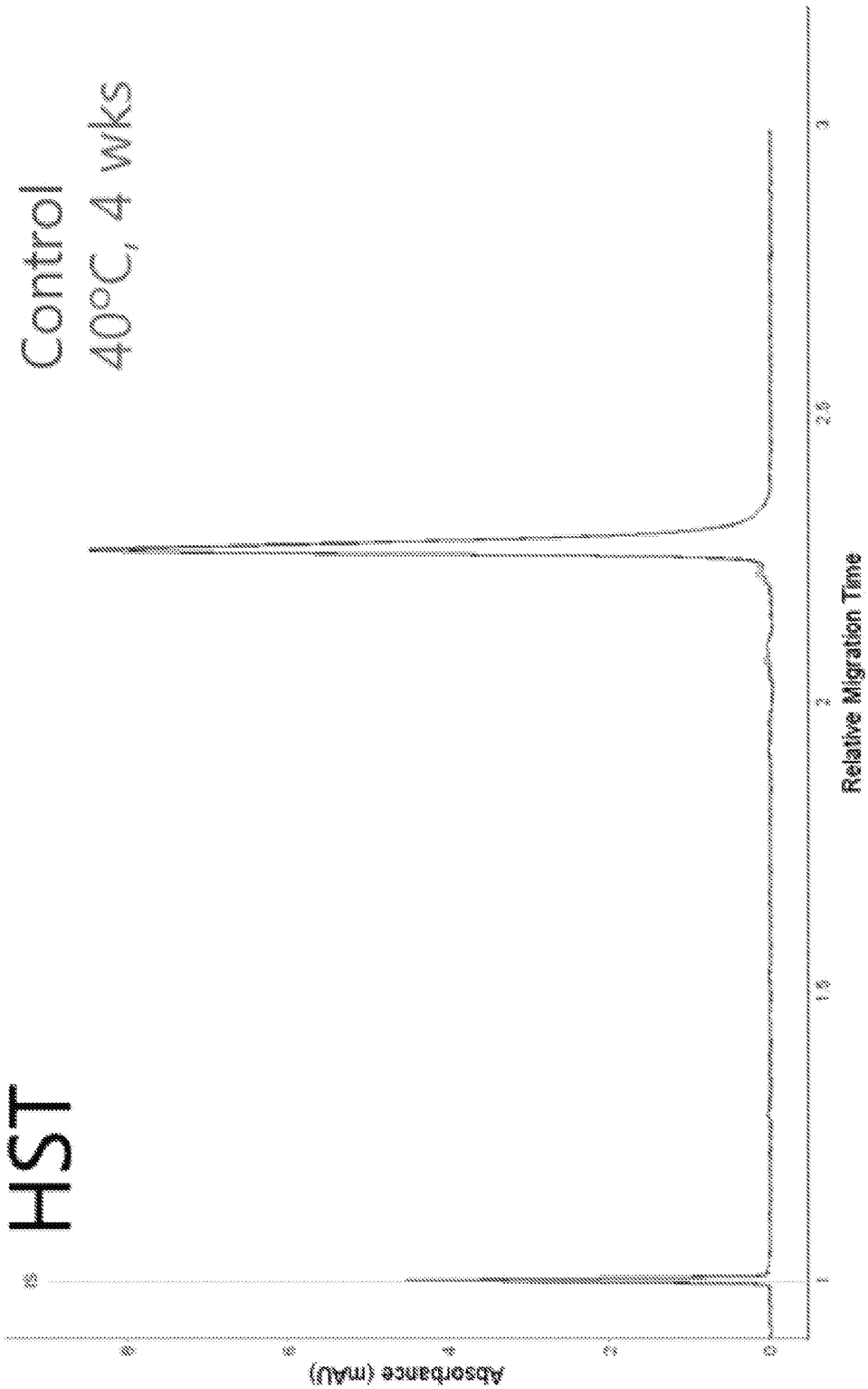

HST
Control
40°C, 4 wks
Absorbance (mAU)
Relative Migration Time

FIG. 252

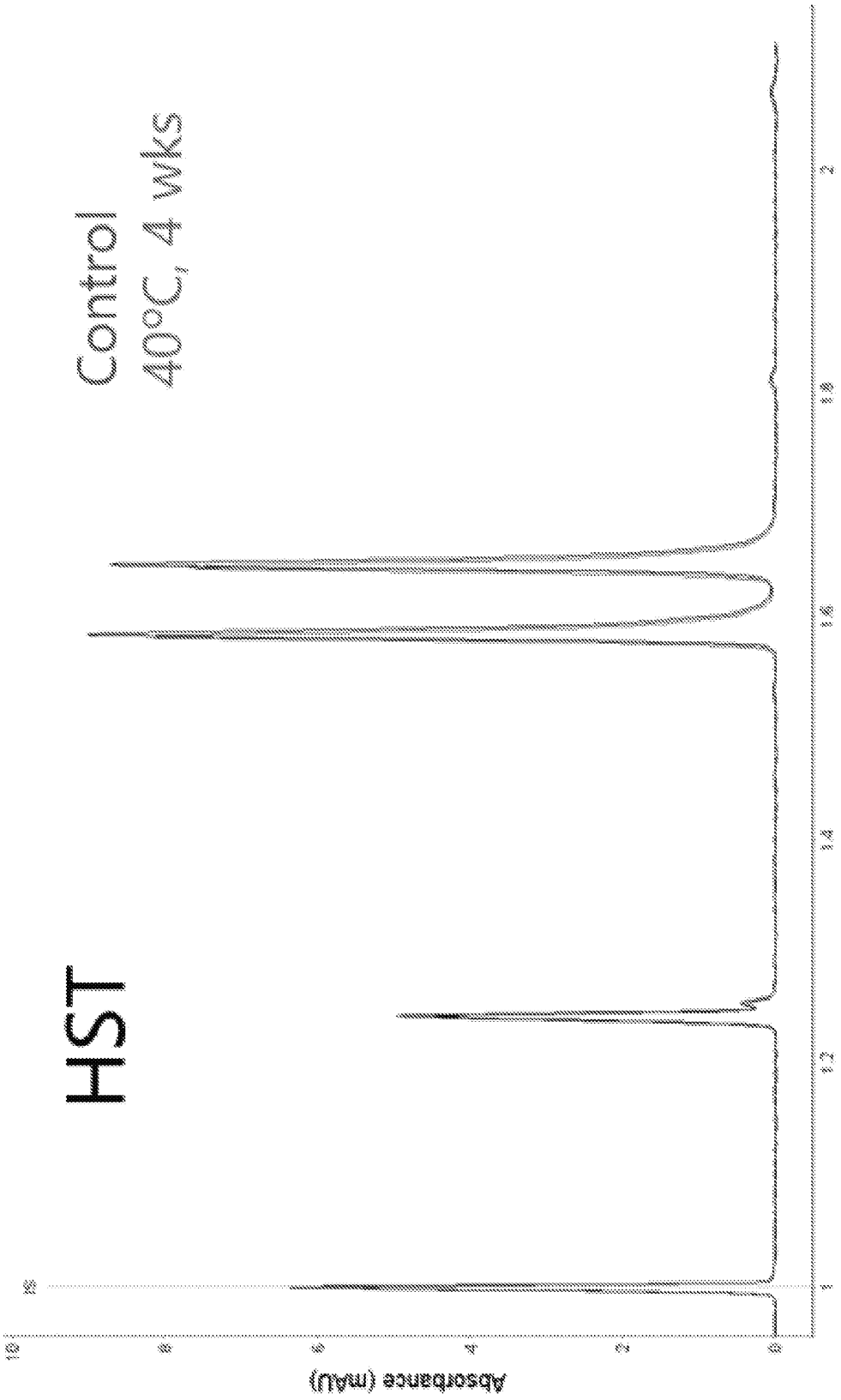
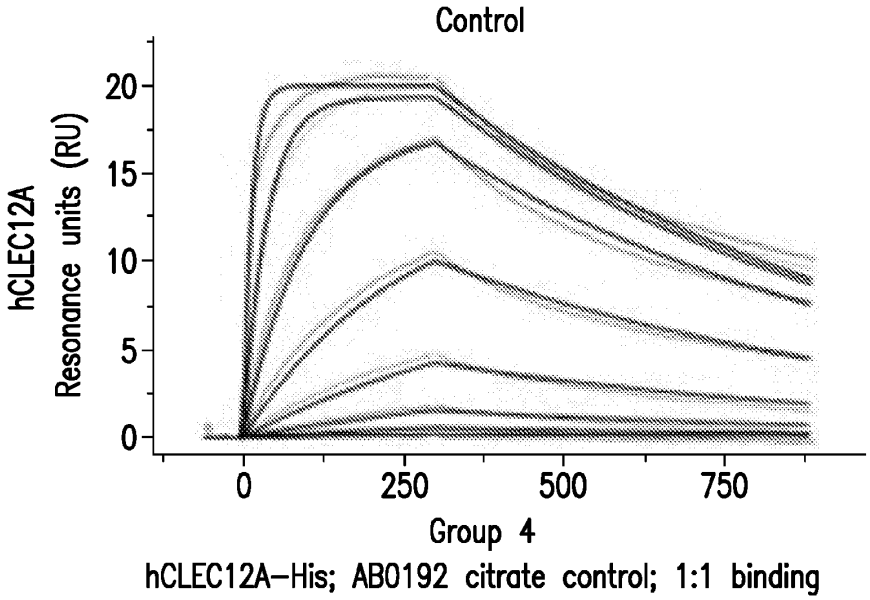


FIG. 252 Continued


Control
hCLEC12A
Resonance units (RU)
20
15
10
5
0
0
250
500
750
Group 4
hCLEC12A–His; AB0192 citrate control; 1:1 binding

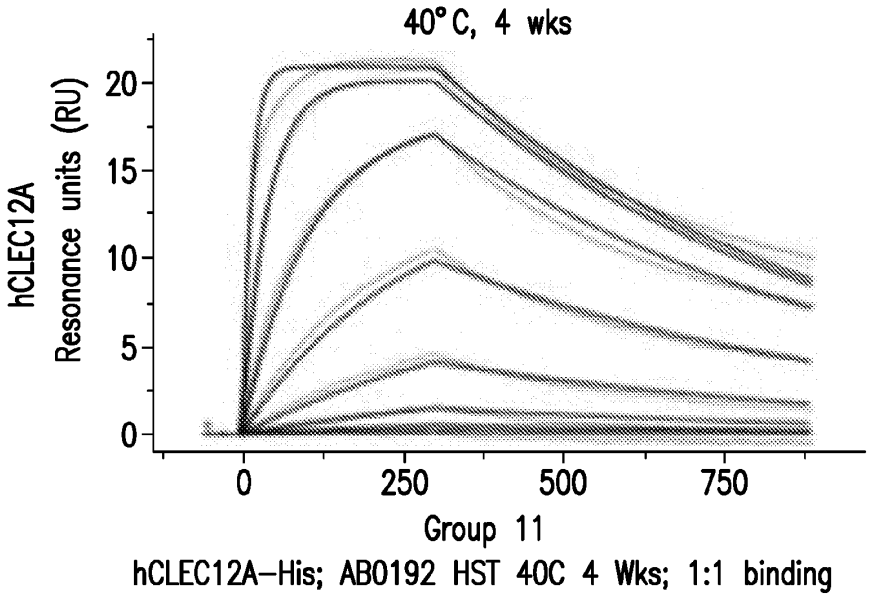
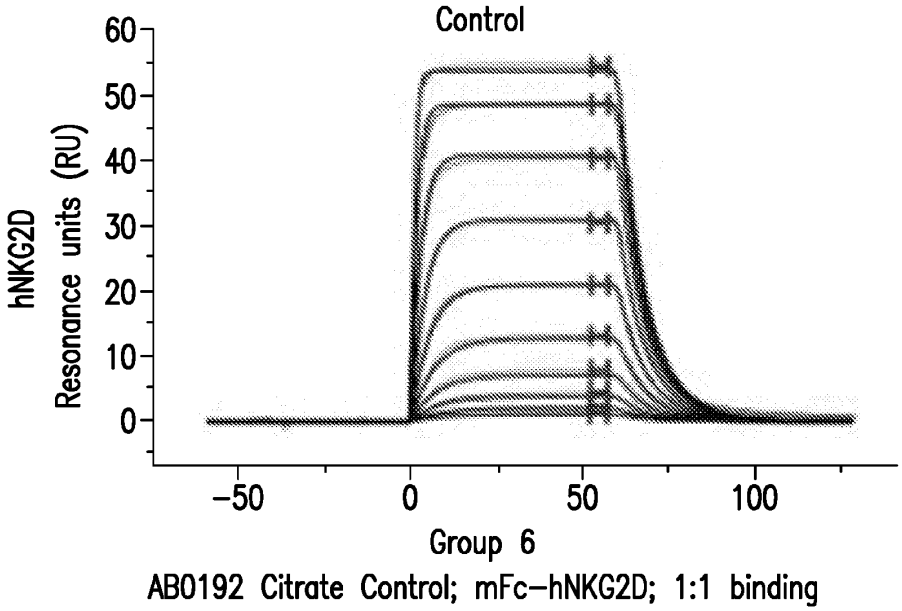

40° C, 4 wks
hCLEC12A
Resonance units (RU)
20
15
10
5
0
0
250
500
750
Group 11
hCLEC12A–His; AB0192 HST 40C 4 Wks; 1:1 binding

FIG. 253



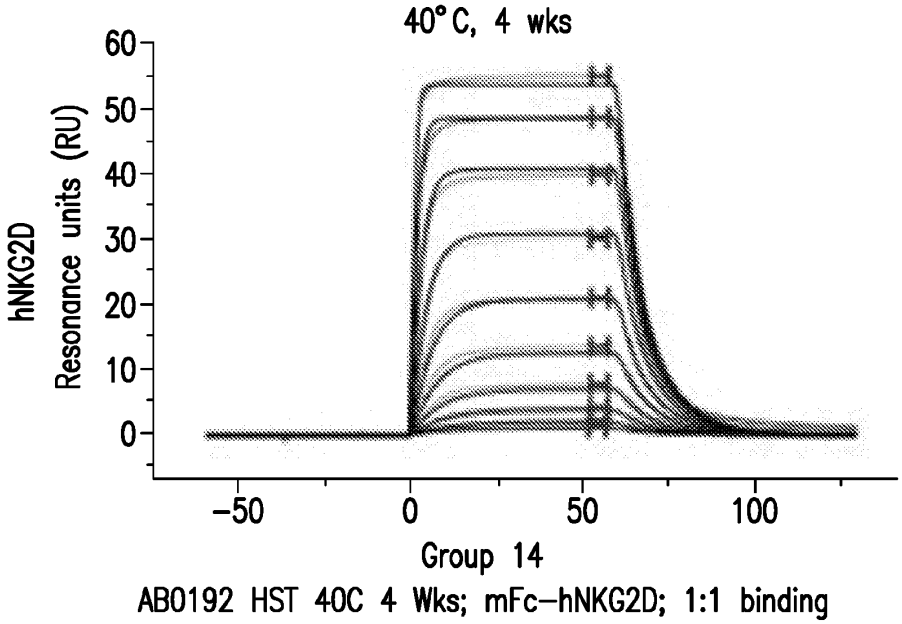
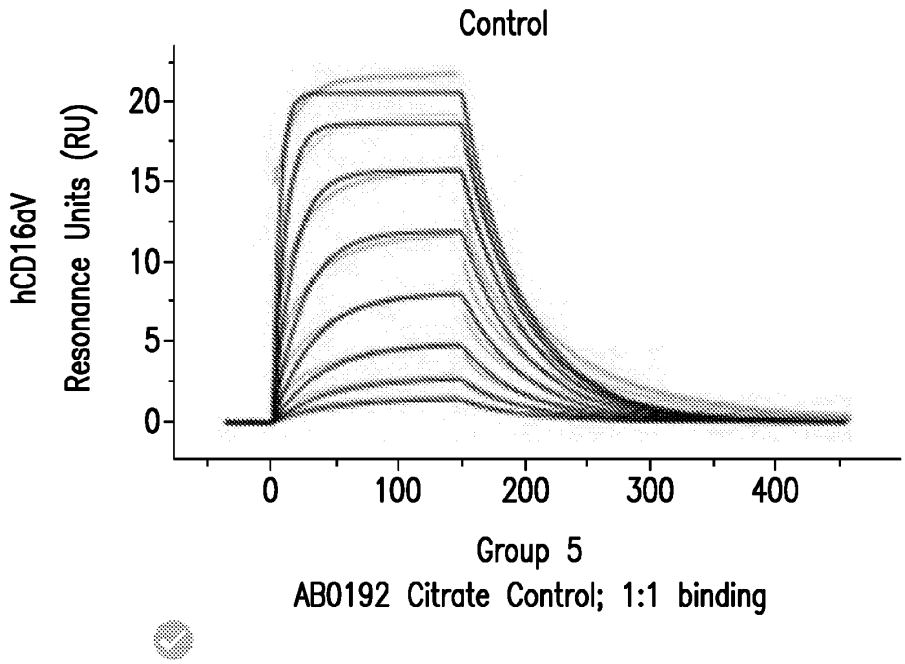


FIG. 253 Continued



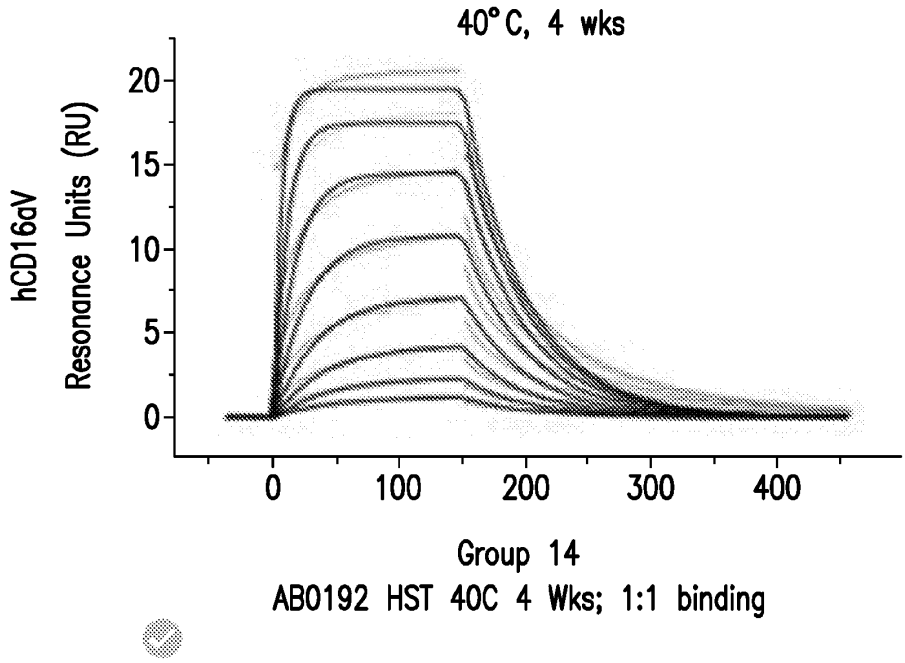
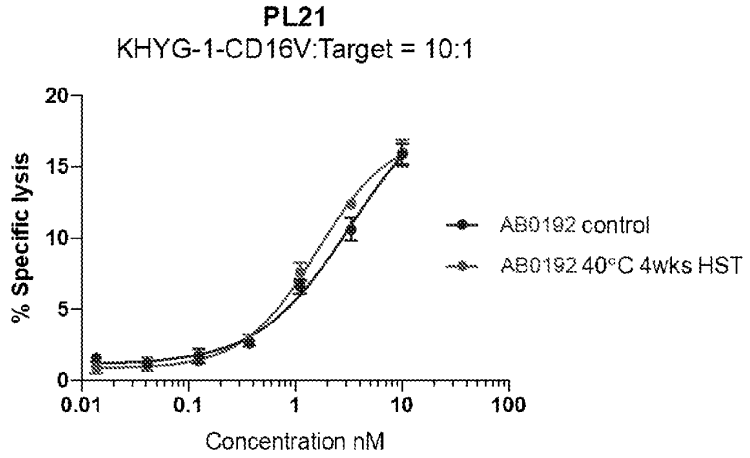


FIG. 253 Continued

FIG. 254


PL21
KHYG-1-CD16V:Target = 10:1
% Specific lysis
20
15
10
5
0
0.01
0.1
1
10
100
Concentration nM
AB0192 control
AB0192 40°C 4wks HST

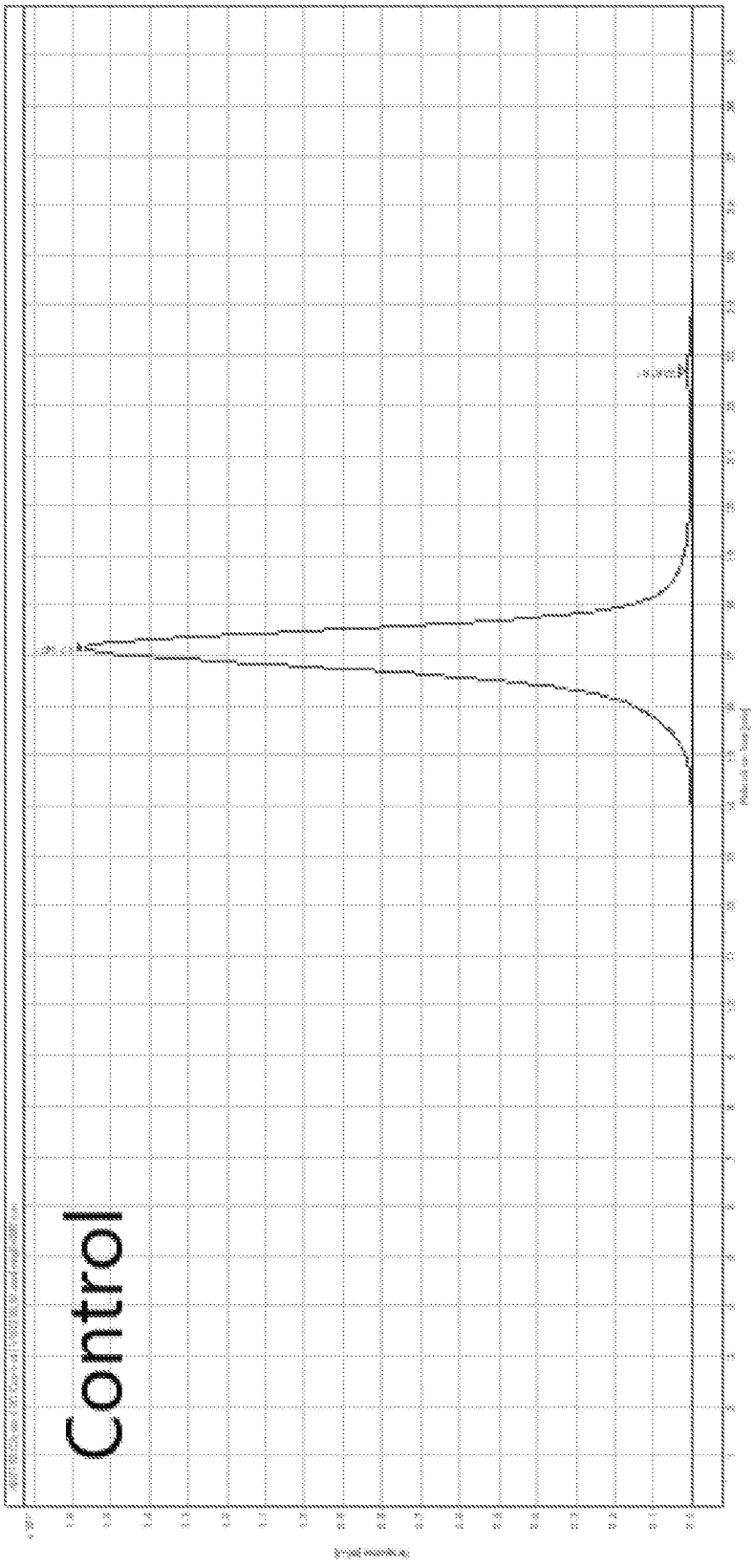


FIG. 255

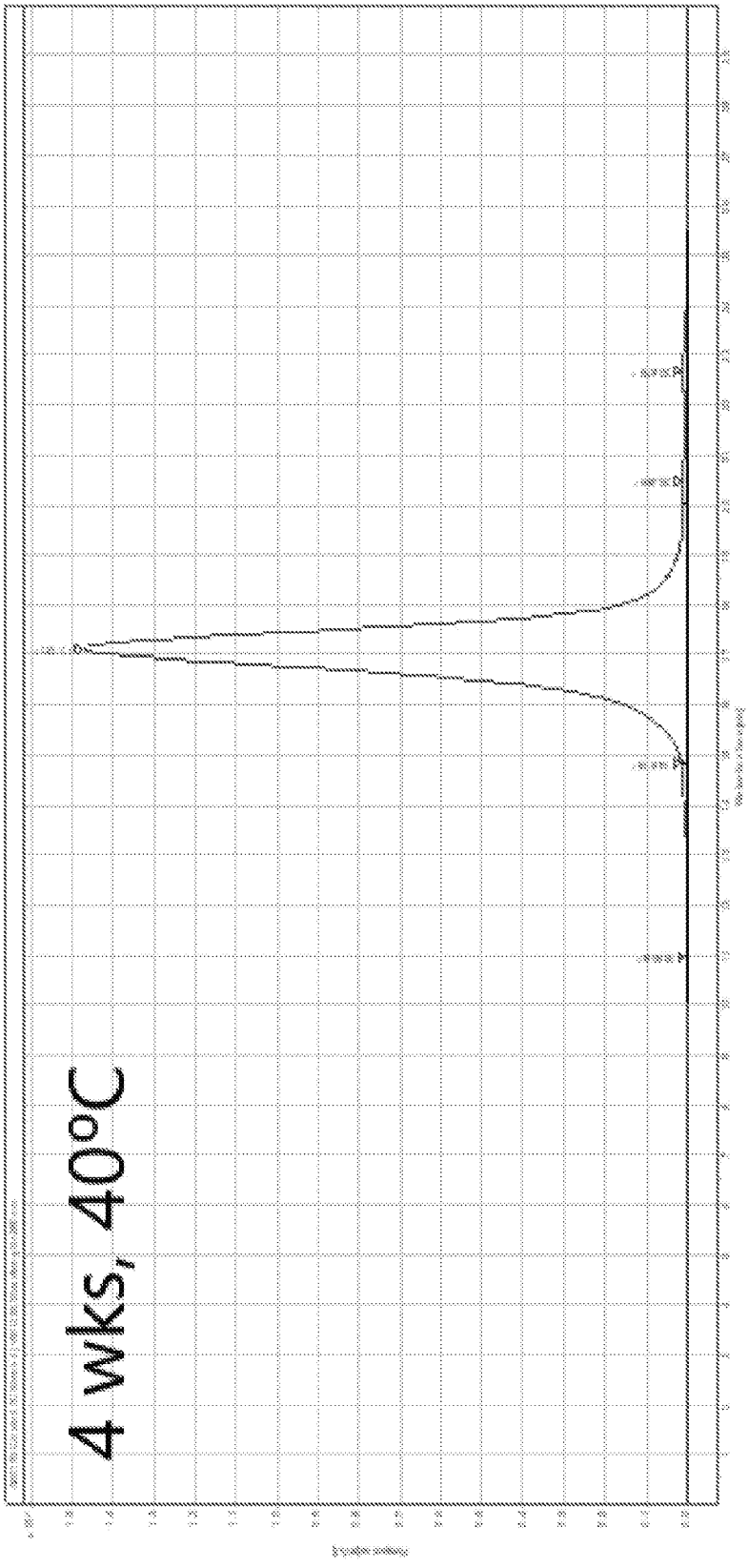


FIG. 255 Continued

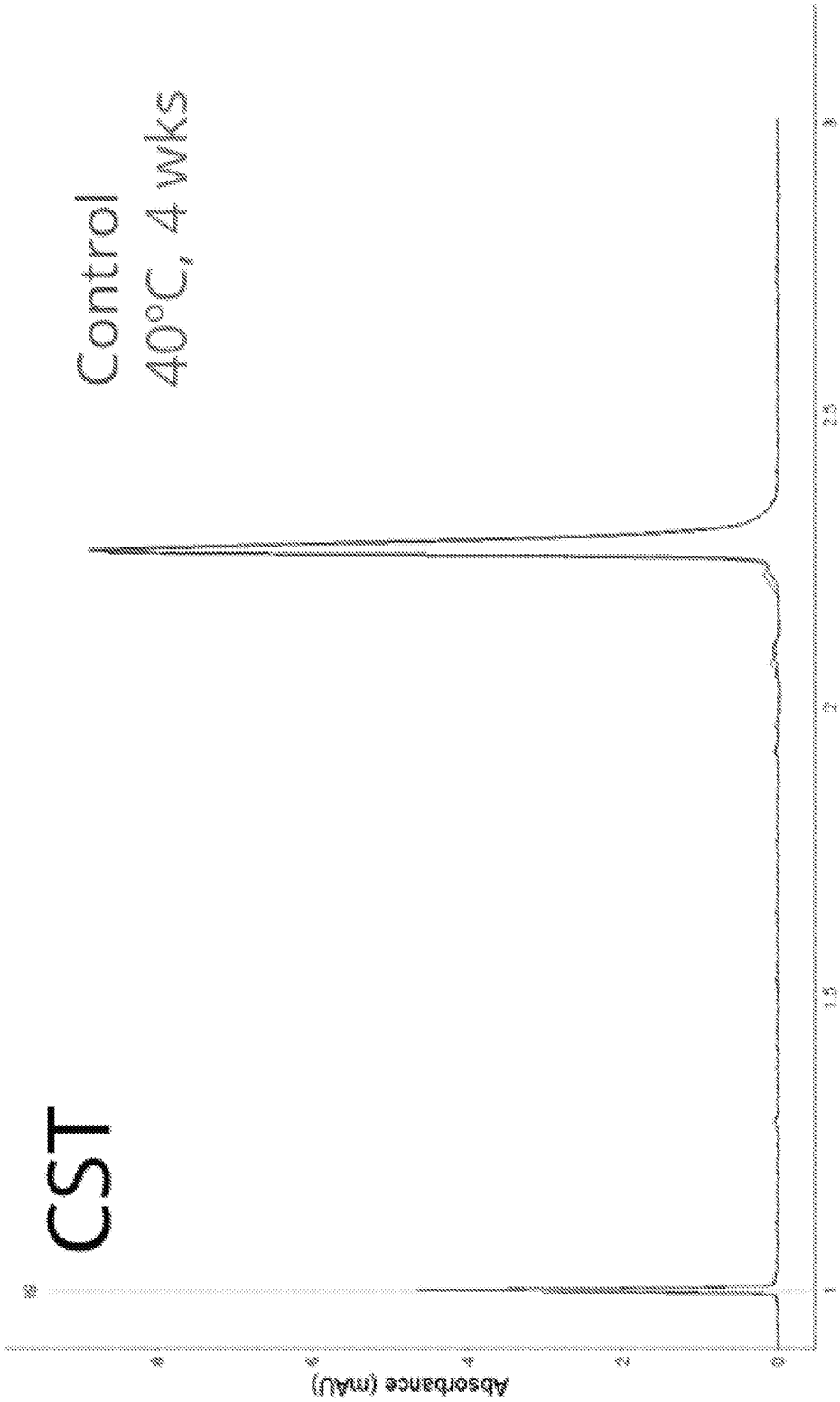

CST
Control
40°C, 4 wks
Absorbance (mAU)
Relative Migration Time

FIG. 256

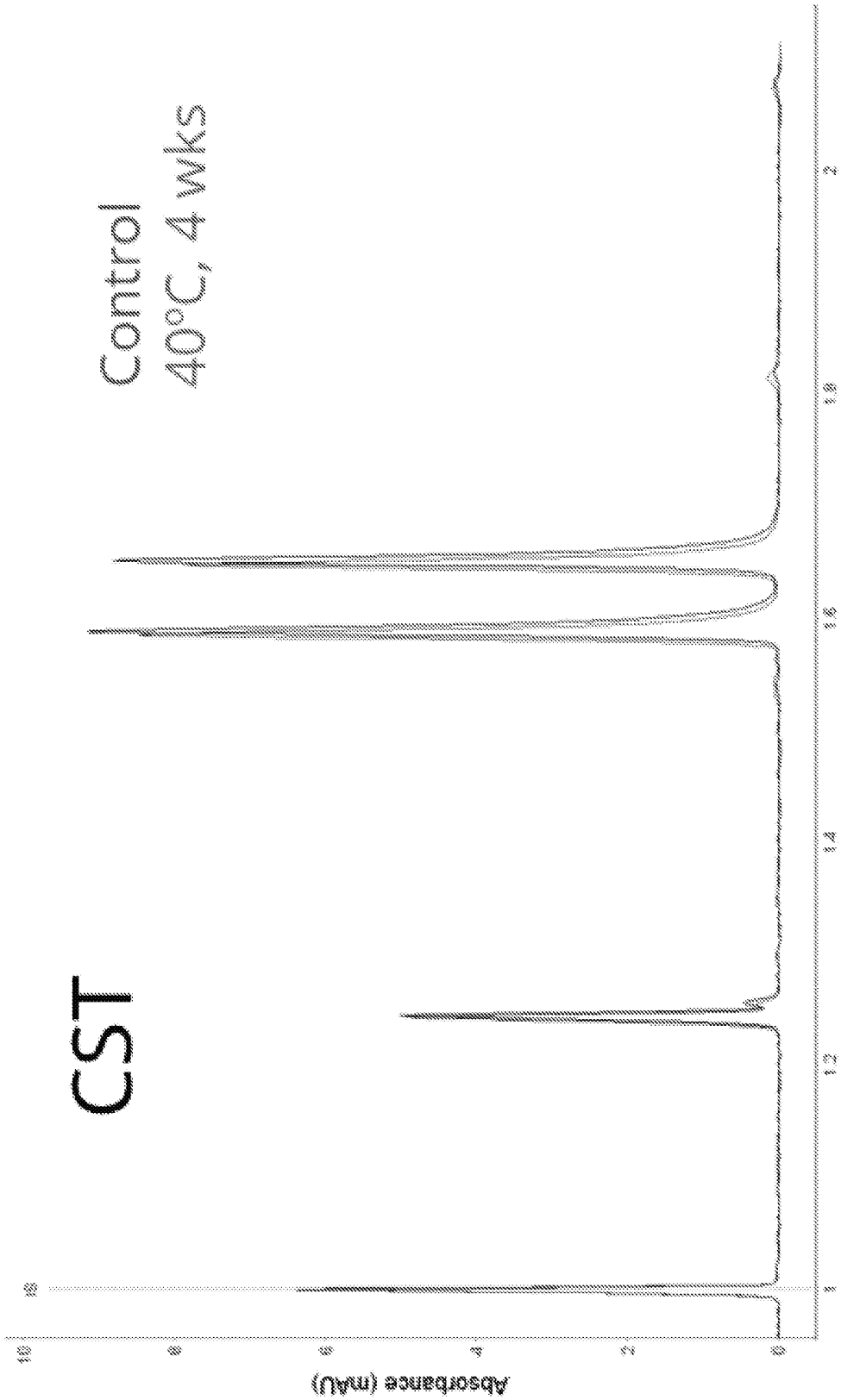


FIG. 256 Continued

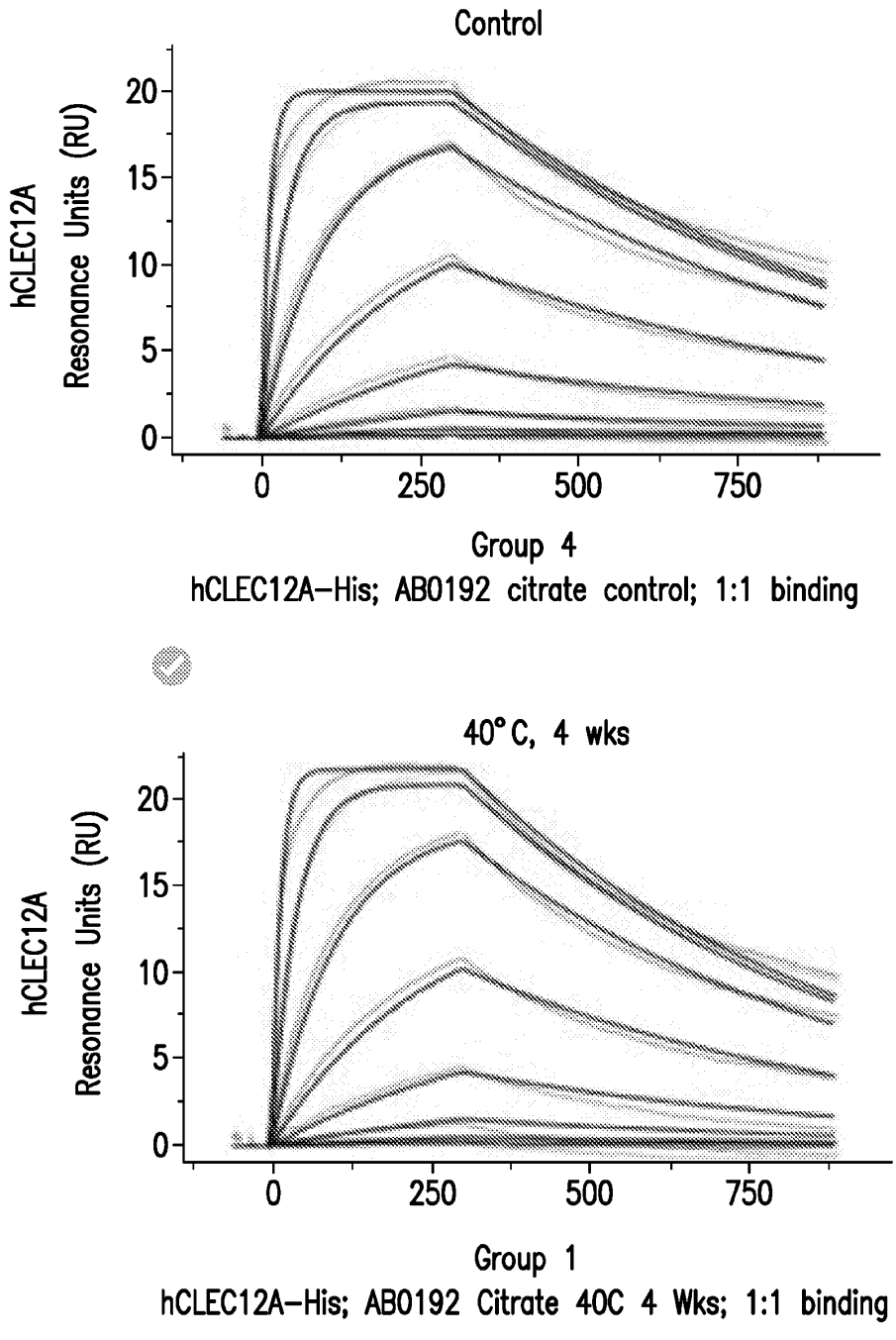
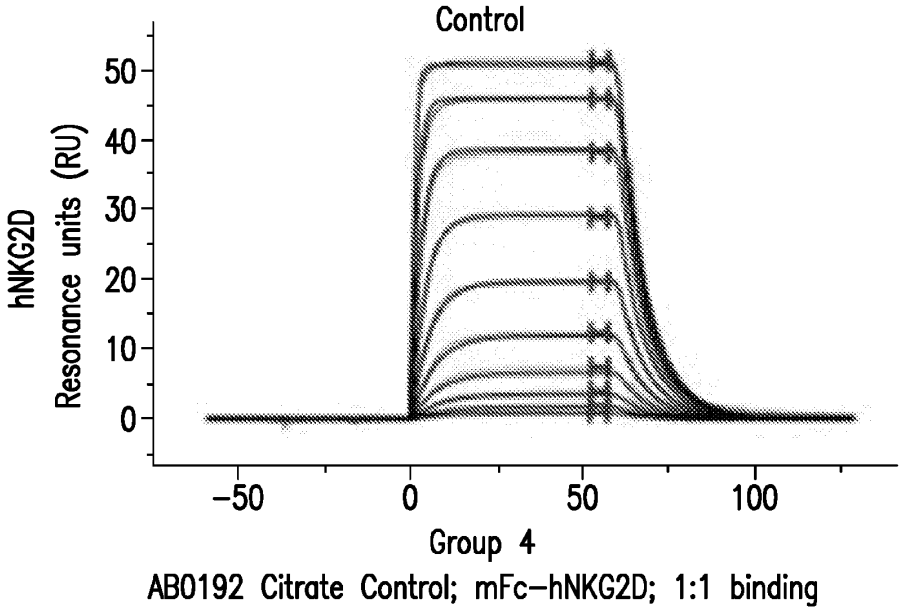

Control
hCLEC12A
Resonance Units (RU)
20
15
10
5
0
0
250
500
750
Group 4
hCLEC12A-His; AB0192 citrate control; 1:1 binding
40° C, 4 wks
hCLEC12A
Resonance Units (RU)
20
15
10
5
0
0
250
500
750
Group 1
hCLEC12A-His; AB0192 Citrate 40C 4 Wks; 1:1 binding

FIG. 257



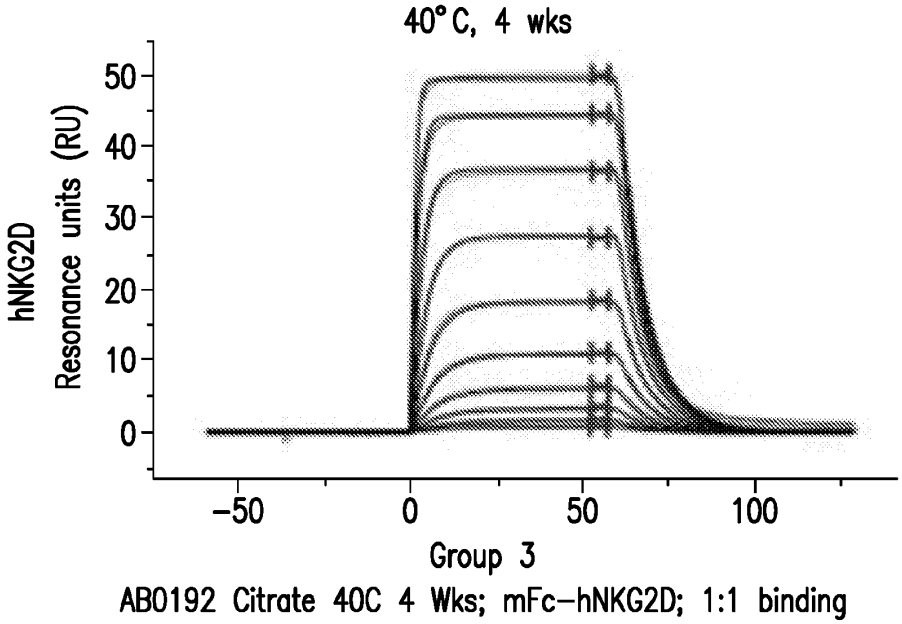
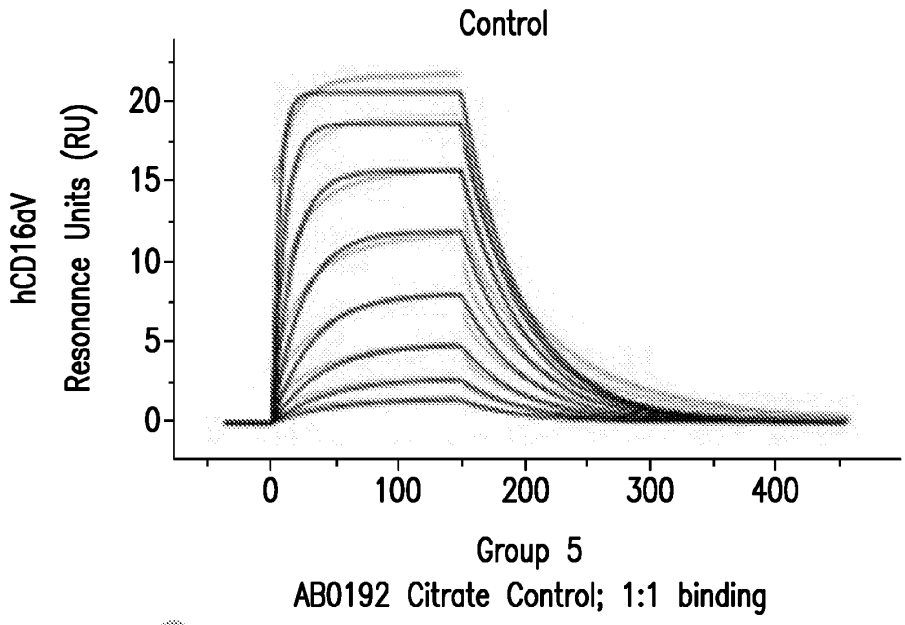


FIG. 257 Continued



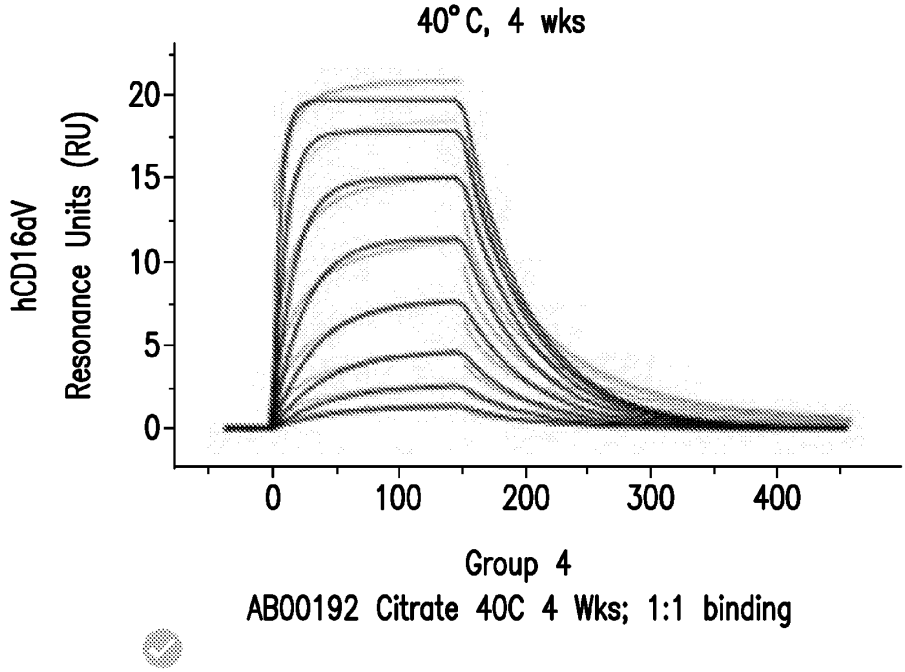
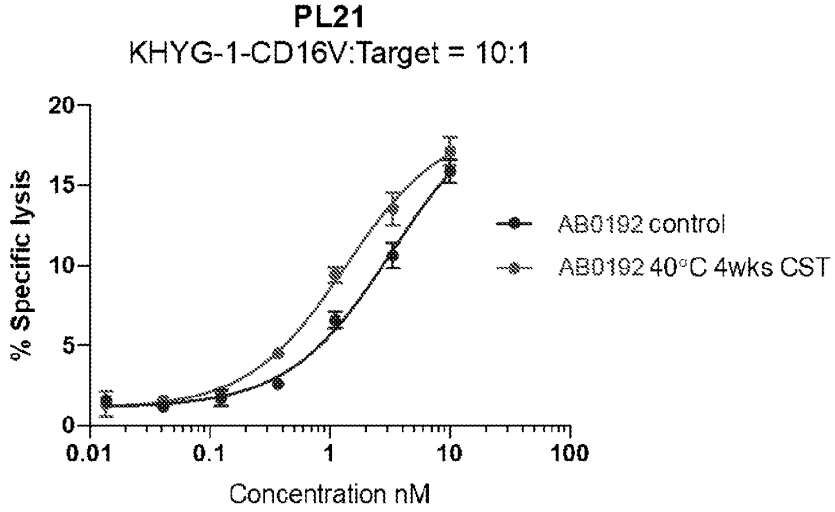


FIG. 257 Continued

FIG. 258


PL21
KHYG-1-CD16V:Target = 10:1
% Specific lysis
20
15
10
5
0
0.01
0.1
1
10
100
Concentration nM
AB0192 control
AB0192 40°C 4wks CST

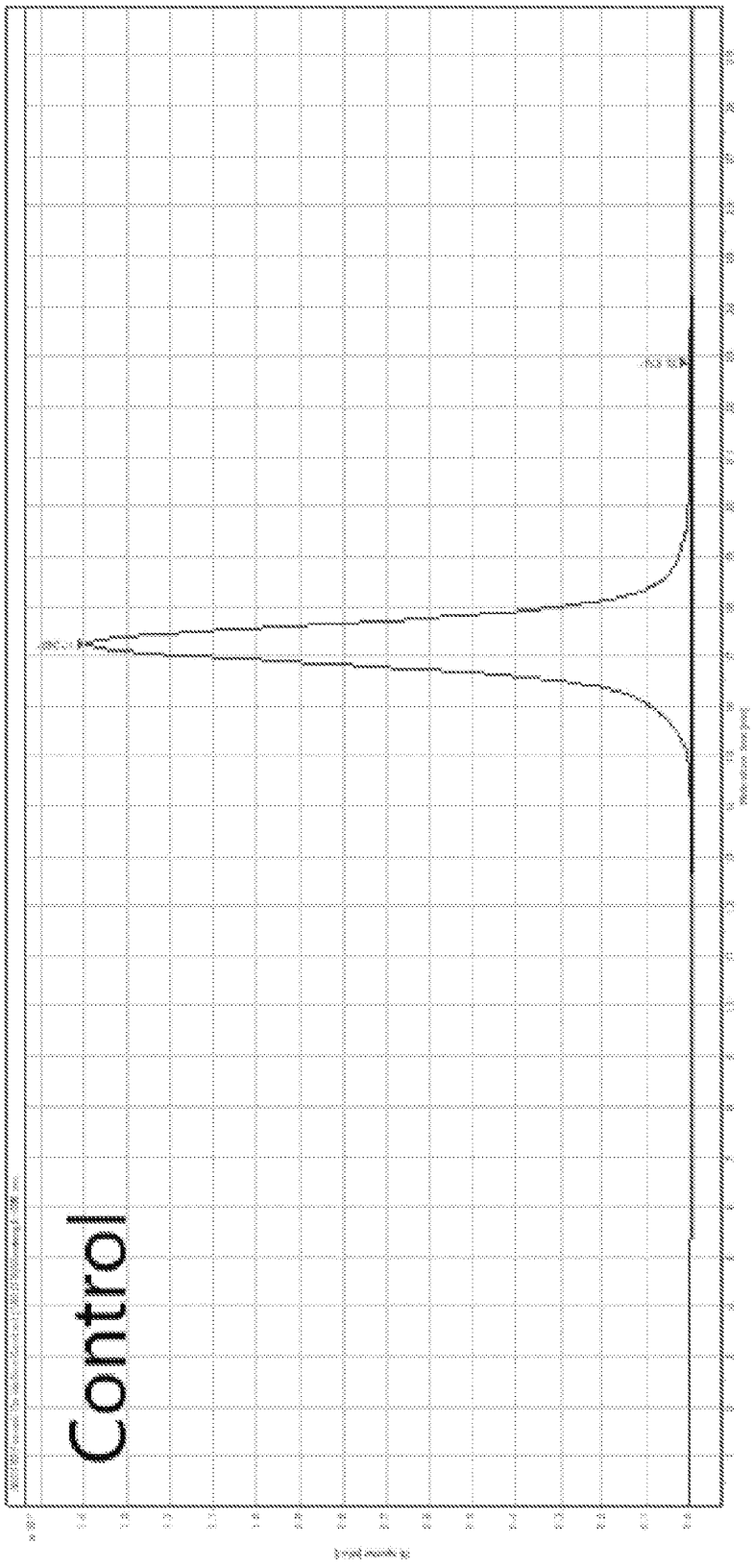


FIG. 259

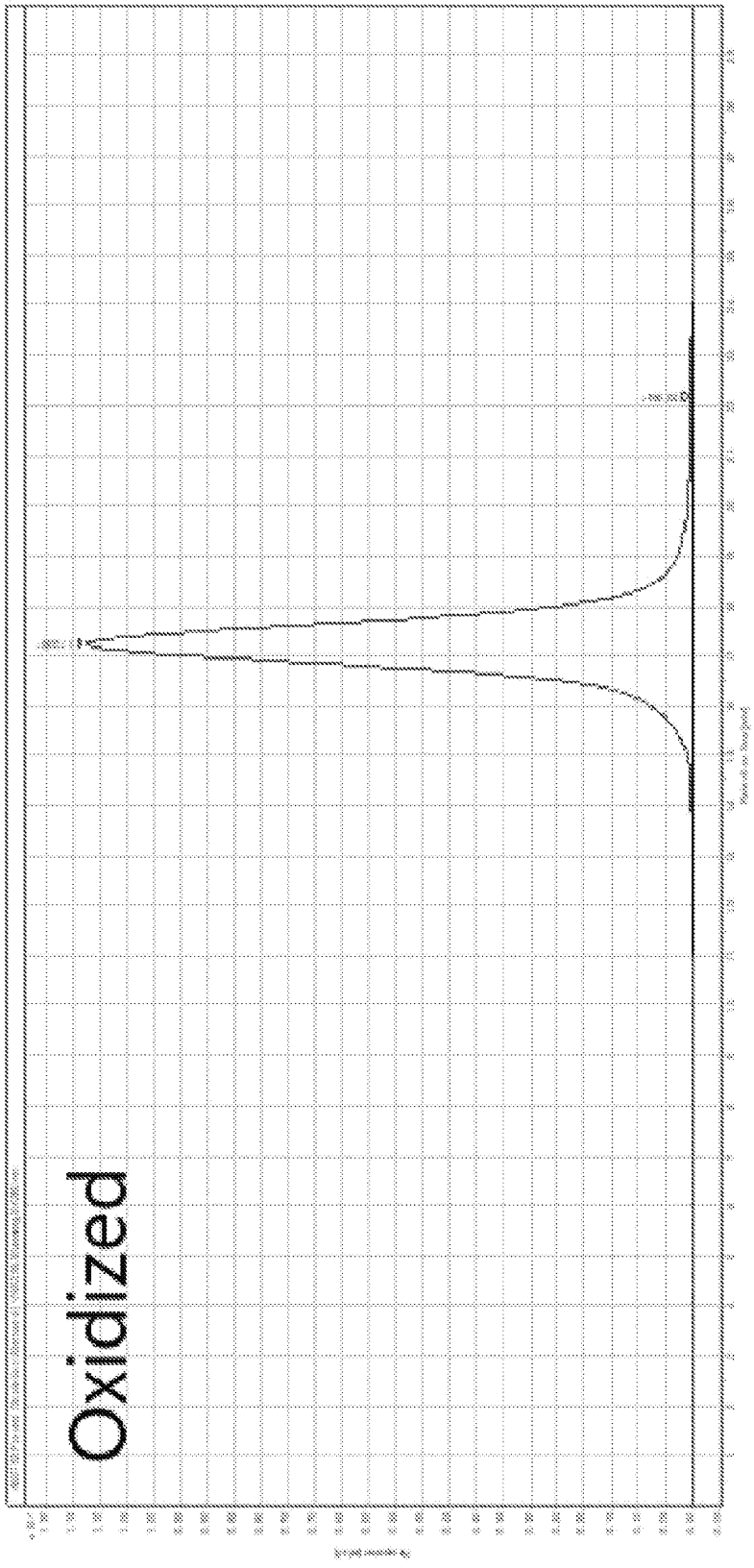


FIG. 259 Continued

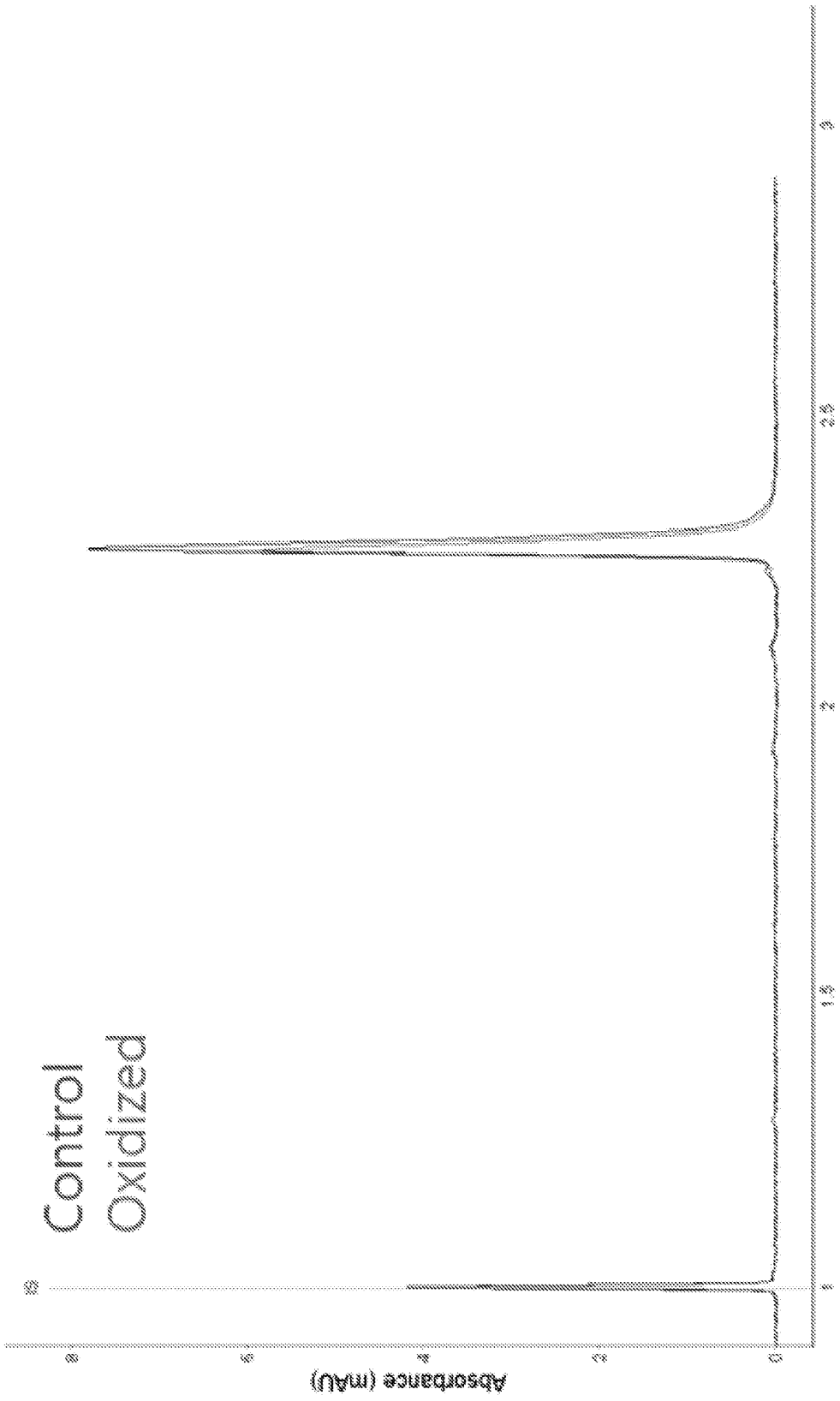


FIG. 260

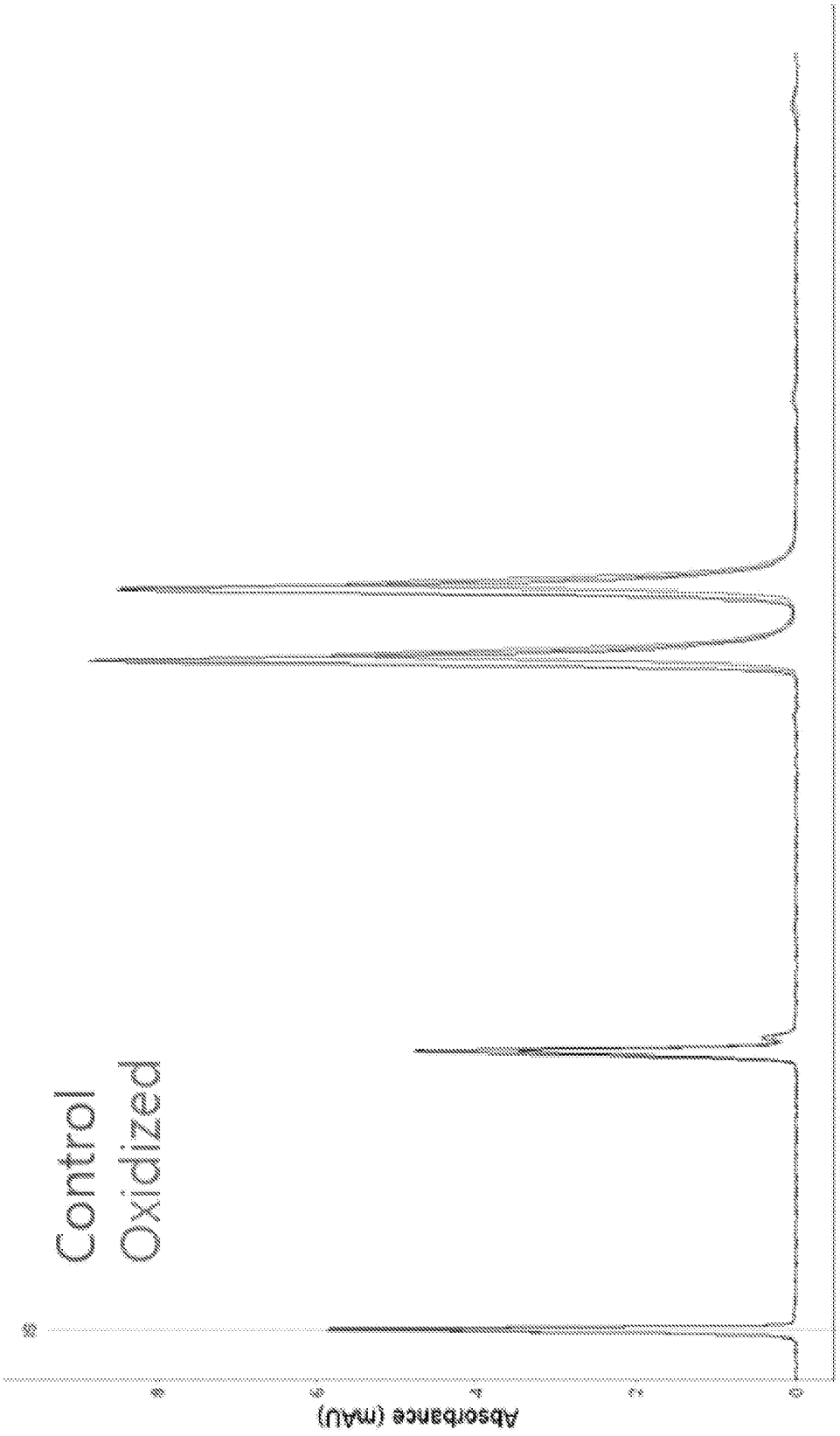
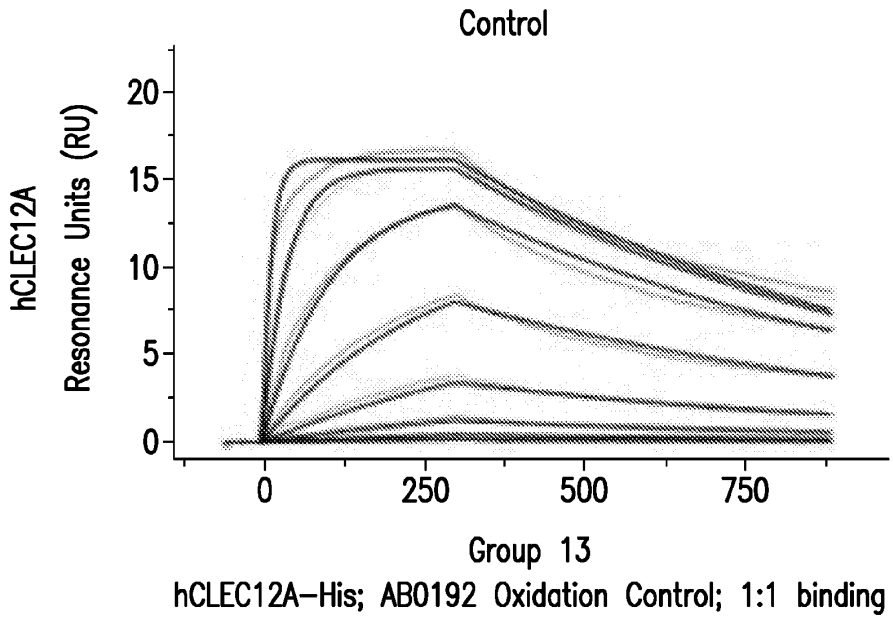


FIG. 260 Continued


Control
hCLEC12A
Resonance Units (RU)
20
15
10
5
0
0
250
500
750
Group 13
hCLEC12A-His; AB0192 Oxidation Control; 1:1 binding

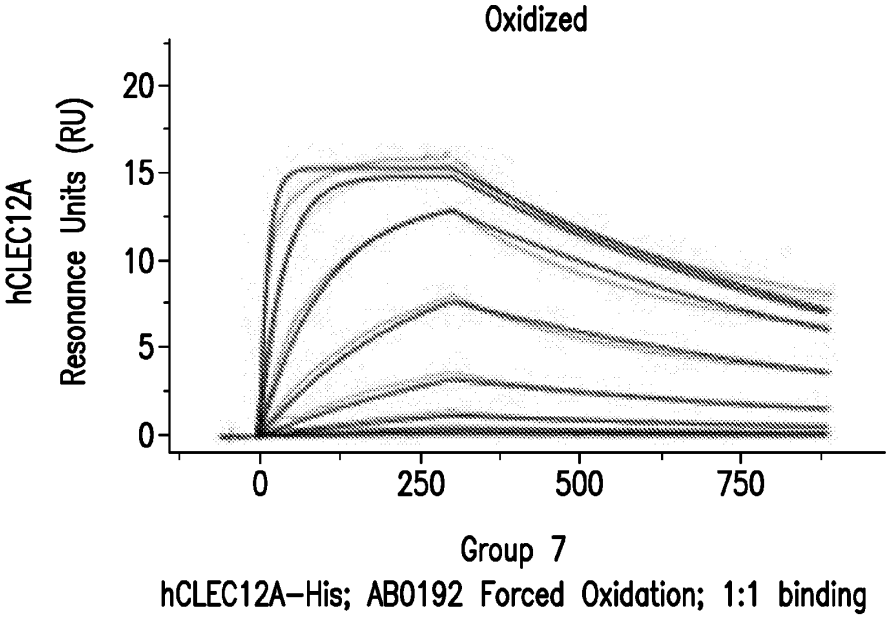
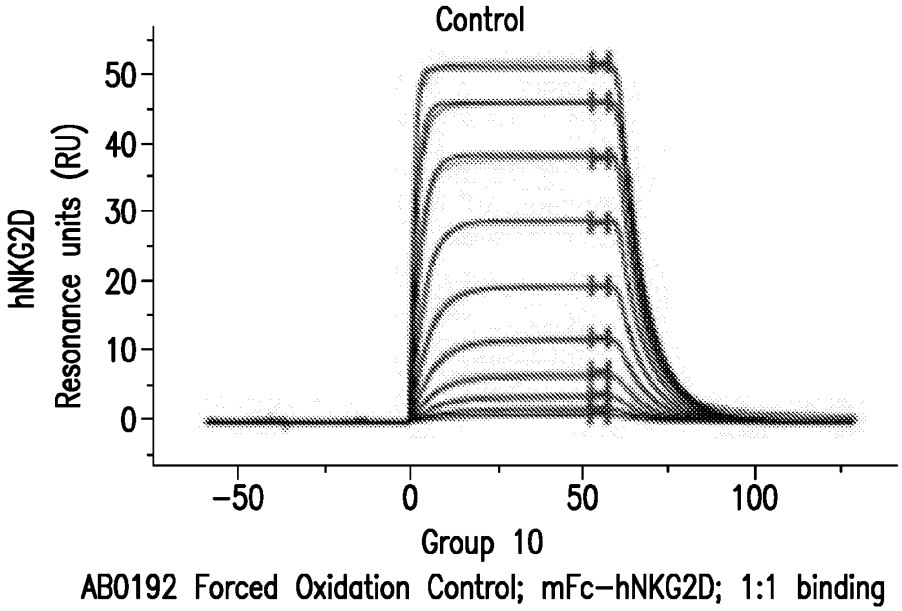

Oxidized
hCLEC12A
Resonance Units (RU)
20
15
10
5
0
0
250
500
750
Group 7
hCLEC12A-His; AB0192 Forced Oxidation; 1:1 binding

FIG. 261



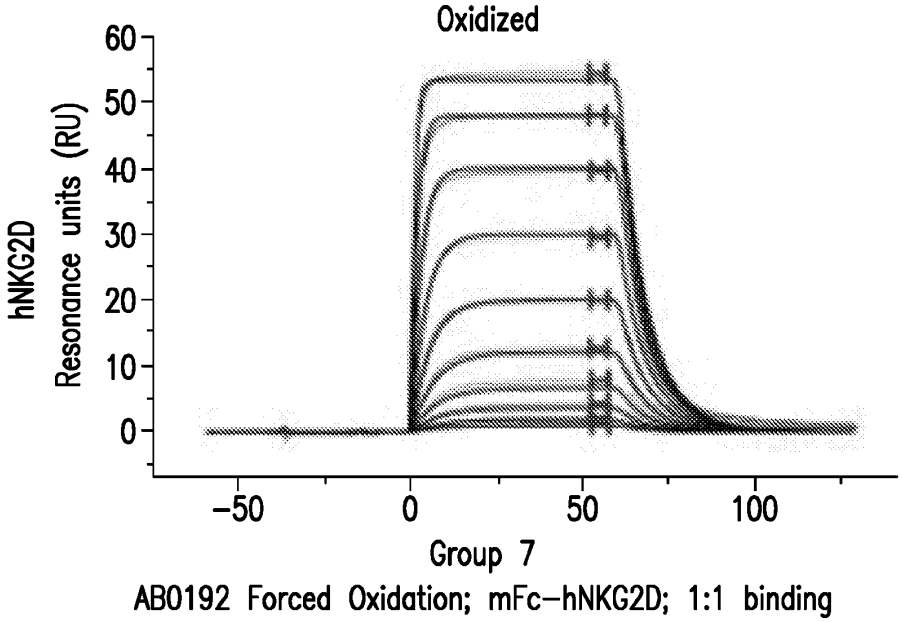
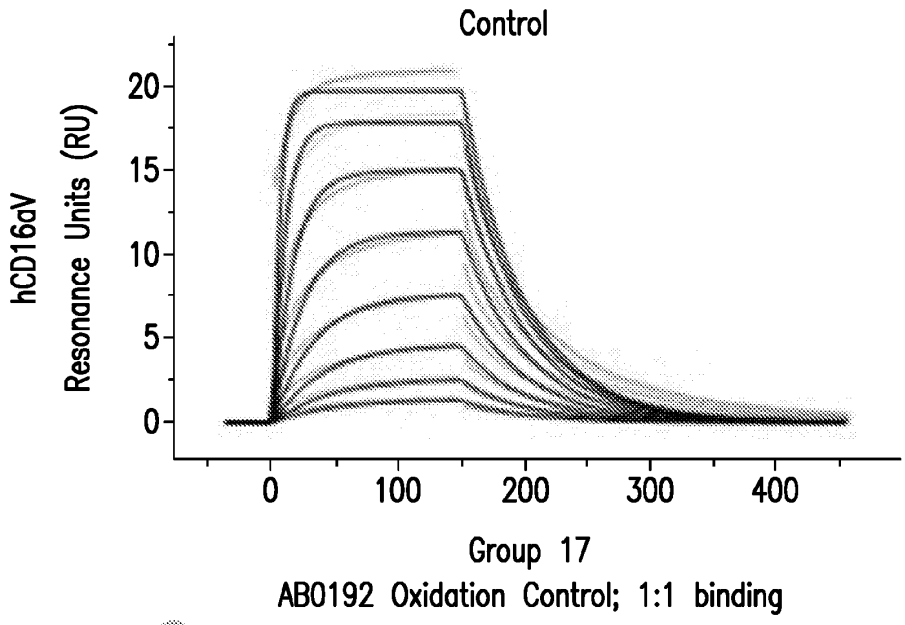


FIG. 261 Continued



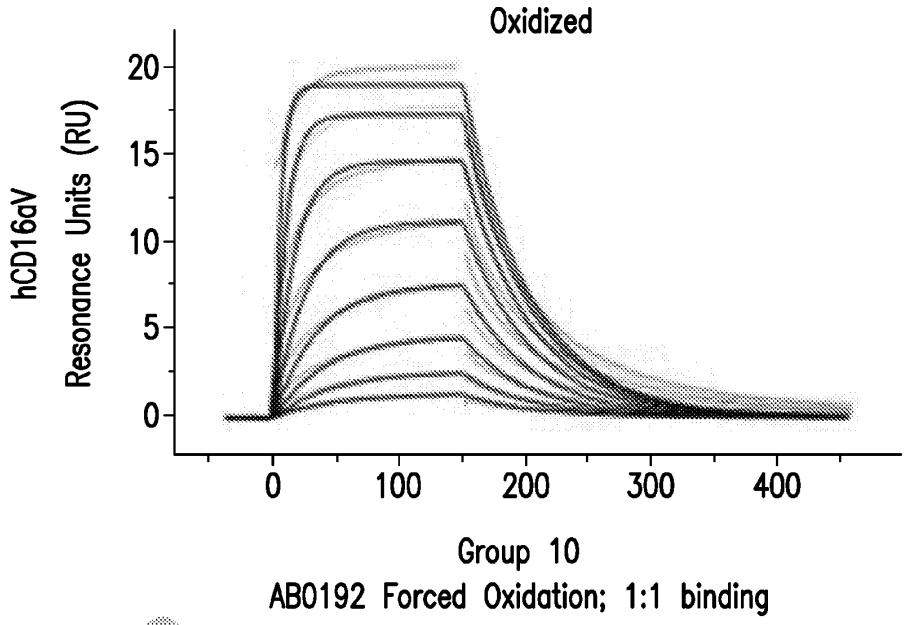
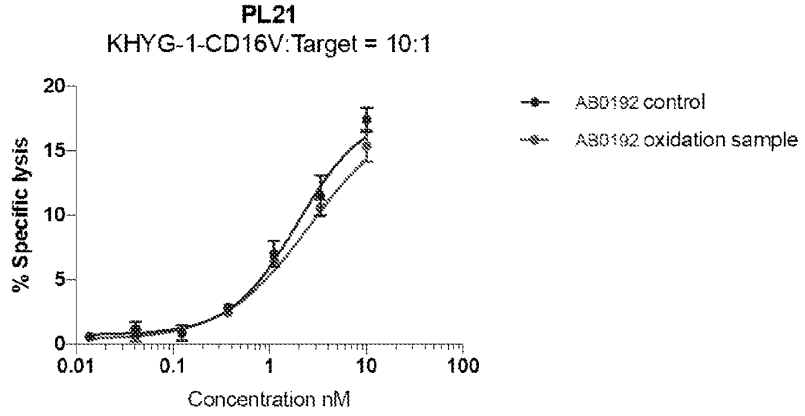


FIG. 261 Continued

FIG. 262


PL21
KHYG-1-CD16V:Target = 10:1
% Specific lysis
20
15
10
5
0
0.01
0.1
1
10
100
Concentration nM
AB0192 control
AB0192 oxidation sample

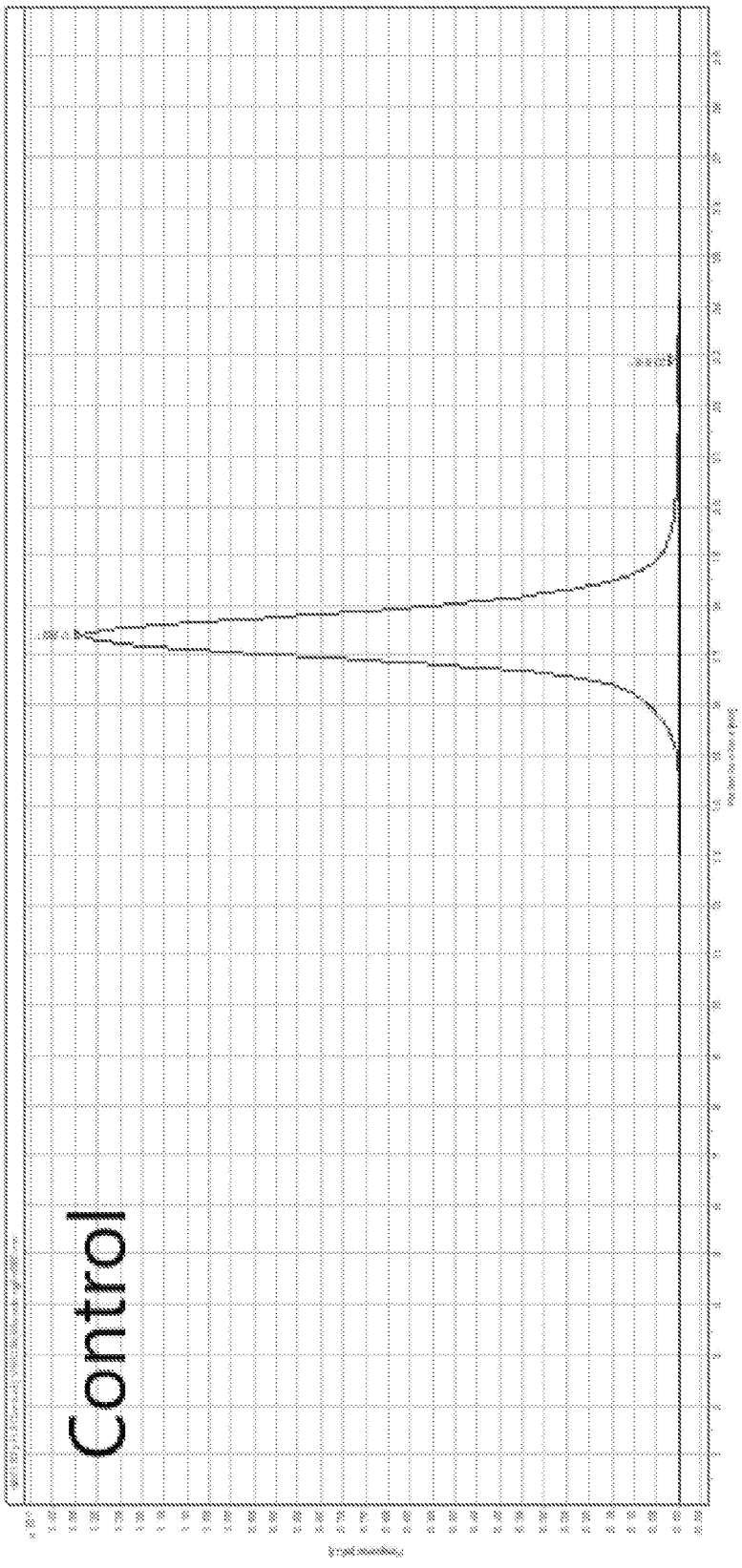


FIG. 263

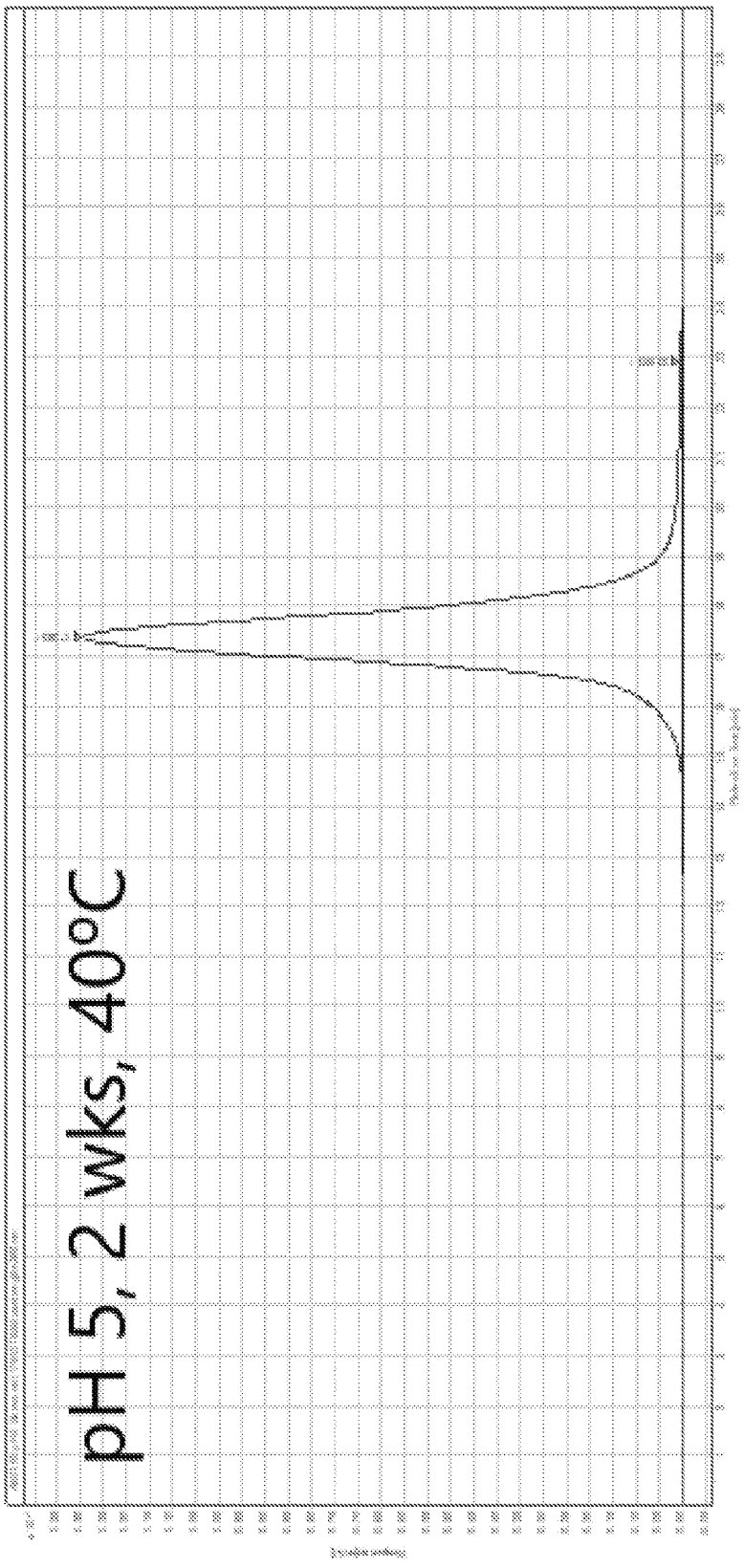


FIG. 263 Continued

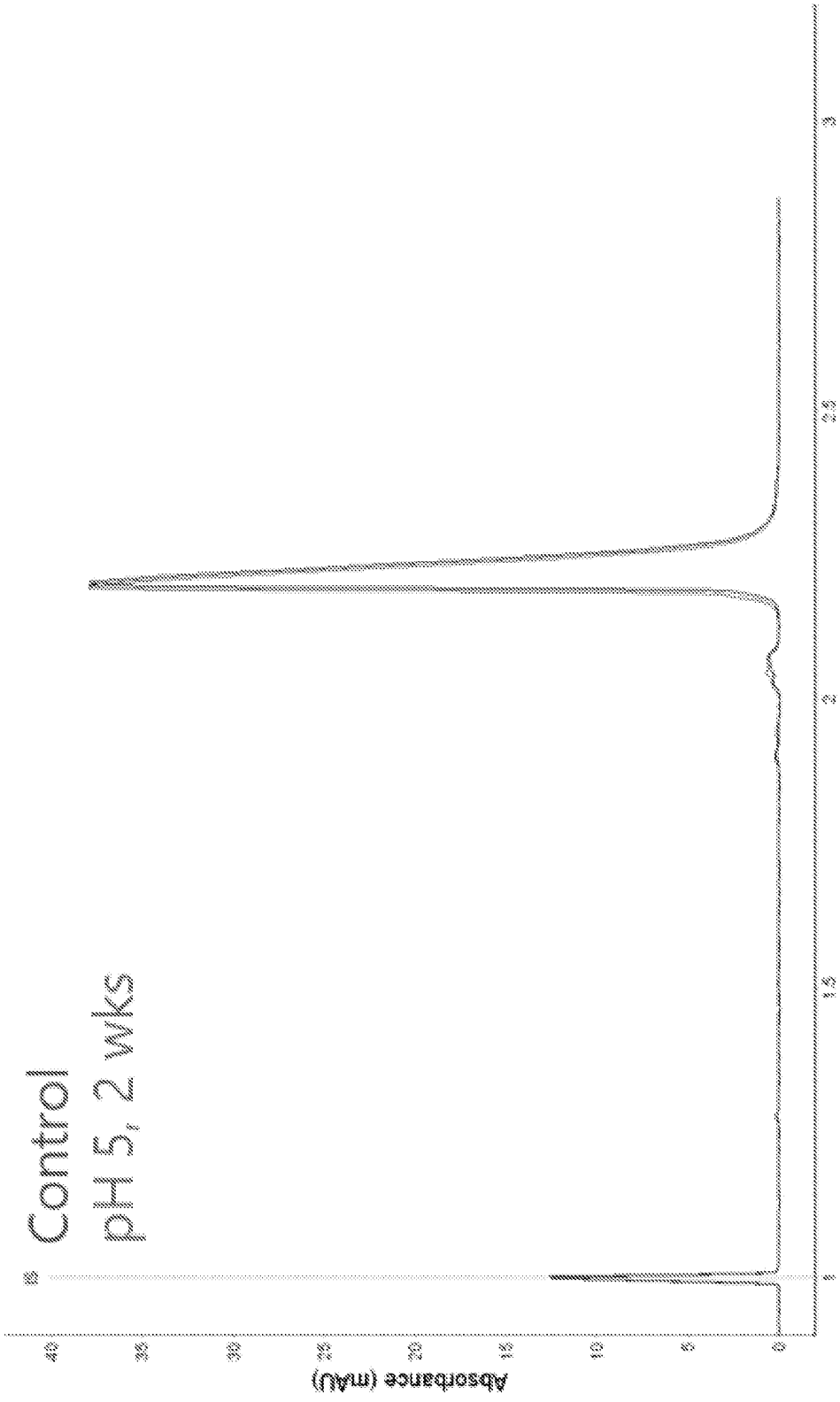


FIG. 264

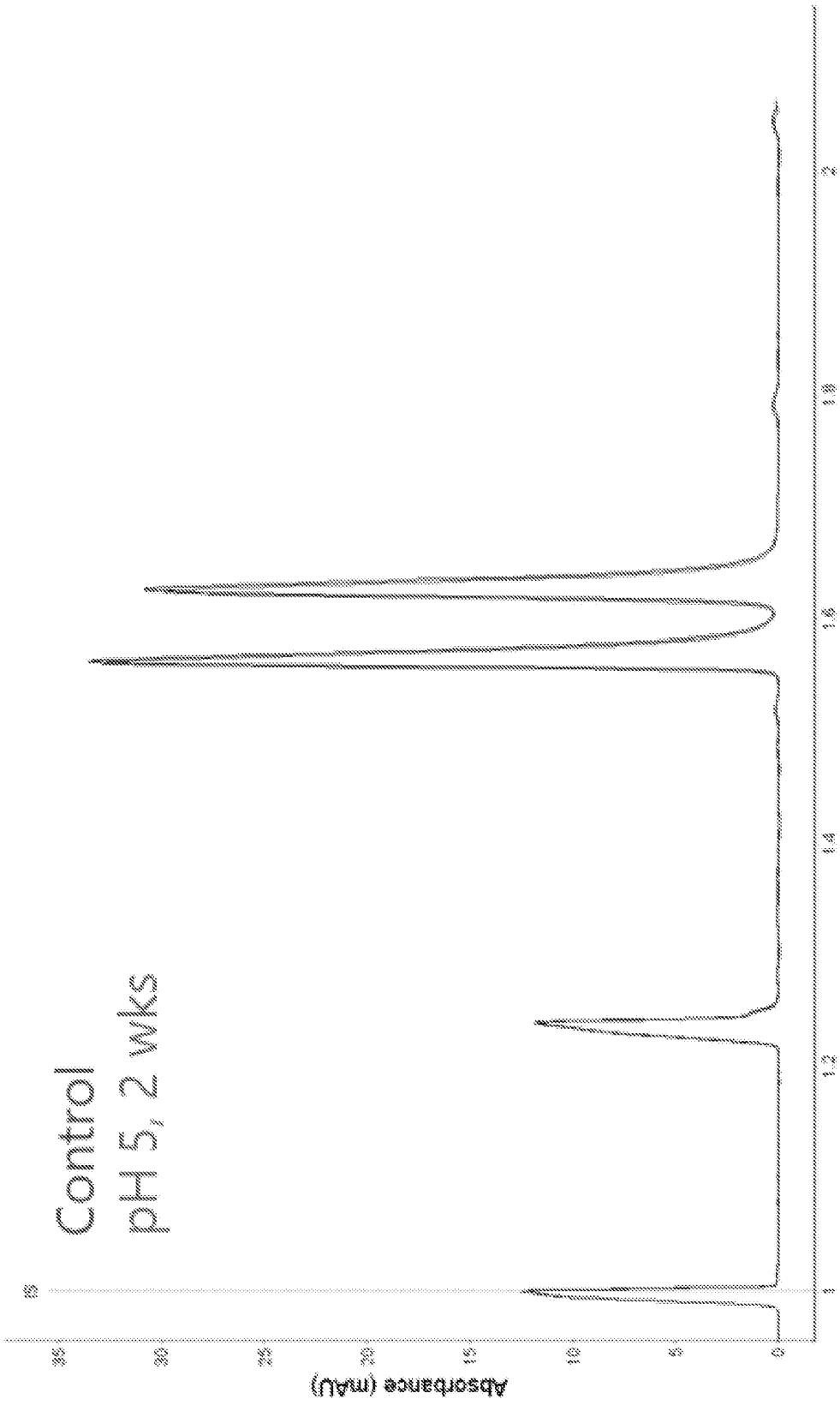


FIG. 264 Continued

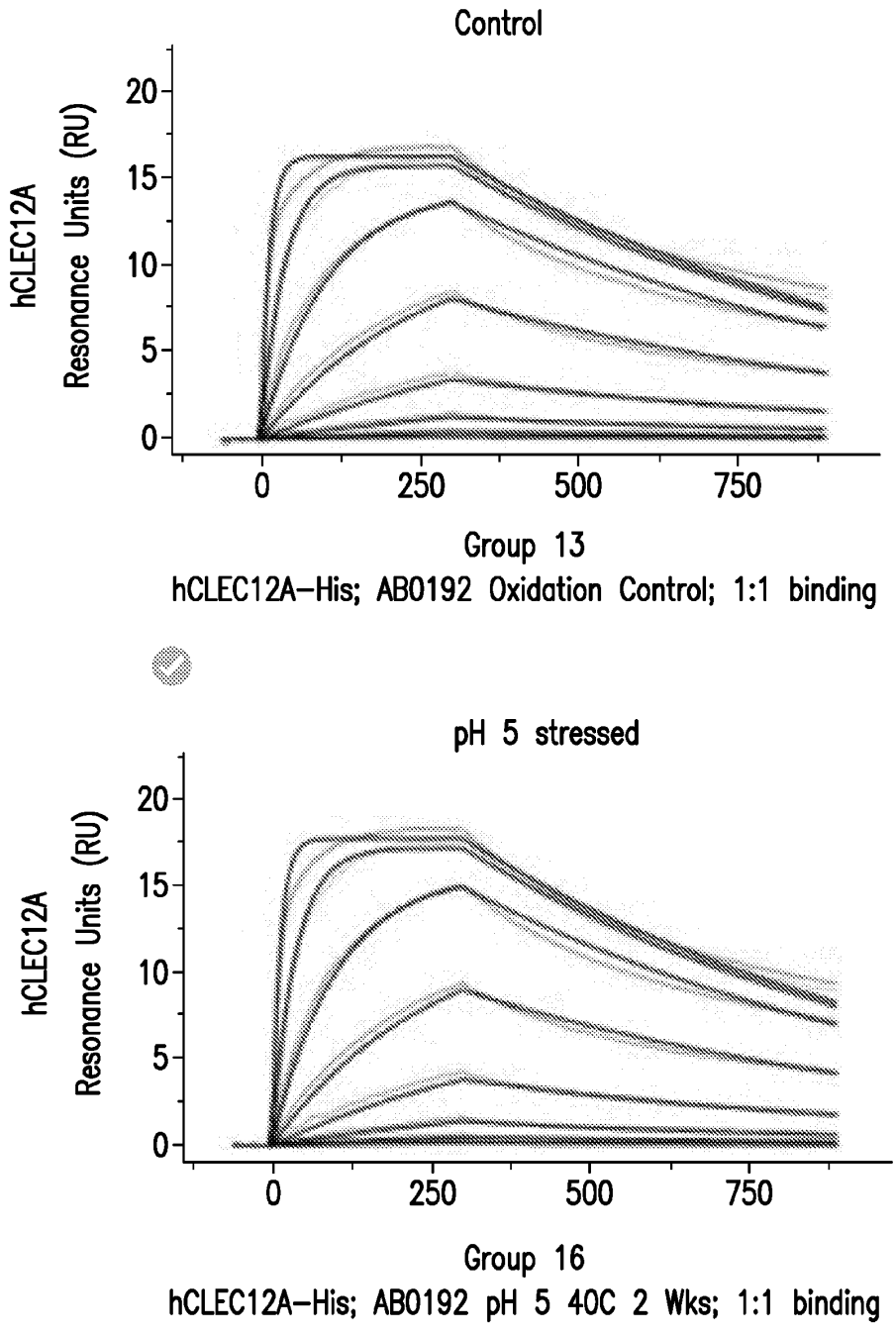
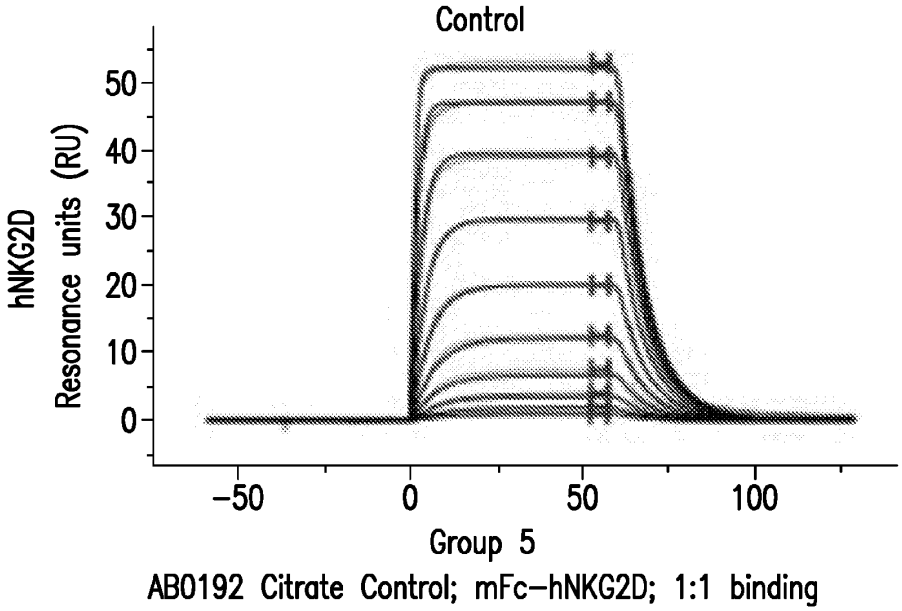

Control
hCLEC12A
Resonance Units (RU)
20
15
10
5
0
0
250
500
750
Group 13
hCLEC12A-His; AB0192 Oxidation Control; 1:1 binding
pH 5 stressed
hCLEC12A
Resonance Units (RU)
20
15
10
5
0
0
250
500
750
Group 16
hCLEC12A-His; AB0192 pH 5 40C 2 Wks; 1:1 binding

FIG. 265



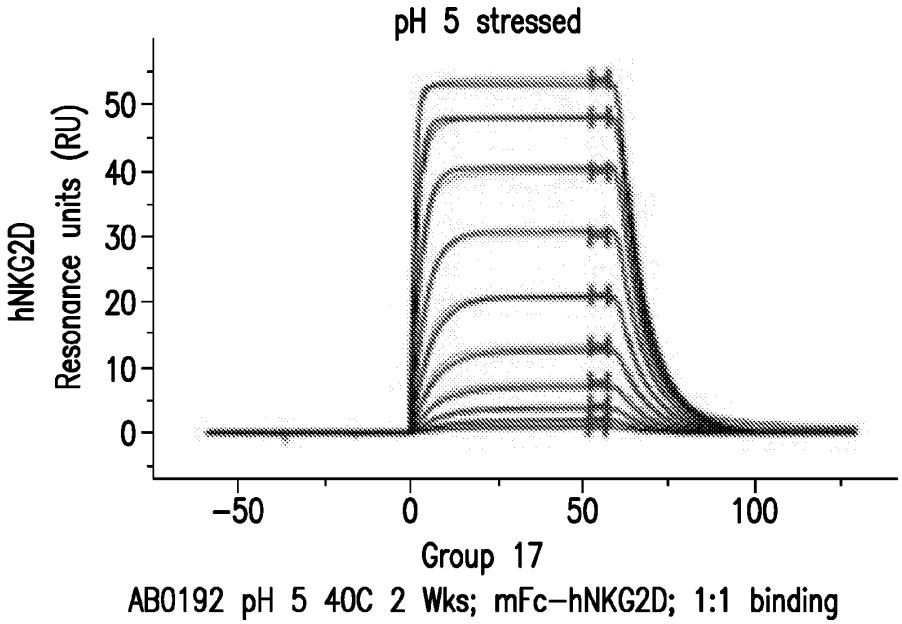
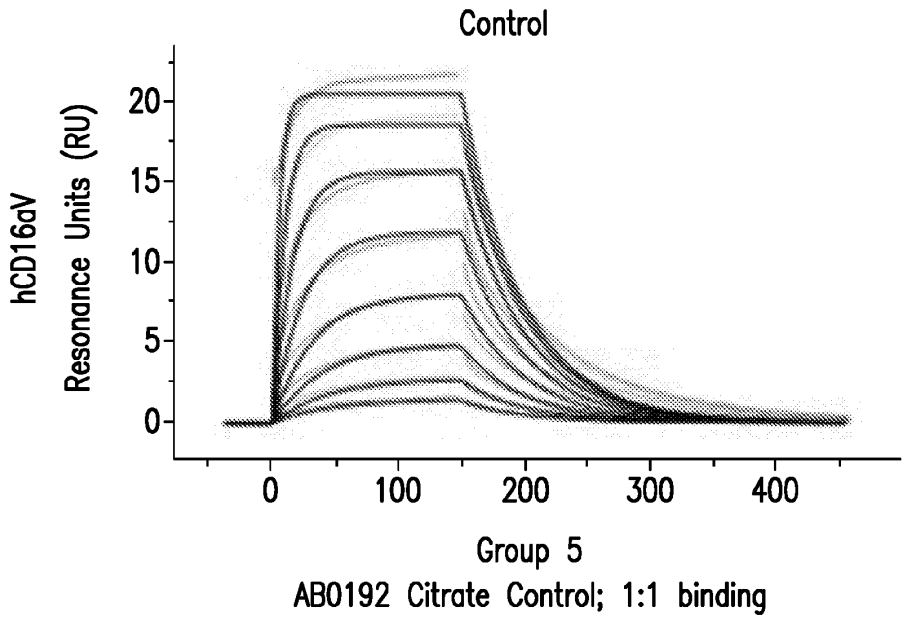


FIG. 265 Continued



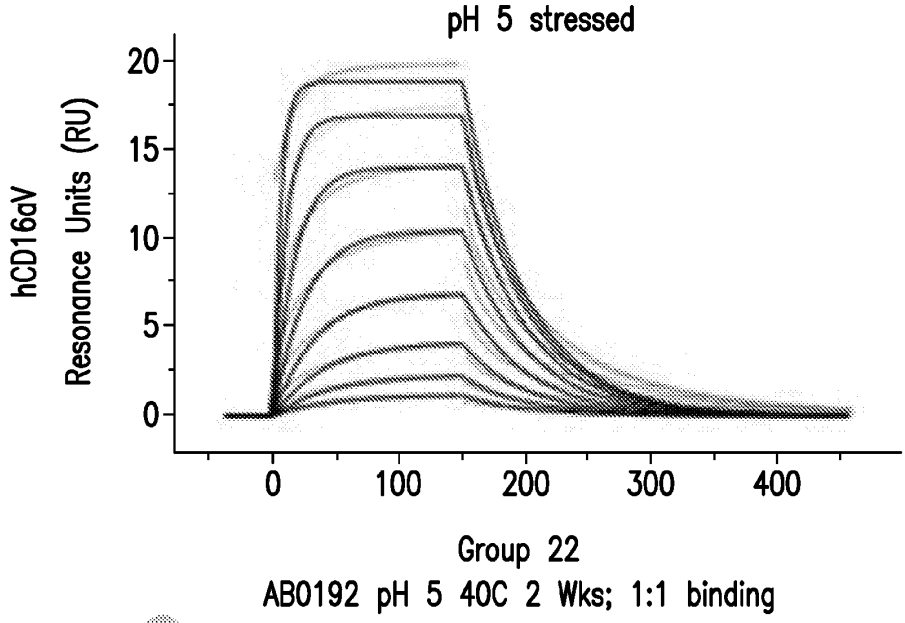


FIG. 265 Continued

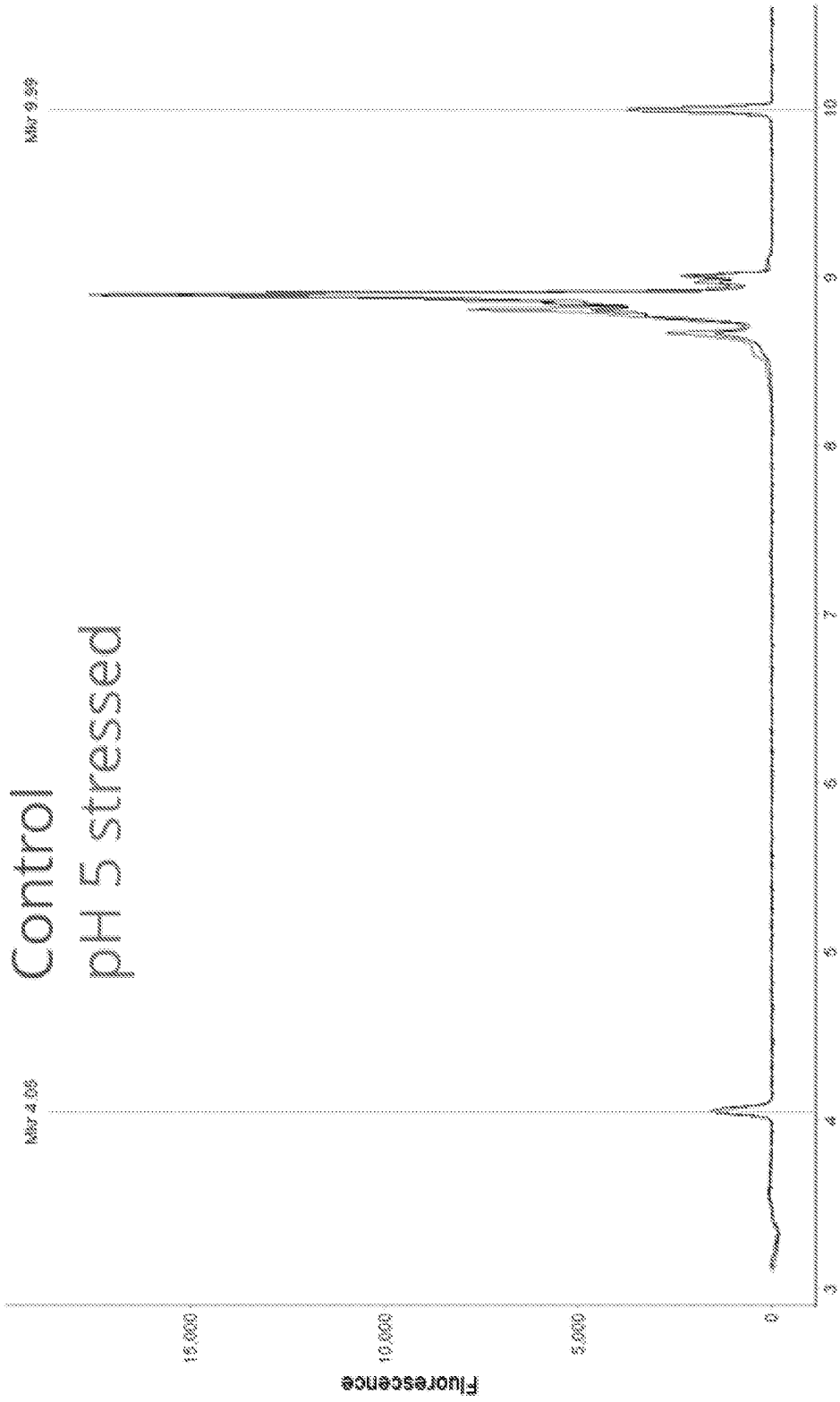
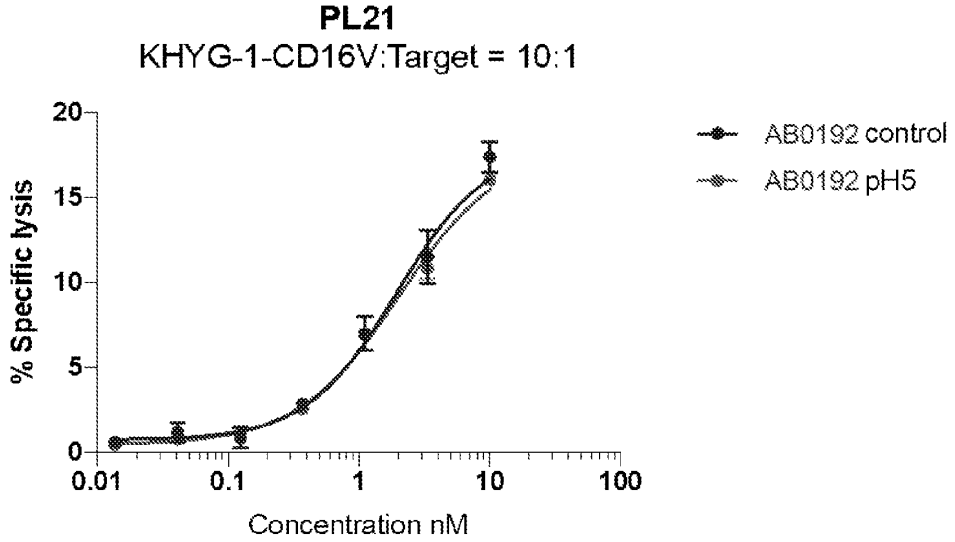

Control
pH 5 stressed
Mkr 4.06
Mkr 9.99
15,000
10,000
5,000
0
Fluorescence
3
4
5
6
7
8
9
10
pI

FIG. 266

FIG. 267


PL21
KHYG-1-CD16V:Target = 10:1
% Specific lysis
20
15
10
5
0
0.01
0.1
1
10
100
Concentration nM
AB0192 control
AB0192 pH5

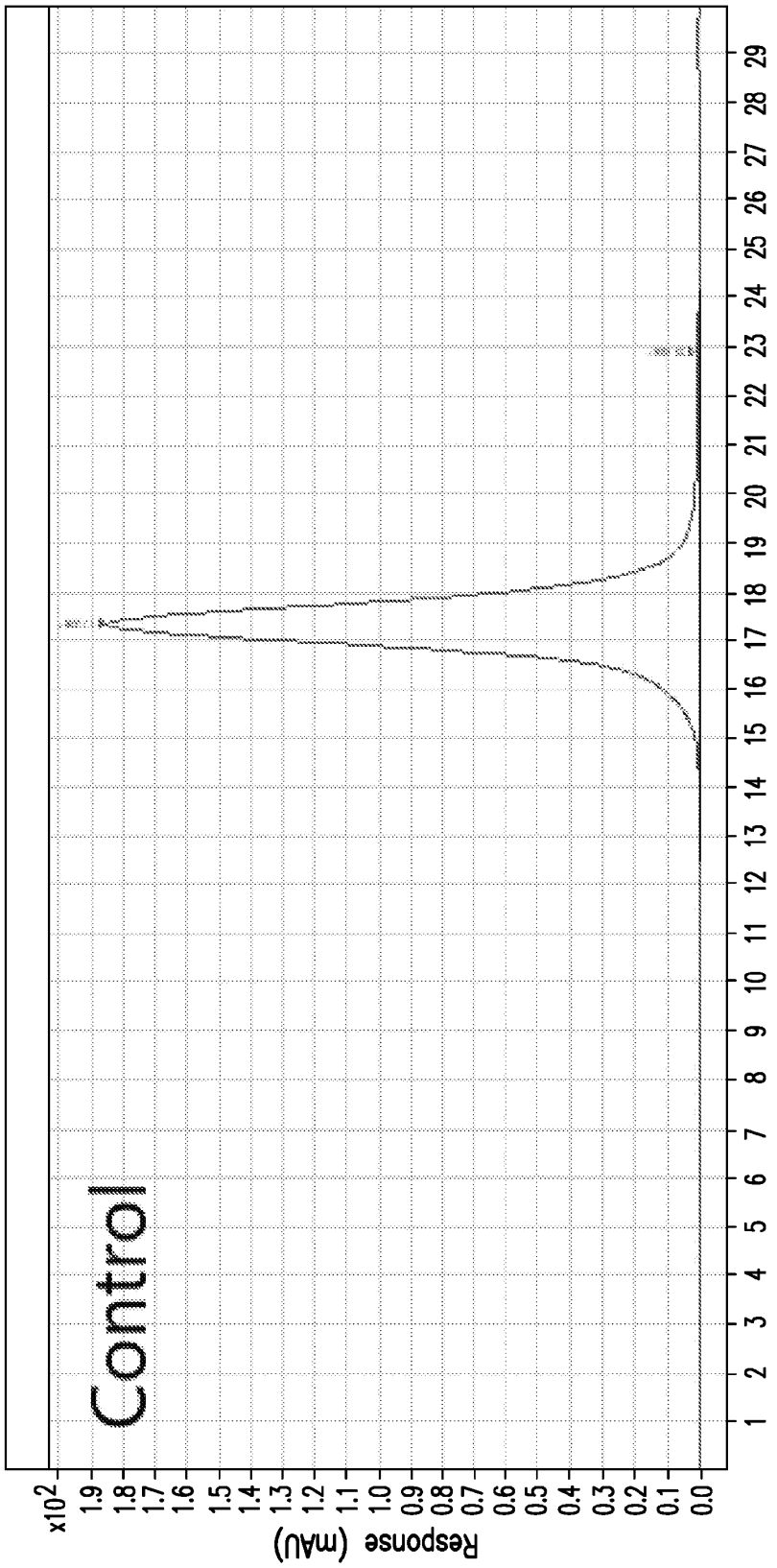

Control
Response (mAU)
x10^2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29

FIG. 268

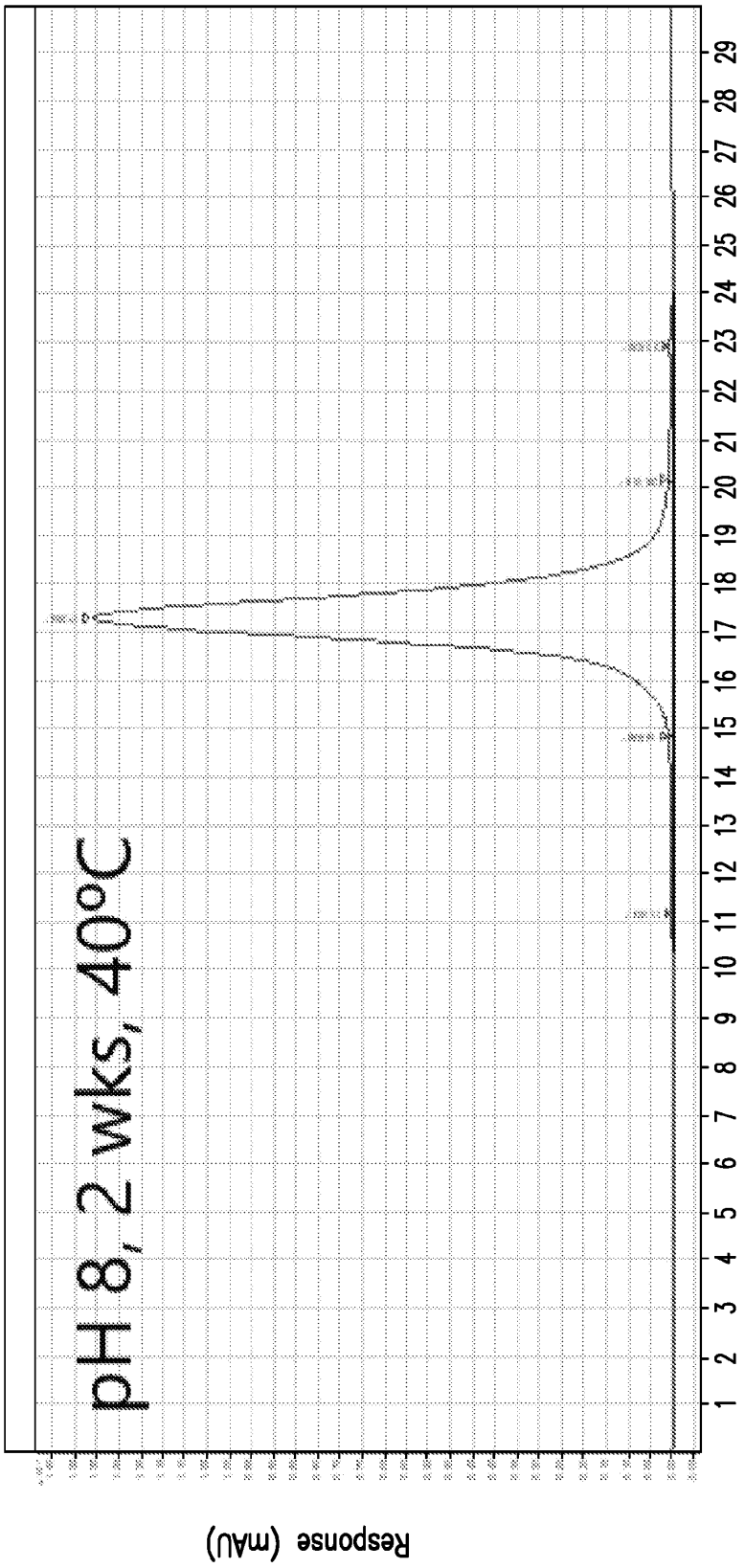


FIG. 268 Continued

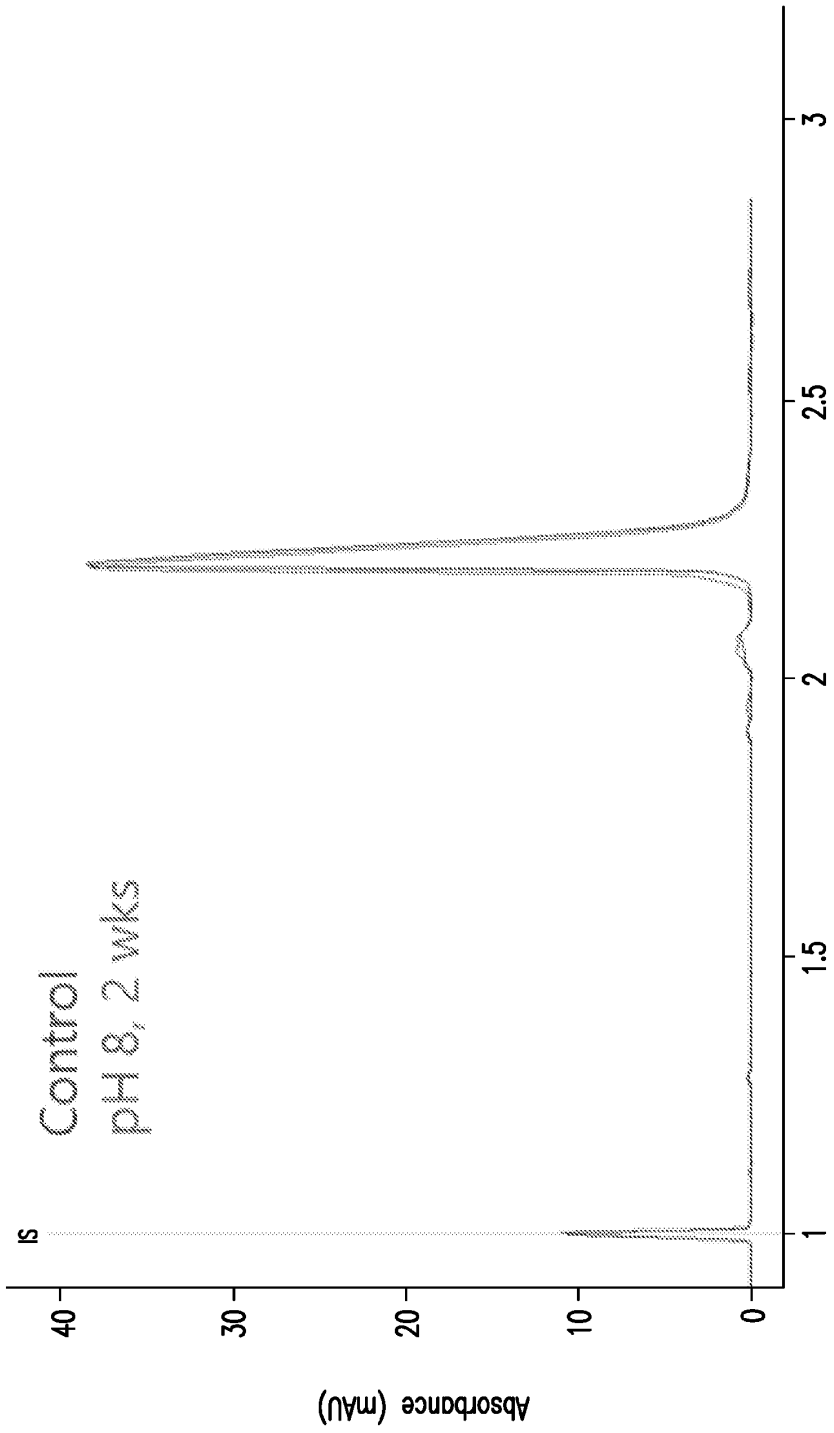

Absorbance (mAU)
40
30
20
10
0
IS
Control
pH 8, 2 wks
1
1.5
2
2.5
3
Relative Migration Time

FIG. 269

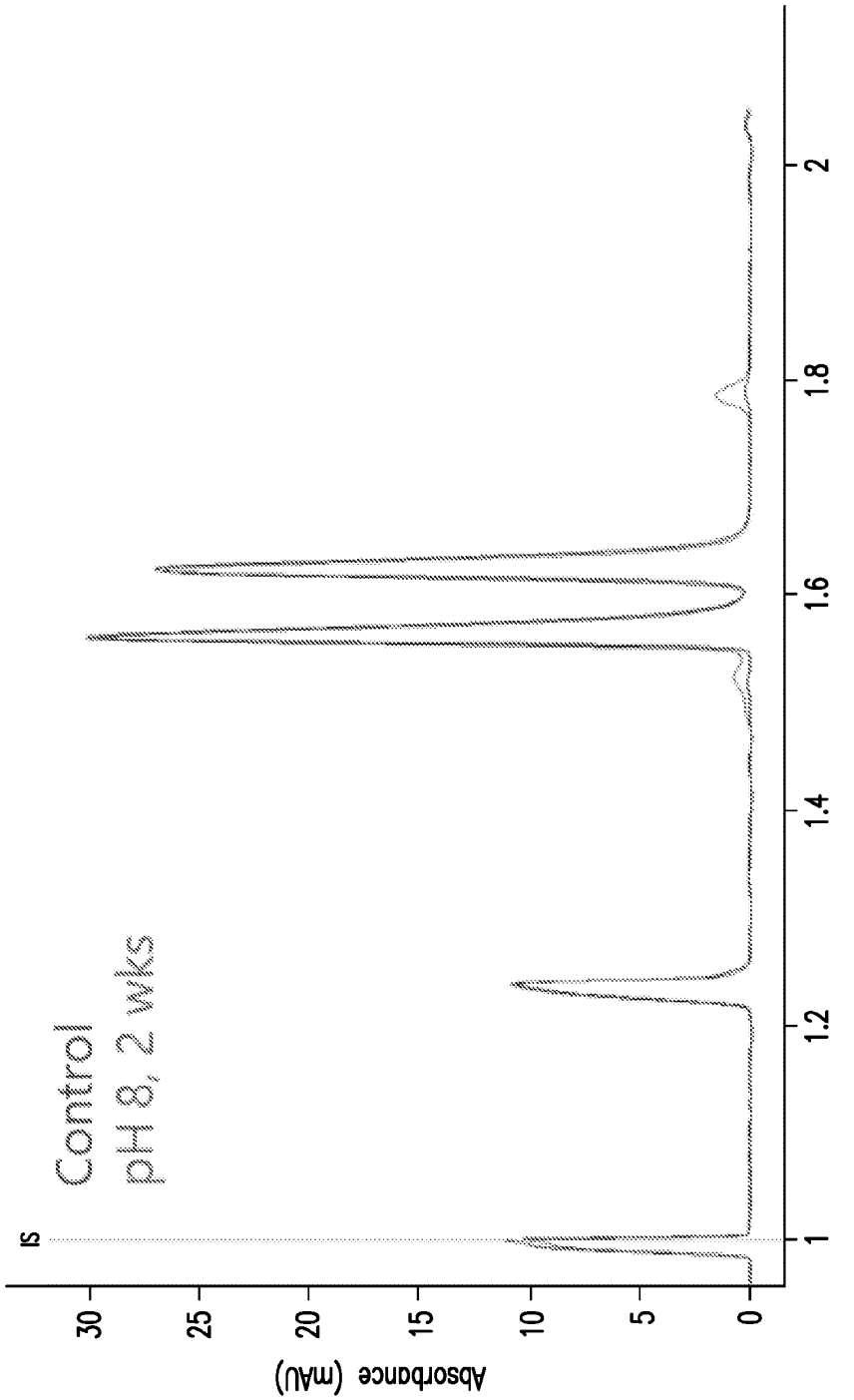


FIG. 269 Continued

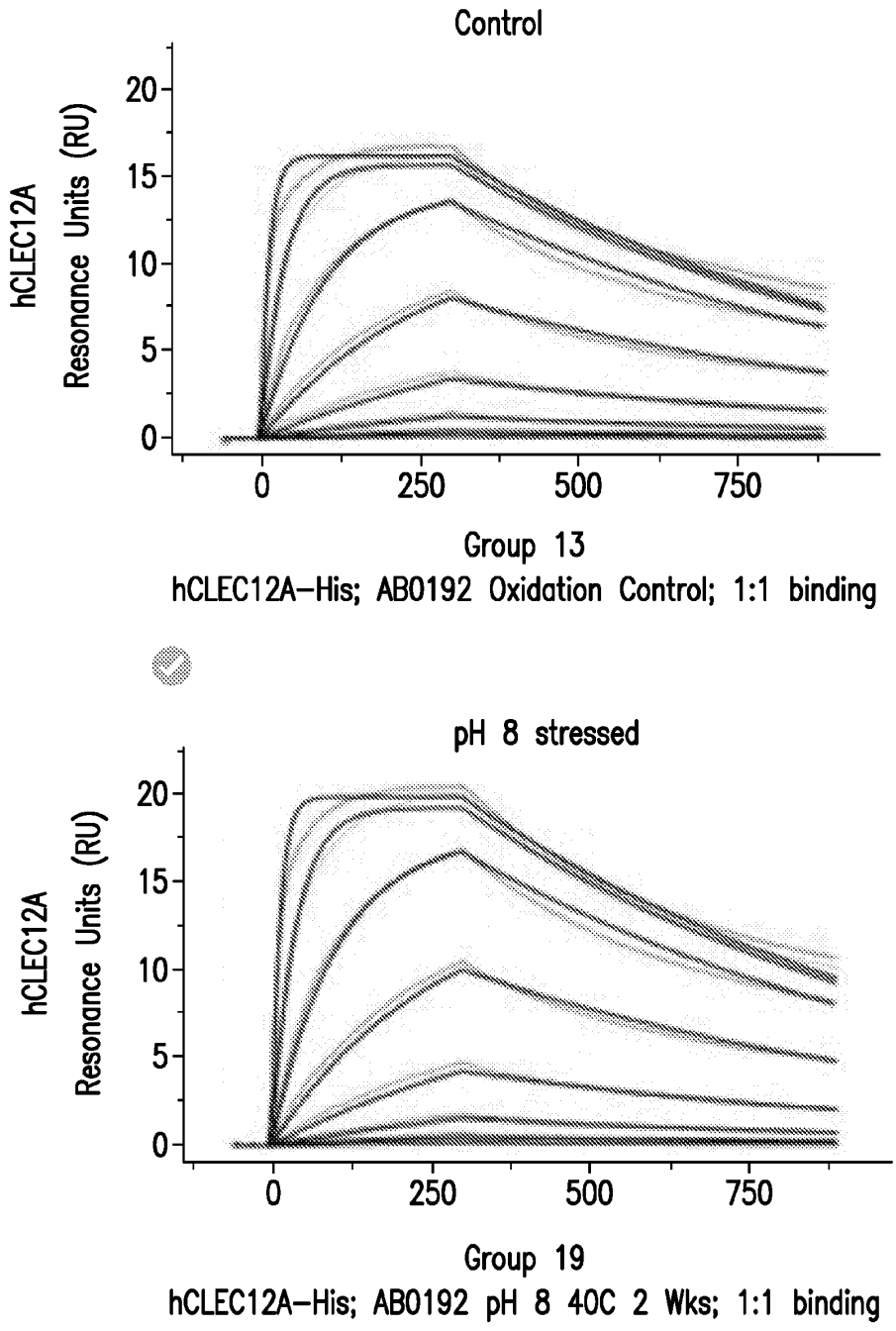
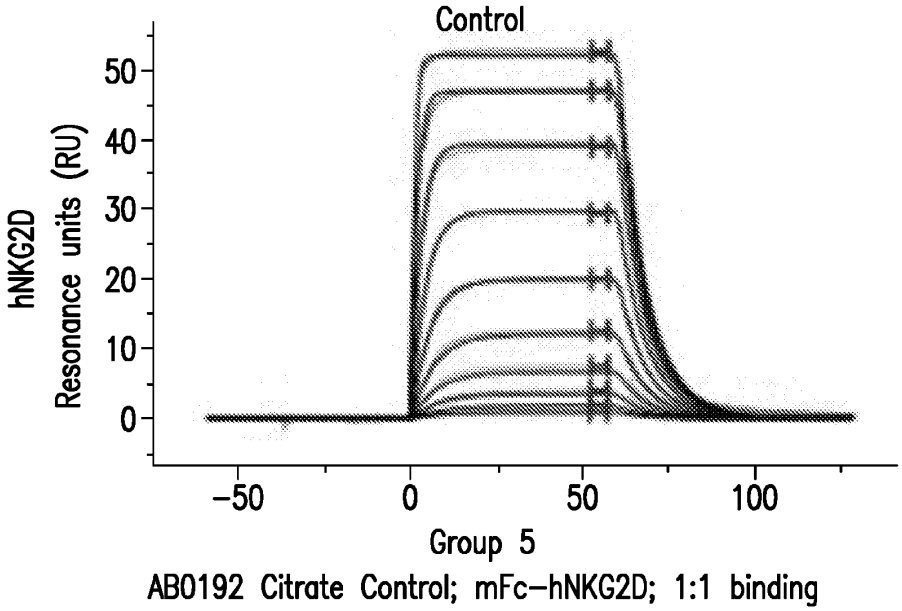

Control
hCLEC12A
Resonance Units (RU)
20
15
10
5
0
0
250
500
750
Group 13
hCLEC12A-His; AB0192 Oxidation Control; 1:1 binding
pH 8 stressed
hCLEC12A
Resonance Units (RU)
20
15
10
5
0
0
250
500
750
Group 19
hCLEC12A-His; AB0192 pH 8 40C 2 Wks; 1:1 binding

FIG. 270



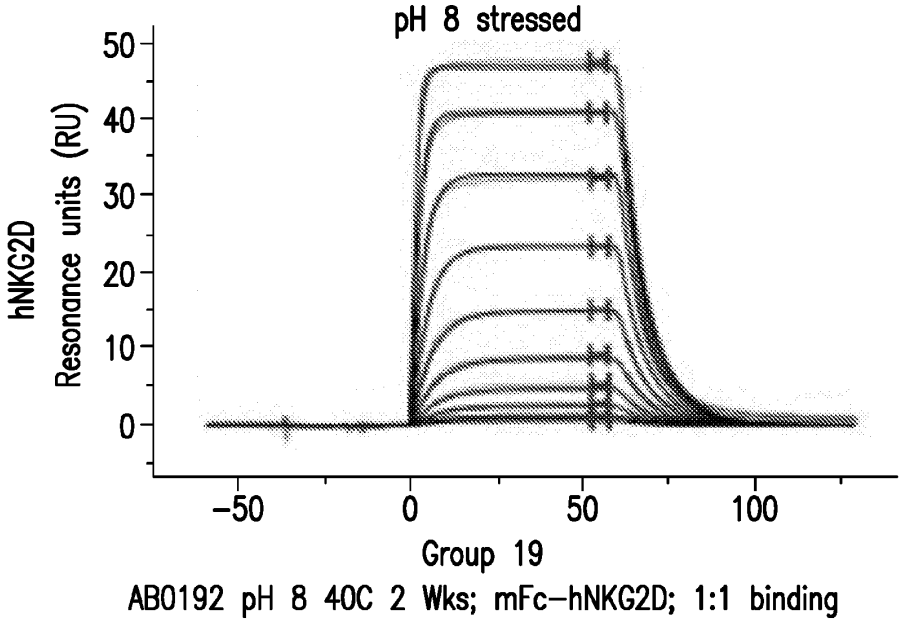
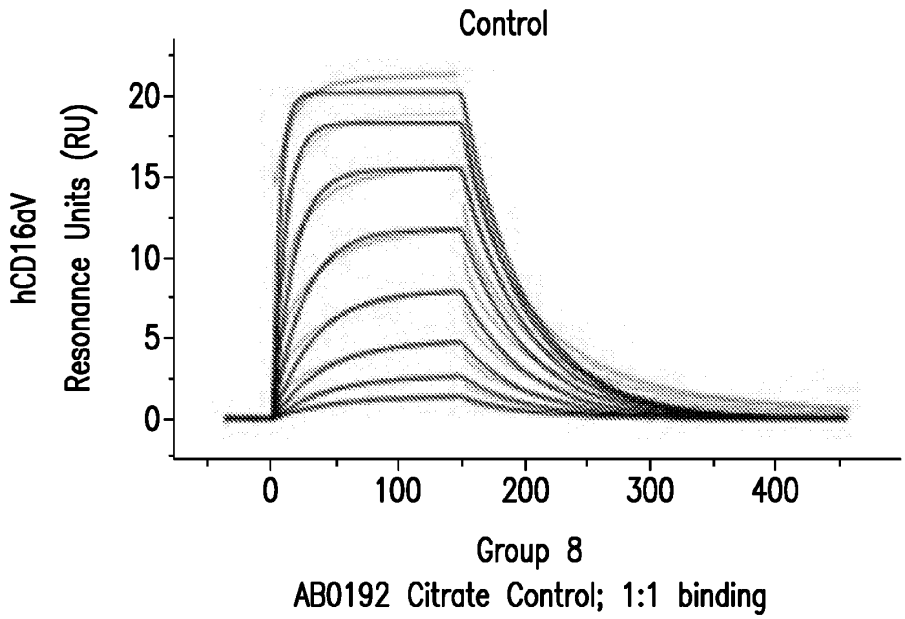


FIG. 270 Continued



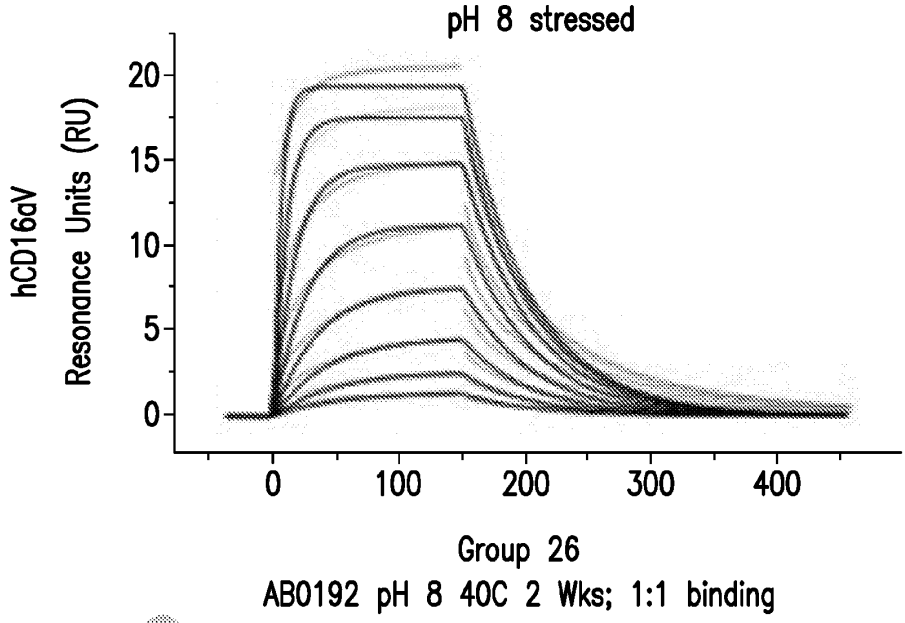


FIG. 270 Continued

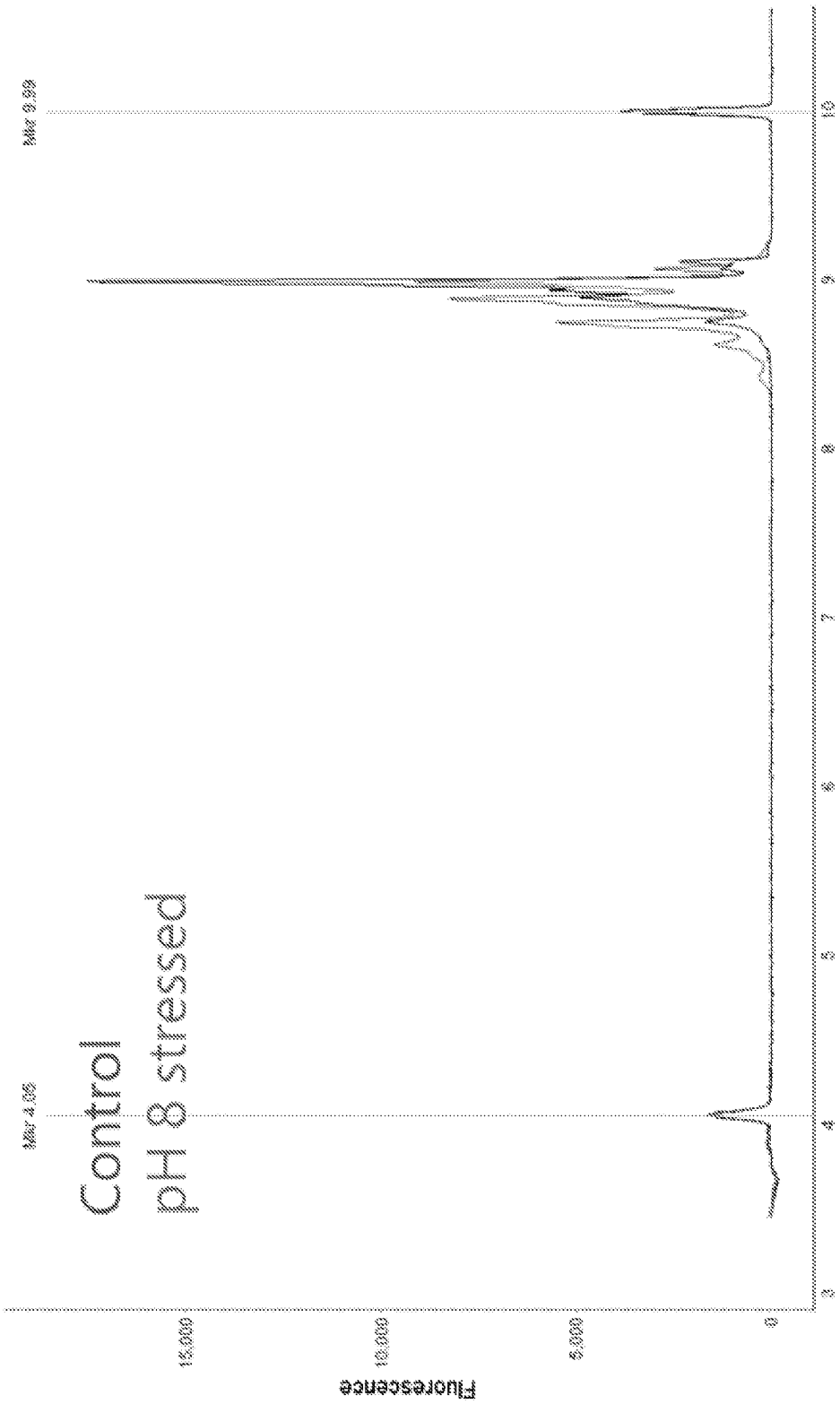
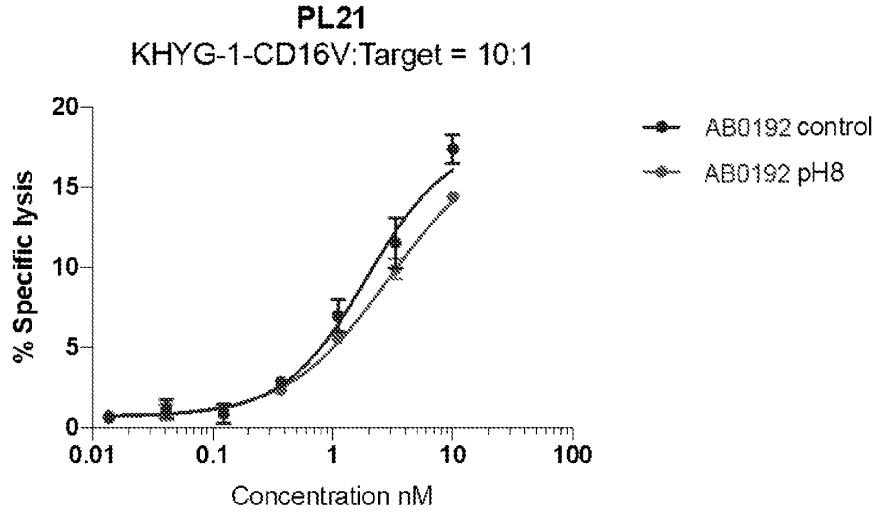

Control
pH 8 stressed
Mkr 4.06
Mkr 9.89
Fluorescence
15,000
10,000
5,000
0
3
4
5
6
7
8
9
10
pI

FIG. 271

FIG. 272


PL21
KHYG-1-CD16V:Target = 10:1
% Specific lysis
20
15
10
5
0
0.01
0.1
1
10
100
Concentration nM
AB0192 control
AB0192 pH8

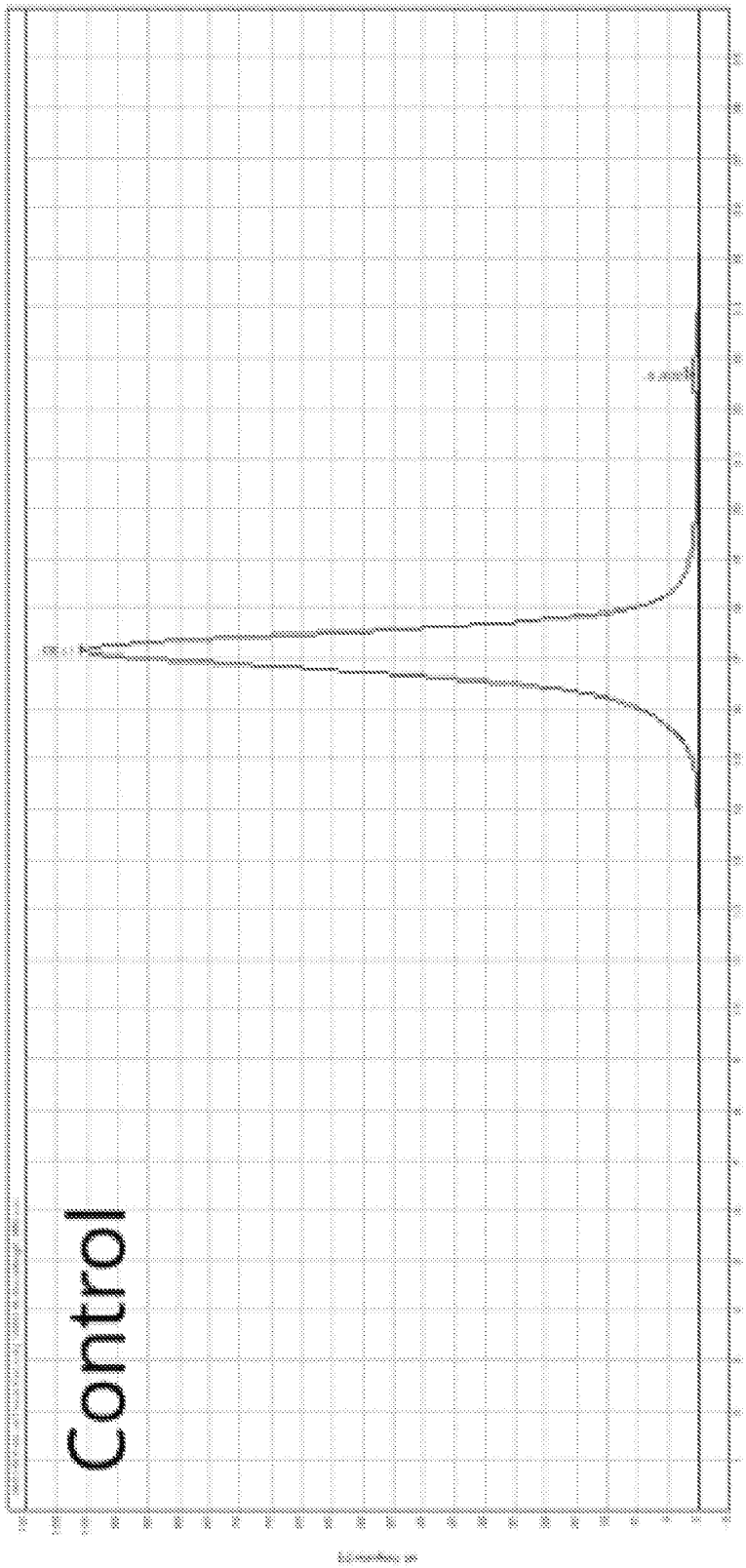


FIG. 273

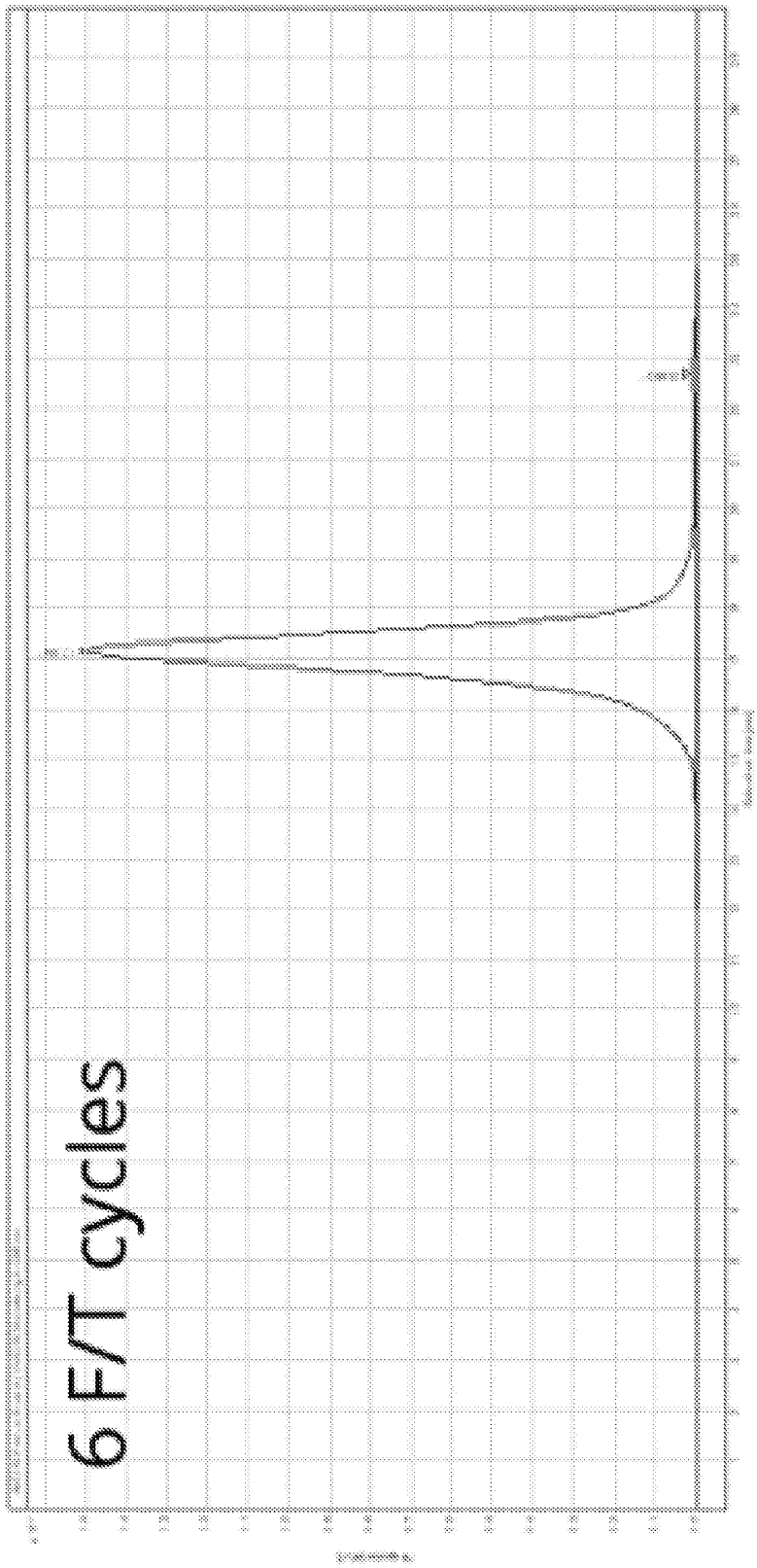


FIG. 273 Continued

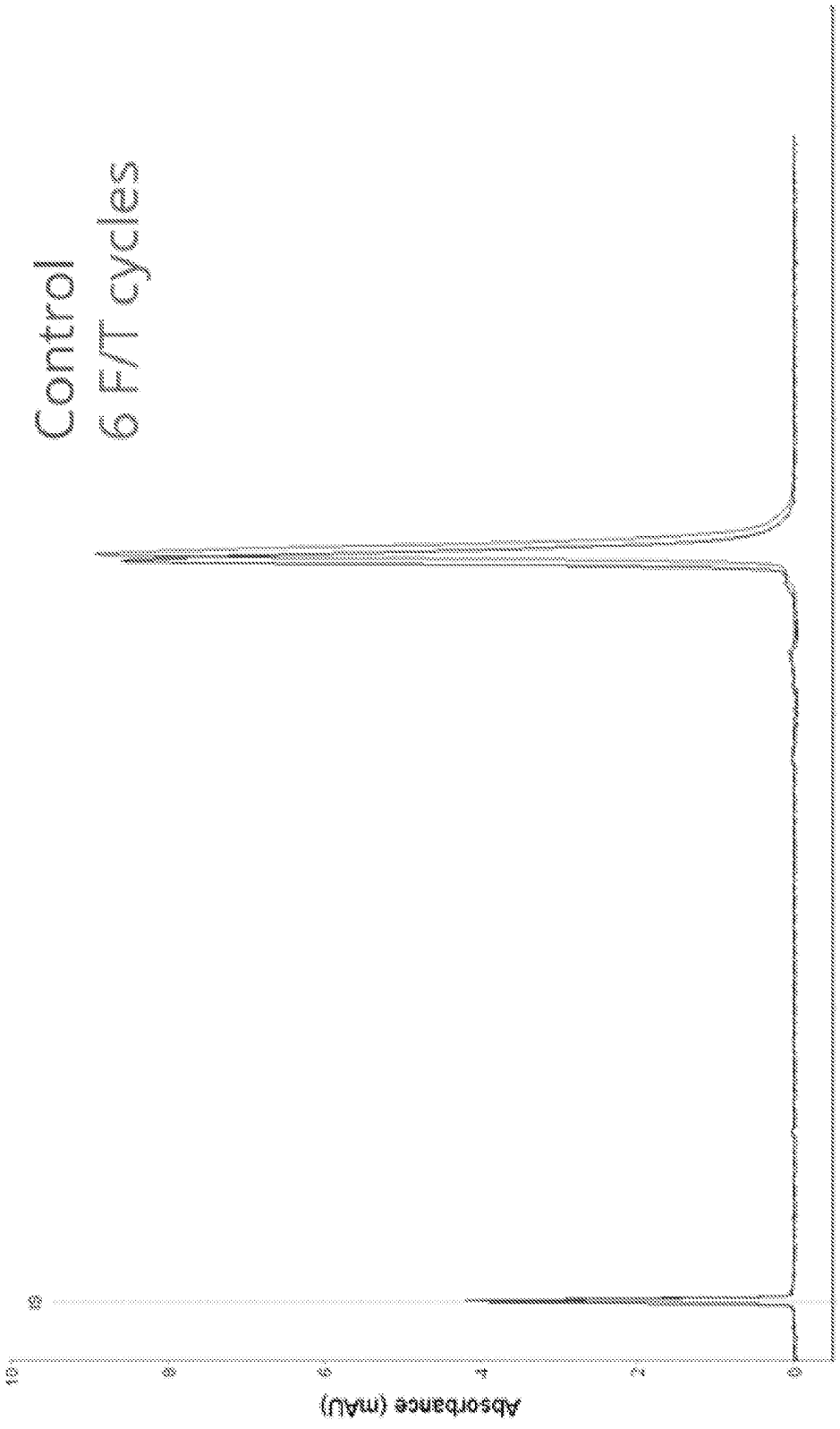

Control
6 F/T cycles
Absorbance (mAU)
Relative Migration Time

FIG. 274

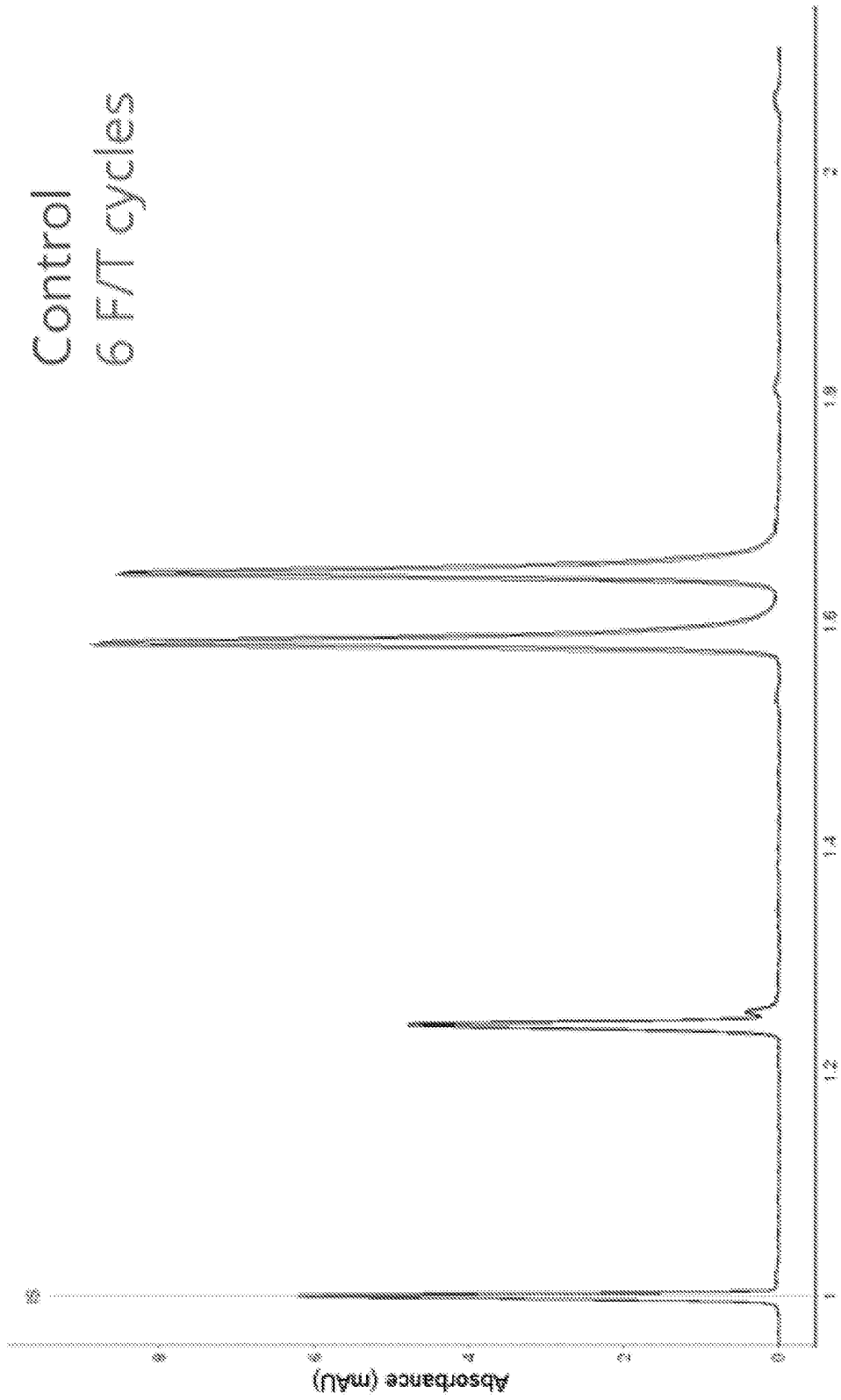


FIG. 274 Continued

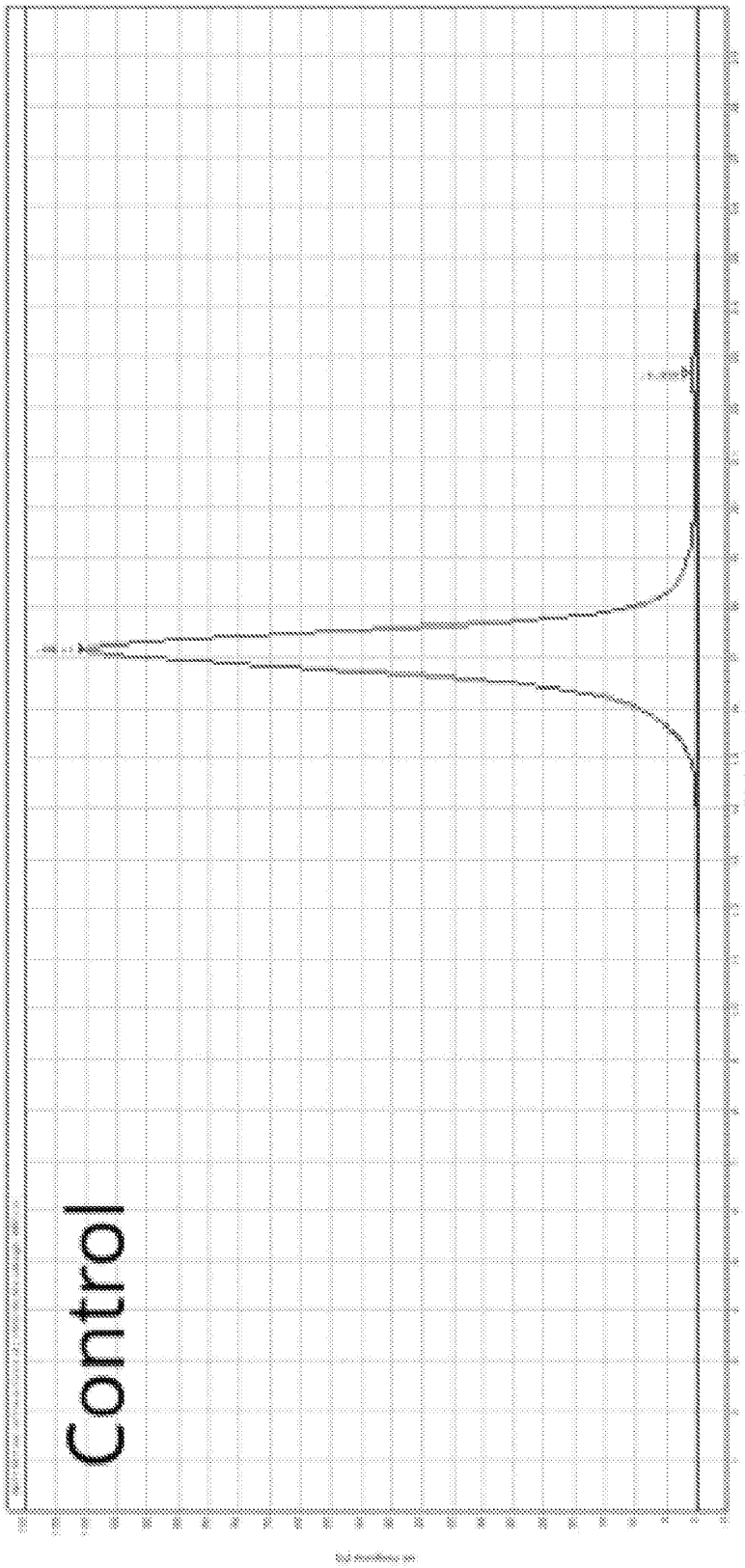


FIG. 275

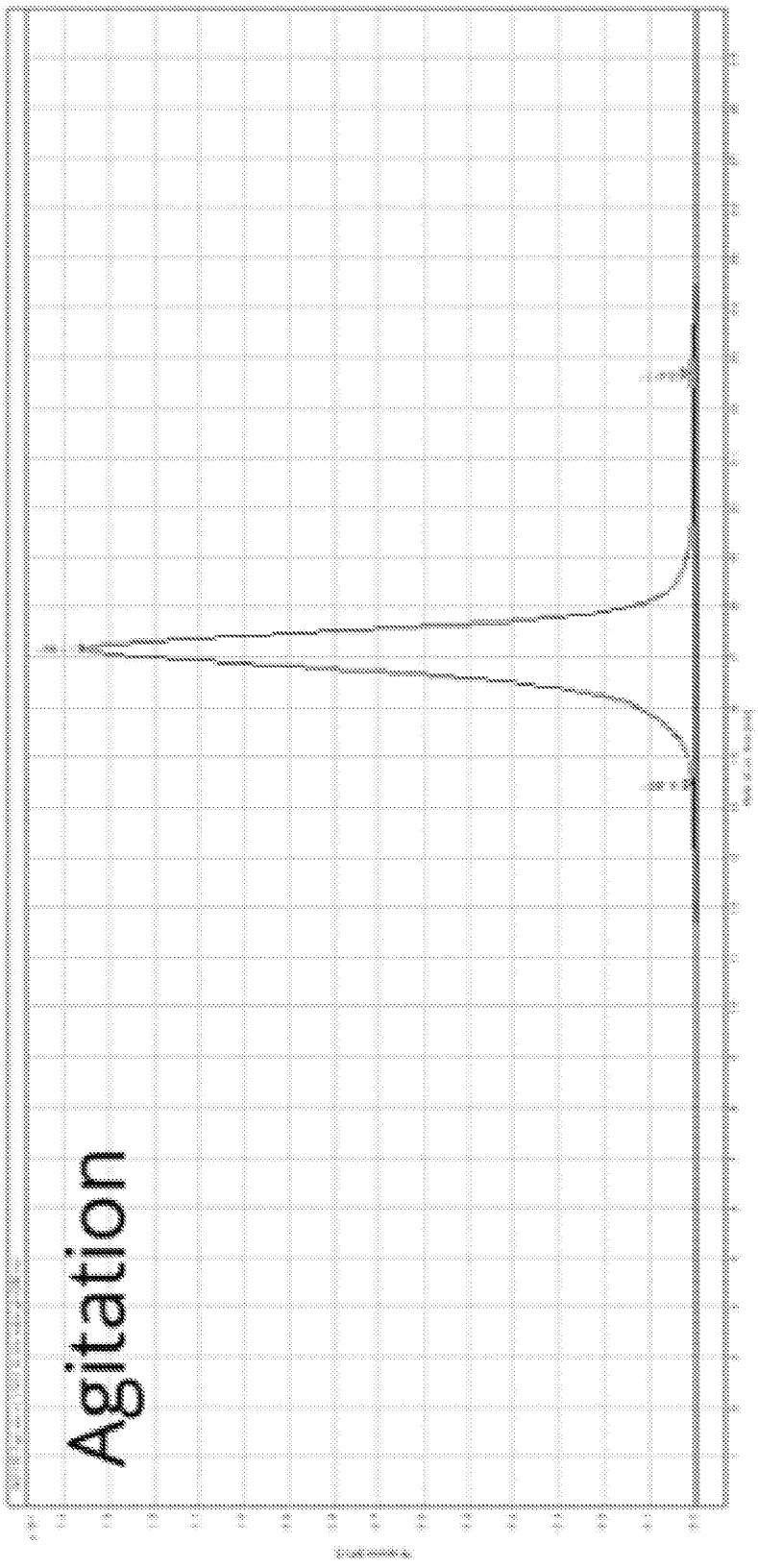


FIG. 275 Continued

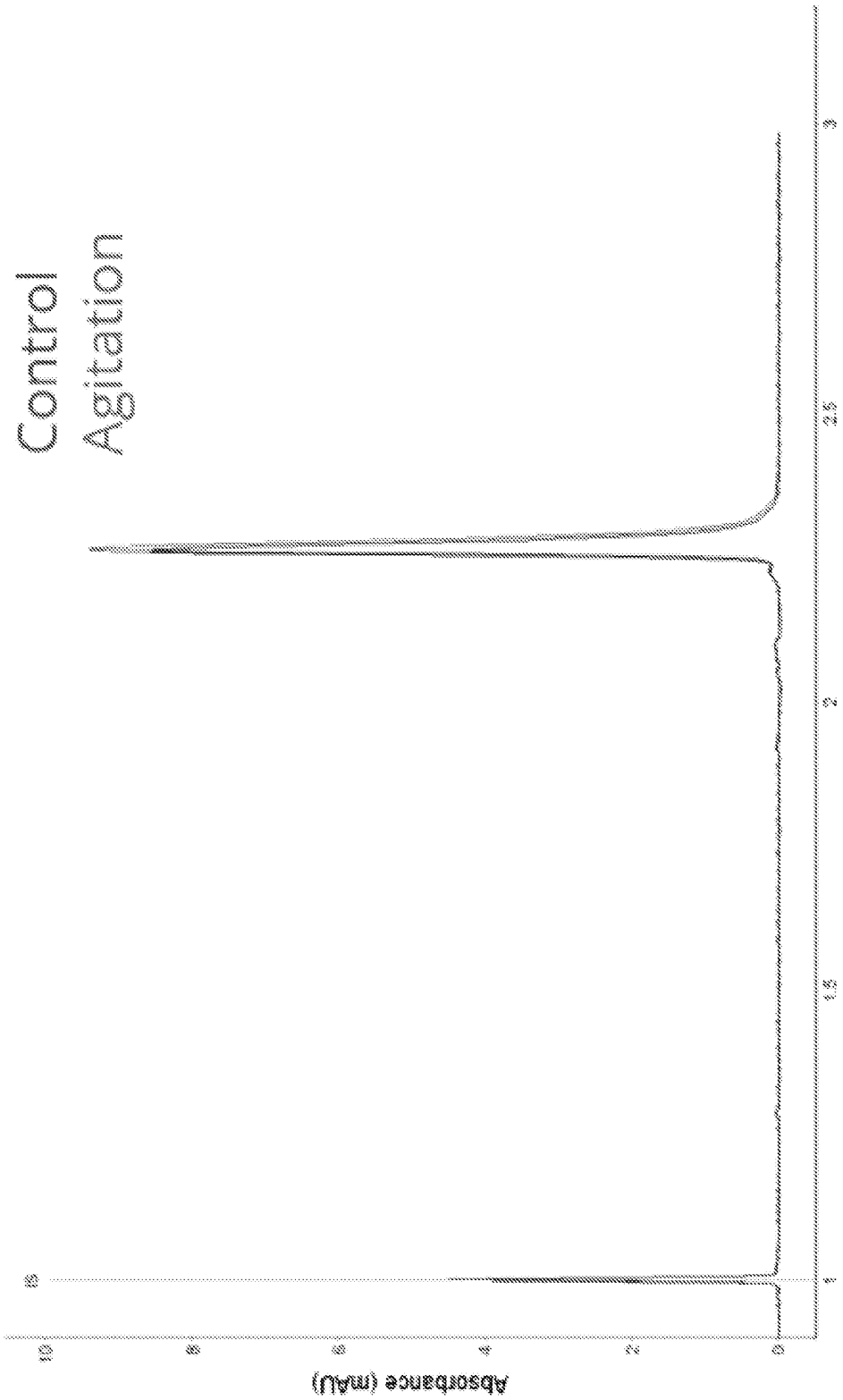

Control
Agitation
Absorbance (mAU)
Relative Migration Time

FIG. 276

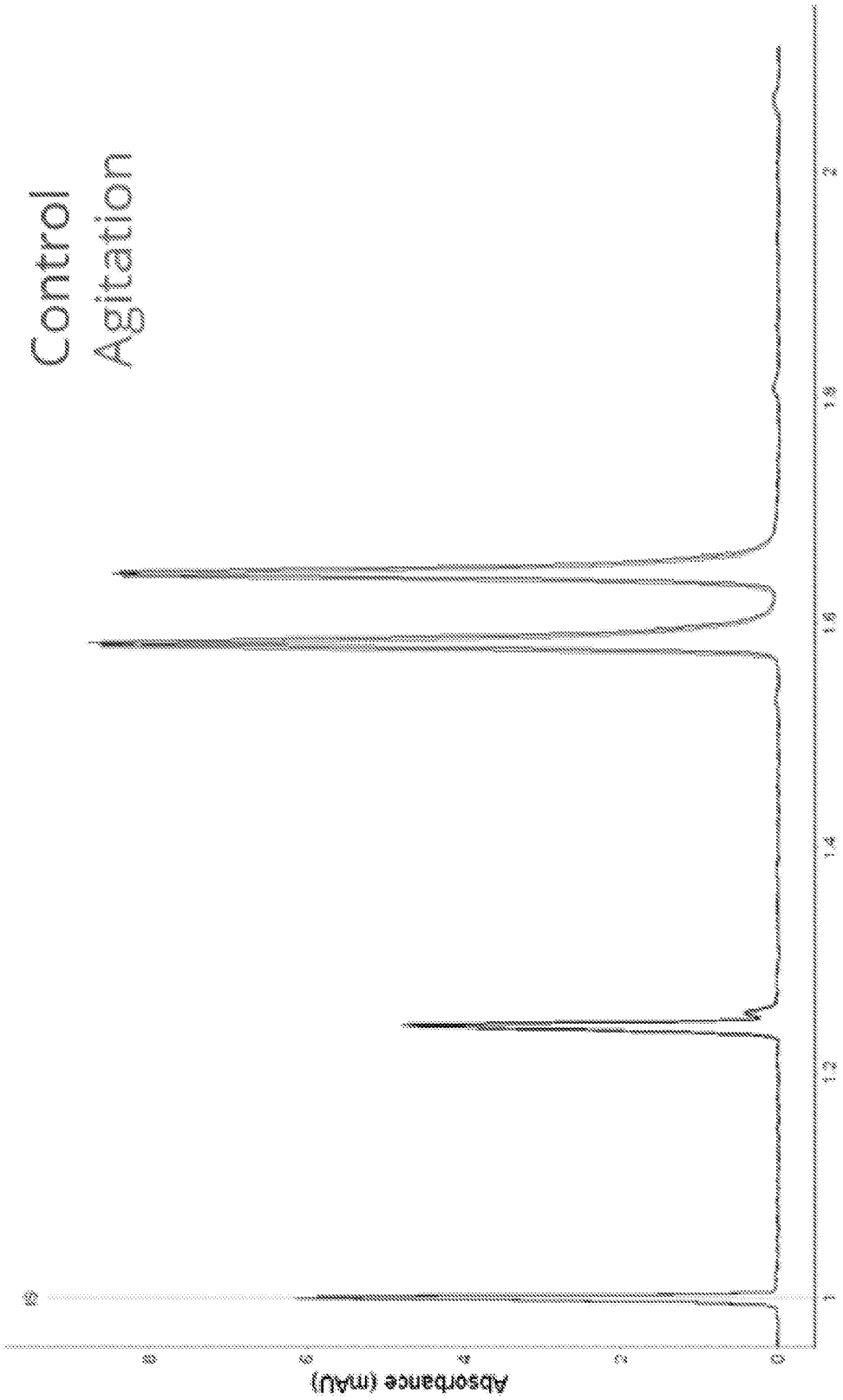


FIG. 276 Continued

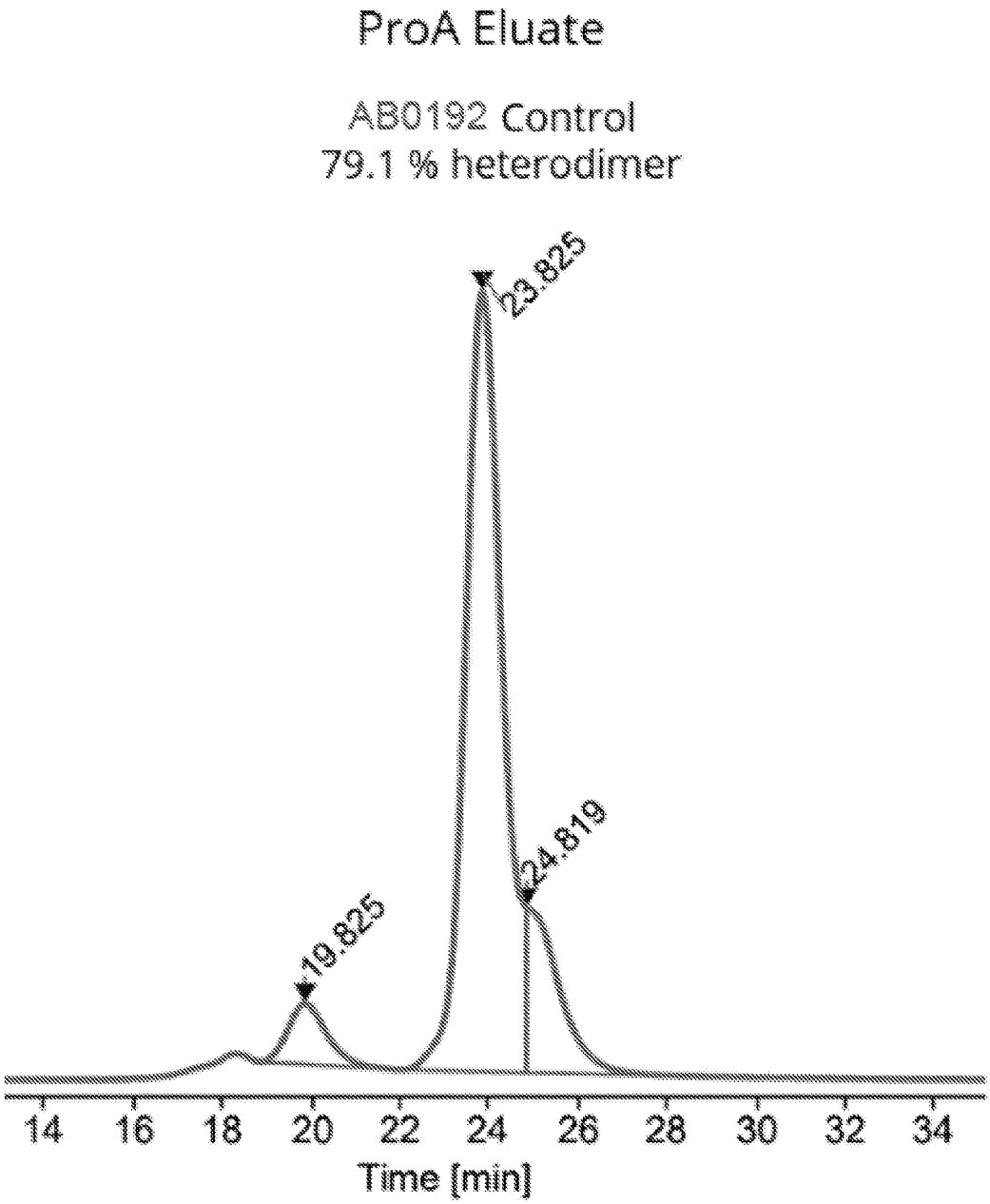

ProA Eluate
AB0192 Control
79.1 % heterodimer
23.825
24.819
19.825
14
16
18
20
22
24
26
28
30
32
34
Time [min]

FIG. 277

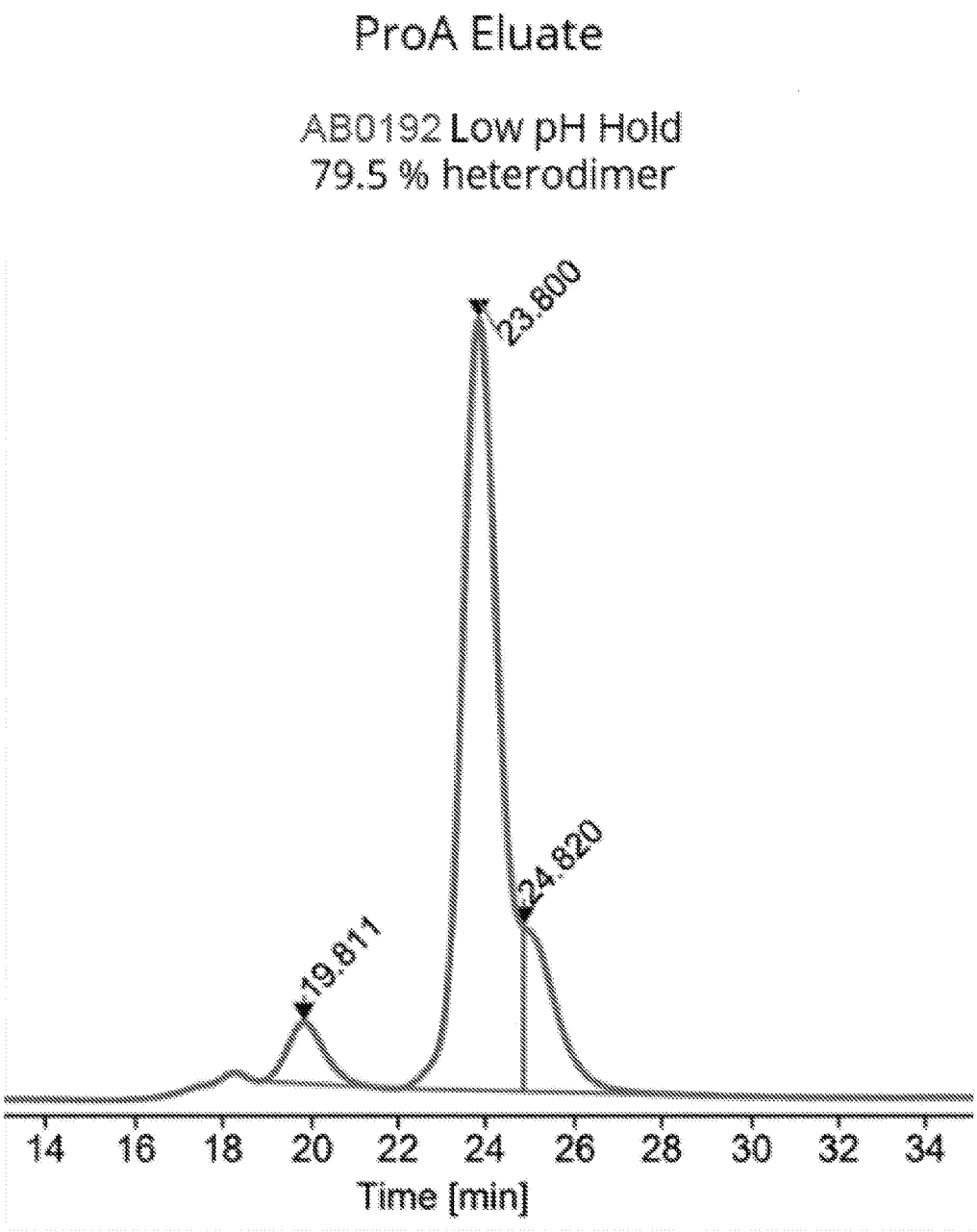
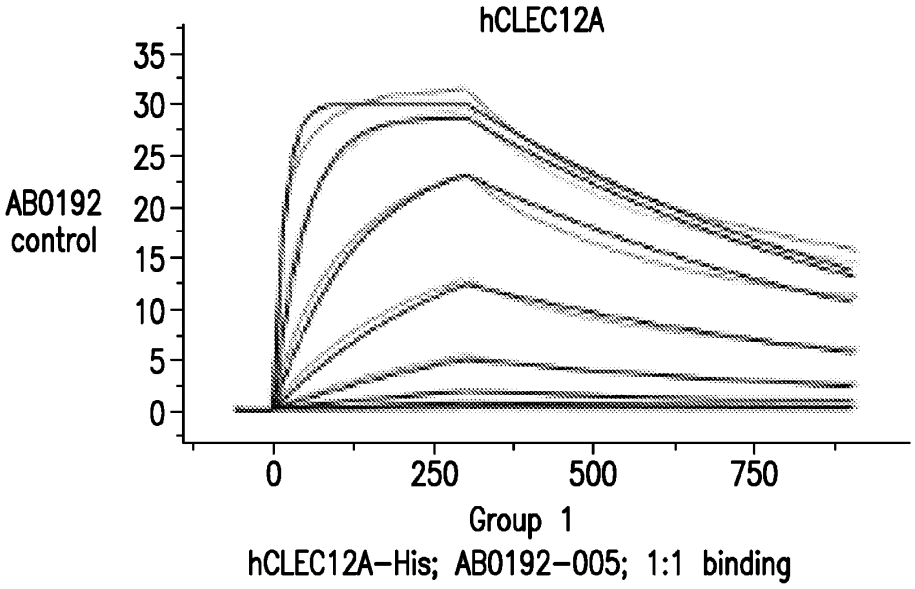


FIG. 277 Continued


Control
Low pH hold
Mkr 4.05
Mkr 9.89
Fluorescence
pI
20,000
15,000
10,000
5,000
0
4
5
6
7
8
9
10

FIG. 278


hCLEC12A
AB0192
control
35
30
25
20
15
10
5
0
0
250
500
750
Group 1
hCLEC12A-His; AB0192-005; 1:1 binding

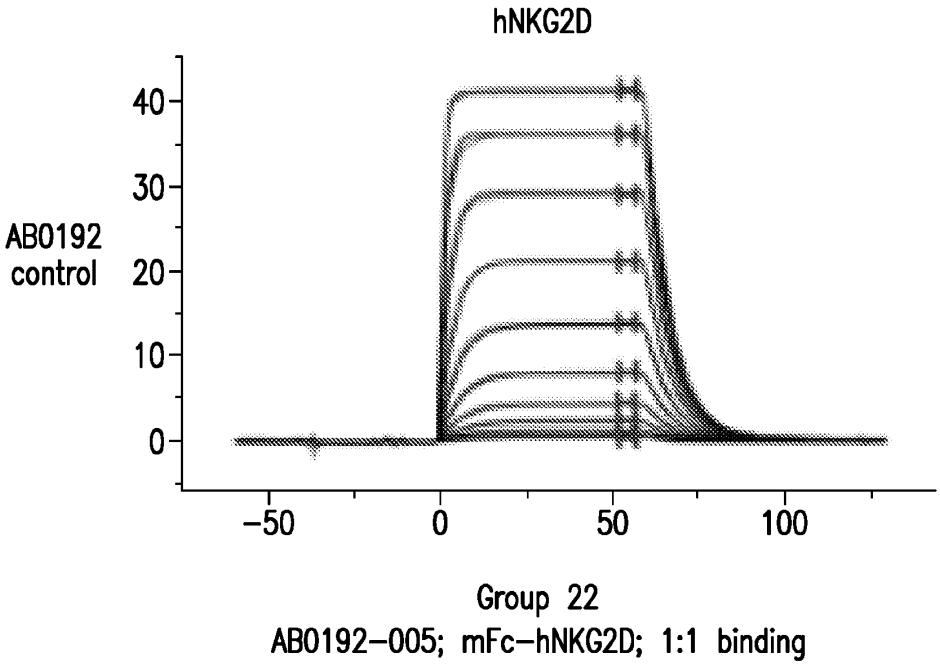
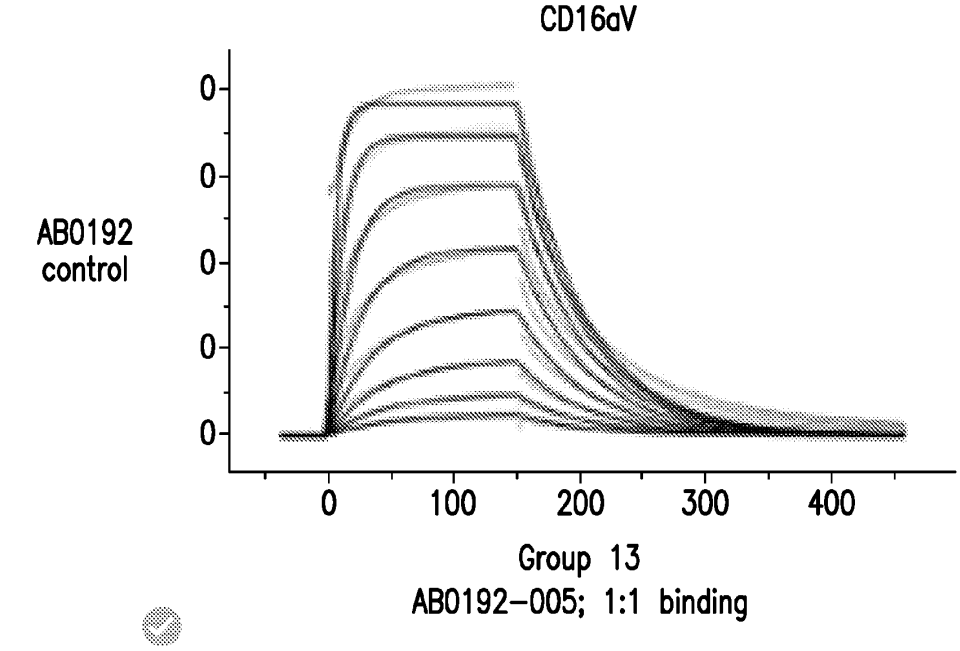

hNKG2D
AB0192
control
40
30
20
10
0
-50
0
50
100
Group 22
AB0192-005; mFc-hNKG2D; 1:1 binding

FIG. 279



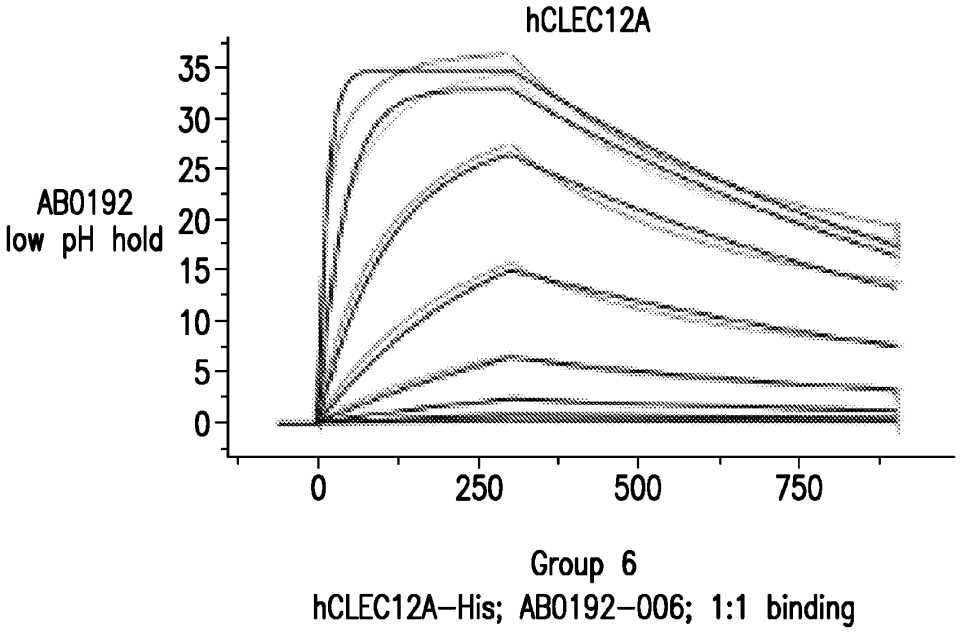
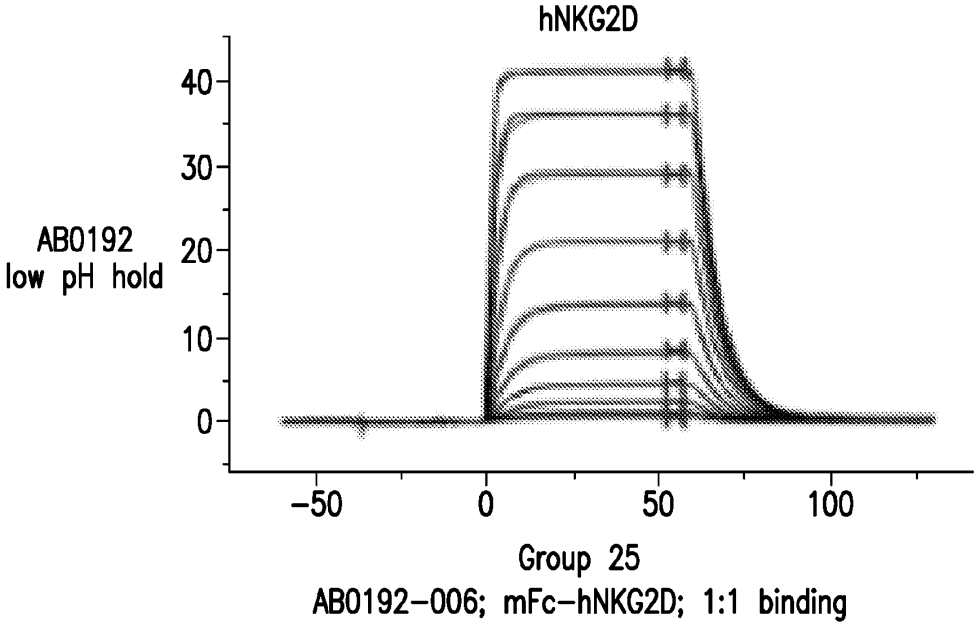


FIG. 279 Continued



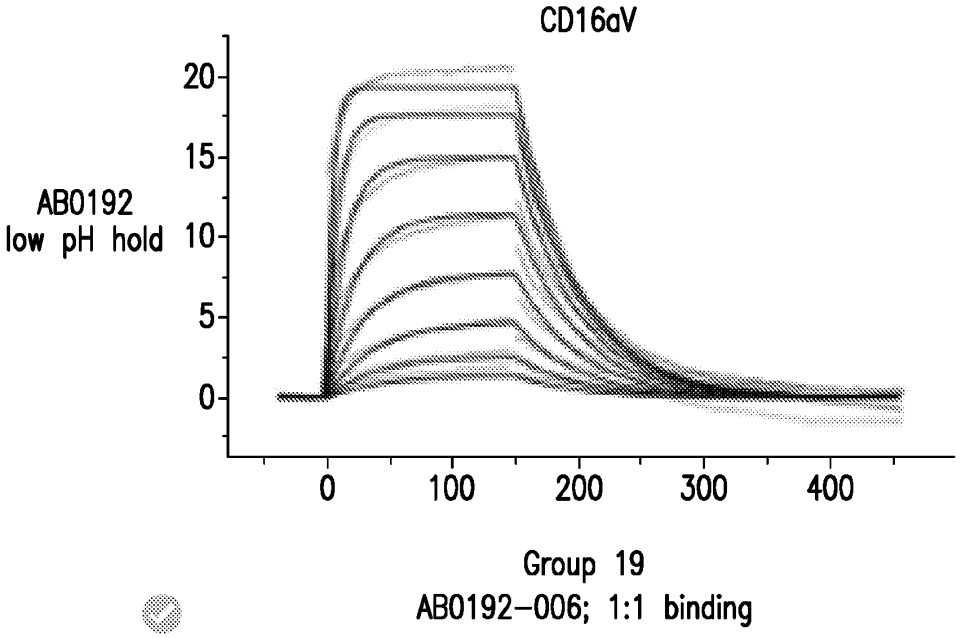
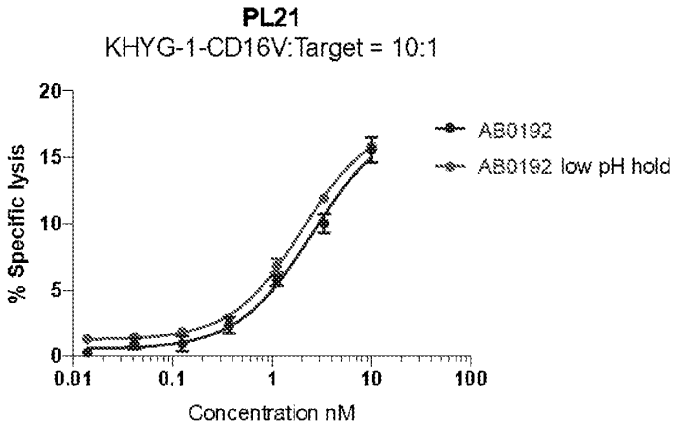
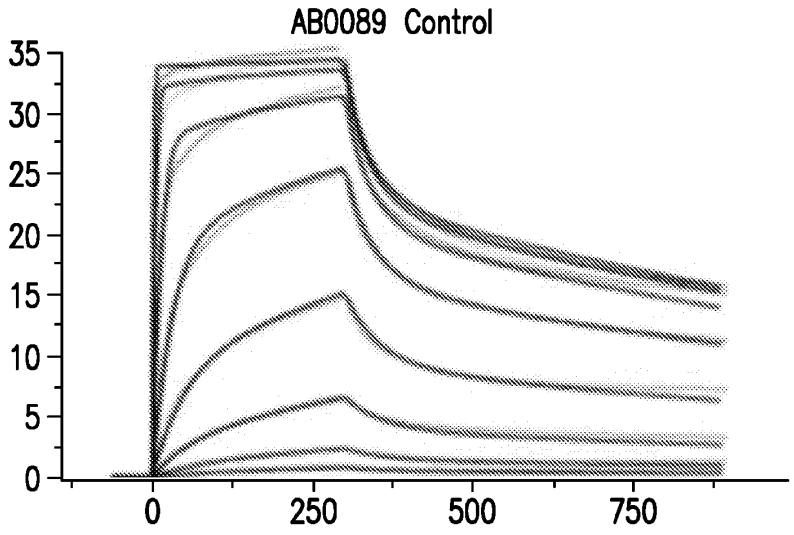


FIG. 279 Continued

FIG. 280


PL21
KHYG-1-CD16V:Target = 10:1
% Specific lysis
20
15
10
5
0
0.01
0.1
1
10
100
Concentration nM
AB0192
AB0192 low pH hold


AB0089 Control
35
30
25
20
15
10
5
0
0
250
500
750

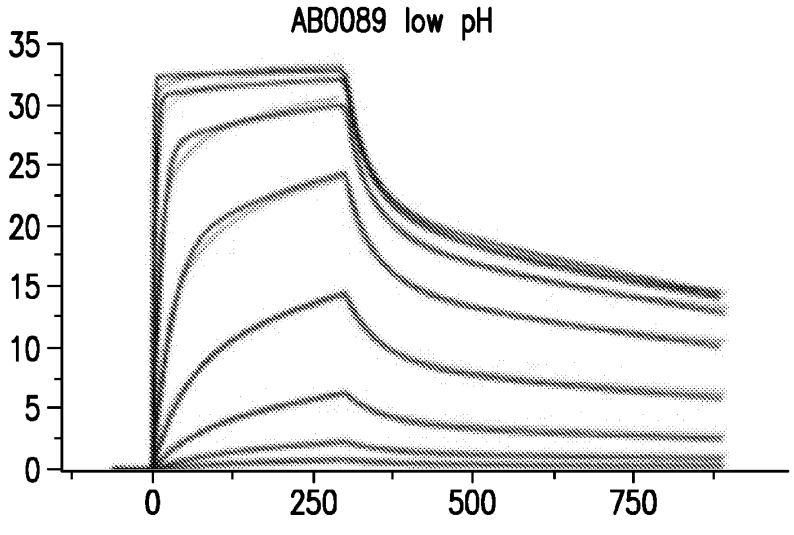
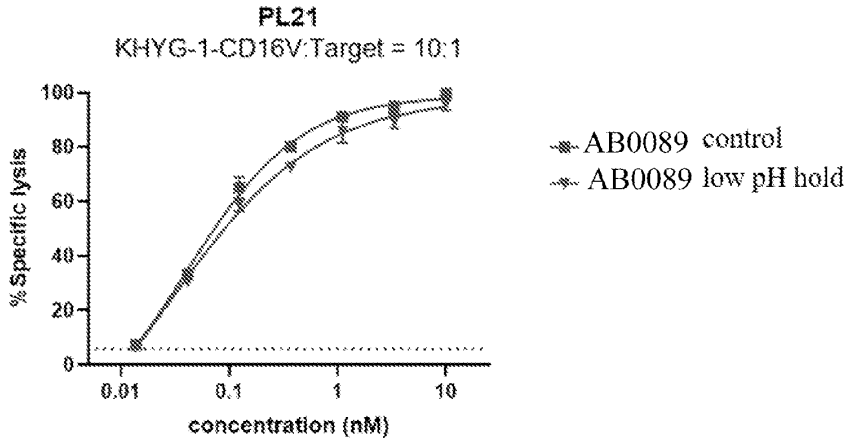

AB0089 low pH
35
30
25
20
15
10
5
0
0
250
500
750

FIG. 281

FIG. 282


PL21
KHYG-1-CD16V:Target = 10:1
% Specific lysis
100
80
60
40
20
0
0.01
0.1
1
10
concentration (nM)
AB0089 control
AB0089 low pH hold