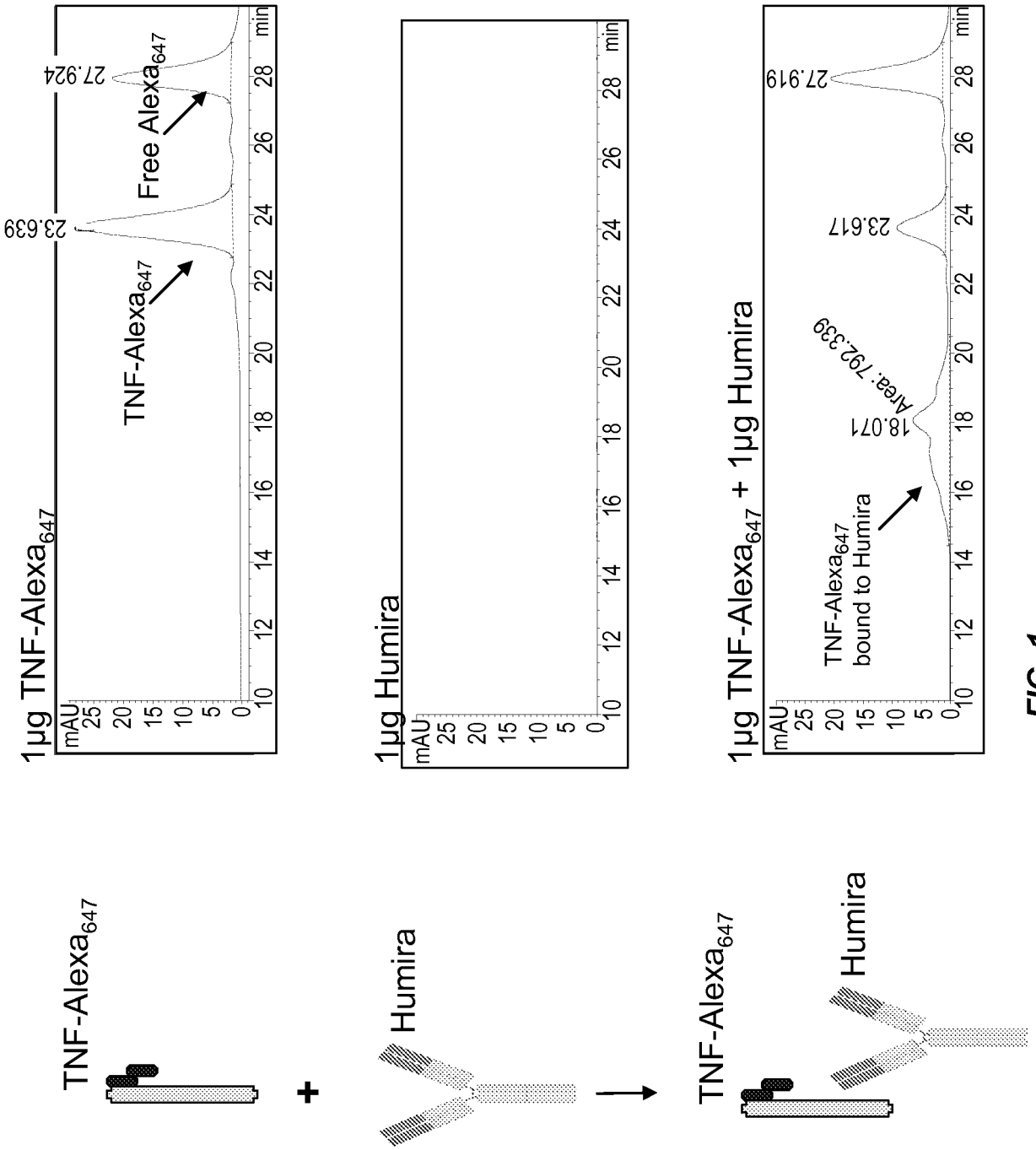

1µg TNF-Alexa647
mAU
min
23.639
27.924
TNF-Alexa647
Free Alexa647
1µg Humira
mAU
min
1µg TNF-Alexa647 + 1µg Humira
mAU
min
27.919
23.617
18.071
Area: 792.339
TNF-Alexa647 bound to Humira
TNF-Alexa647
Humira
TNF-Alexa647
Humira

FIG. 1

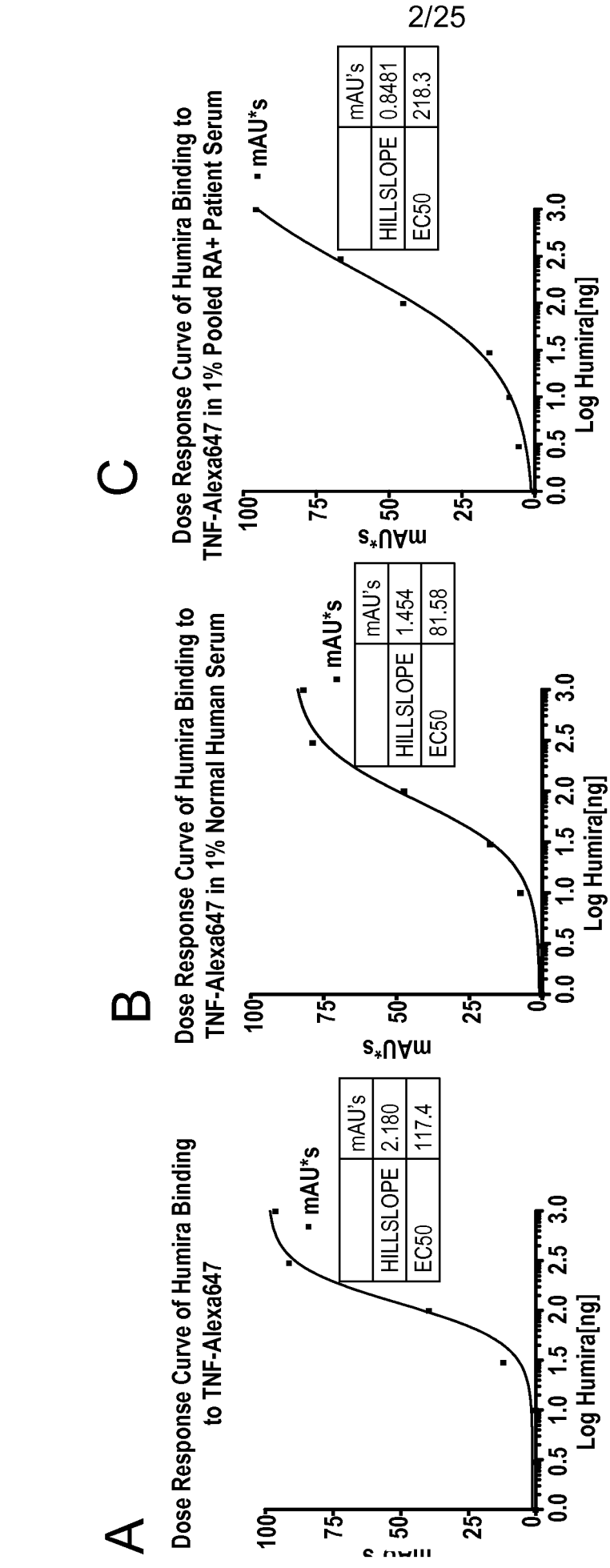

A
Dose Response Curve of Humira Binding to TNF-Alexa647
mAU*s
HILLSLOPE 2.180
EC50 117.4
mAU's
Log Humira[ng]
B
Dose Response Curve of Humira Binding to TNF-Alexa647 in 1% Normal Human Serum
mAU*s
HILLSLOPE 1.454
EC50 81.58
mAU's
Log Humira[ng]
C
Dose Response Curve of Humira Binding to TNF-Alexa647 in 1% Pooled RA+ Patient Serum
mAU*s
HILLSLOPE 0.8481
EC50 218.3
mAU's
Log Humira[ng]

FIG. 2

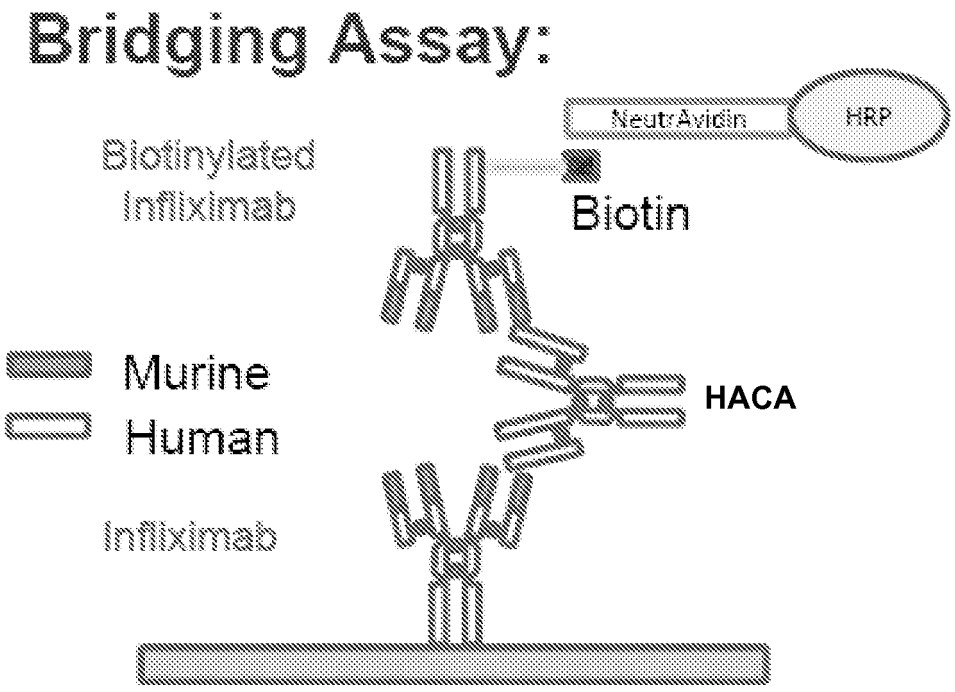

Bridging Assay:
NeutrAvidin
HRP
Biotinylated
Infliximab
Biotin
Murine
Human
HACA
Infliximab

FIG. 3

(1) Preparation of Alexa647-labeled Remicade
(2) Incubation with patient serum (HACA) to form the complex
(3) Quantification of the complex by SE-HPLC
(4) Assay works in the presence of remicade
Alexa647-Remicade
+
HACA
(1)
(2)
Incubation
MW = 150kD
MW = 150kD
HACA
SE-HPLC Analysis
(3)
Alexa647-Remicade
MW = 300kD

FIG. 4

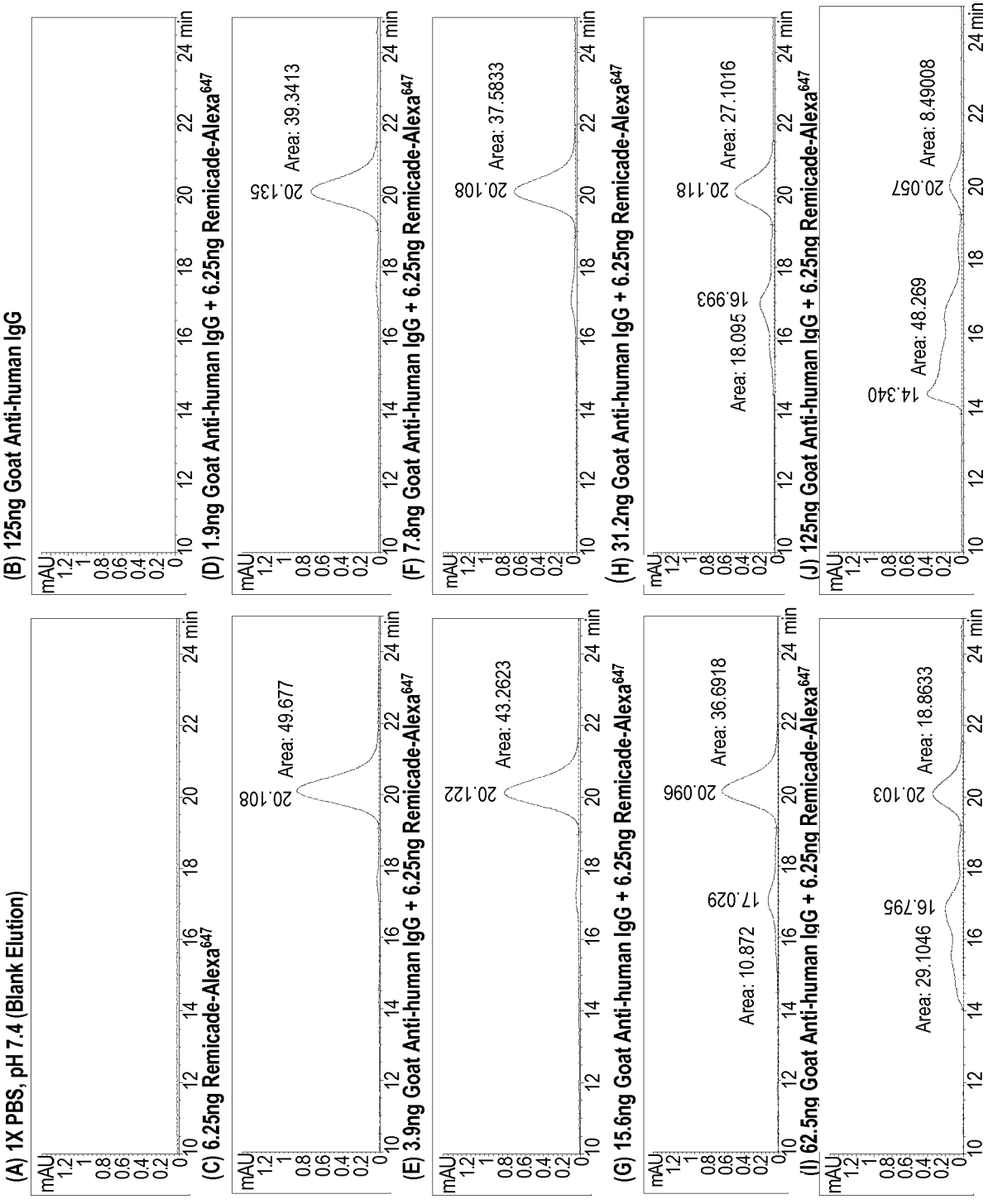

(A) 1X PBS, pH 7.4 (Blank Elution)
(B) 125ng Goat Anti-human IgG
(C) 6.25ng Remicade-Alexa647
(D) 1.9ng Goat Anti-human IgG + 6.25ng Remicade-Alexa647
(E) 3.9ng Goat Anti-human IgG + 6.25ng Remicade-Alexa647
(F) 7.8ng Goat Anti-human IgG + 6.25ng Remicade-Alexa647
(G) 15.6ng Goat Anti-human IgG + 6.25ng Remicade-Alexa647
(H) 31.2ng Goat Anti-human IgG + 6.25ng Remicade-Alexa647
(I) 62.5ng Goat Anti-human IgG + 6.25ng Remicade-Alexa647
(J) 125ng Goat Anti-human IgG + 6.25ng Remicade-Alexa647
mAU
min
Area: 49.677
Area: 39.3413
Area: 43.2623
Area: 37.5833
Area: 36.6918
Area: 27.1016
Area: 10.872
Area: 18.095
Area: 29.1046
Area: 18.8633
Area: 48.269
Area: 8.49008

FIG. 5

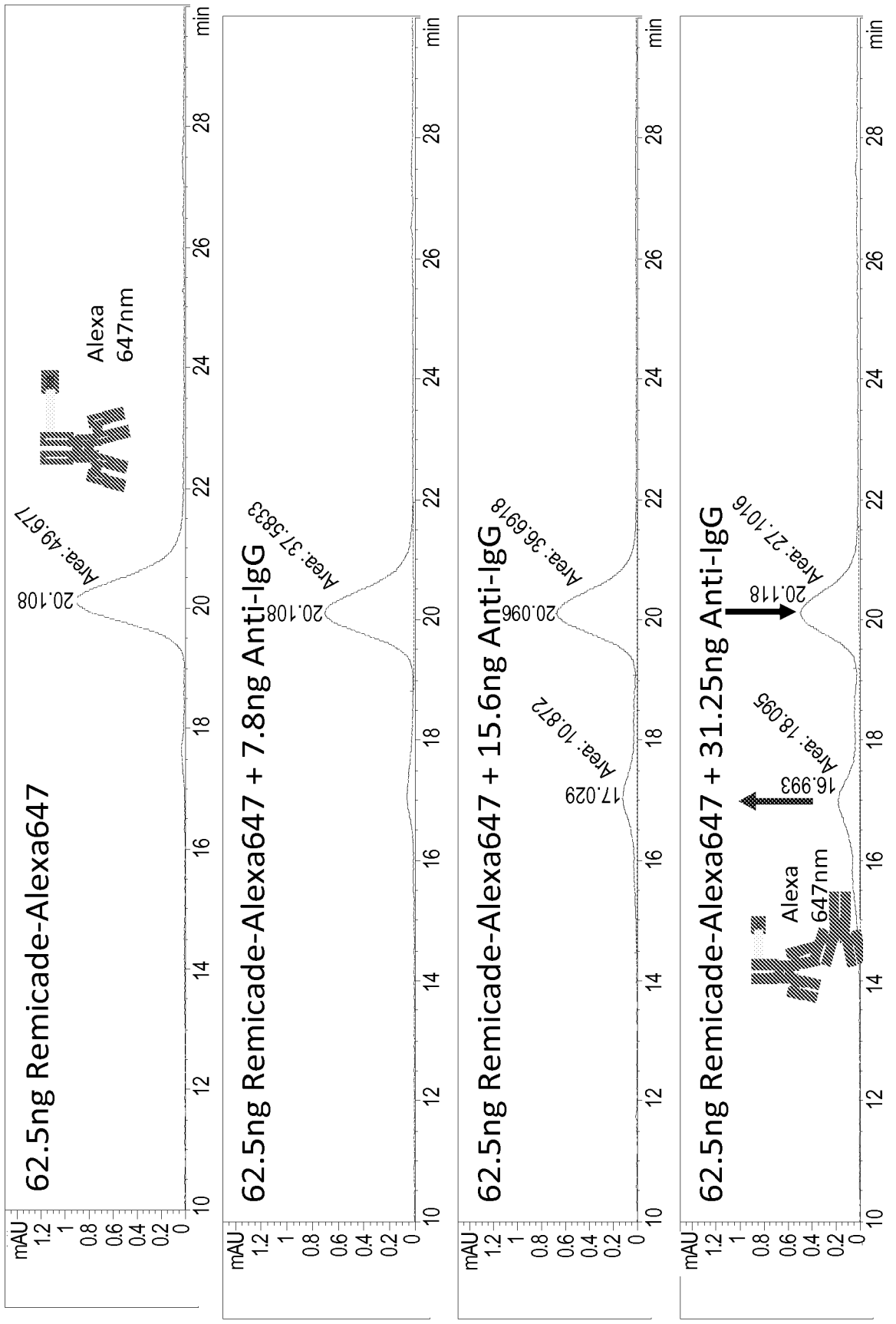

62.5ng Remicade-Alexa647
Area: 49.677
20.108
Alexa
647nm
62.5ng Remicade-Alexa647 + 7.8ng Anti-IgG
Area: 37.5833
20.108
62.5ng Remicade-Alexa647 + 15.6ng Anti-IgG
Area: 36.6978
20.096
Area: 10.872
17.029
62.5ng Remicade-Alexa647 + 31.25ng Anti-IgG
Area: 27.1016
20.118
Area: 18.095
16.993
Alexa
647nm
mAU
min

FIG. 6

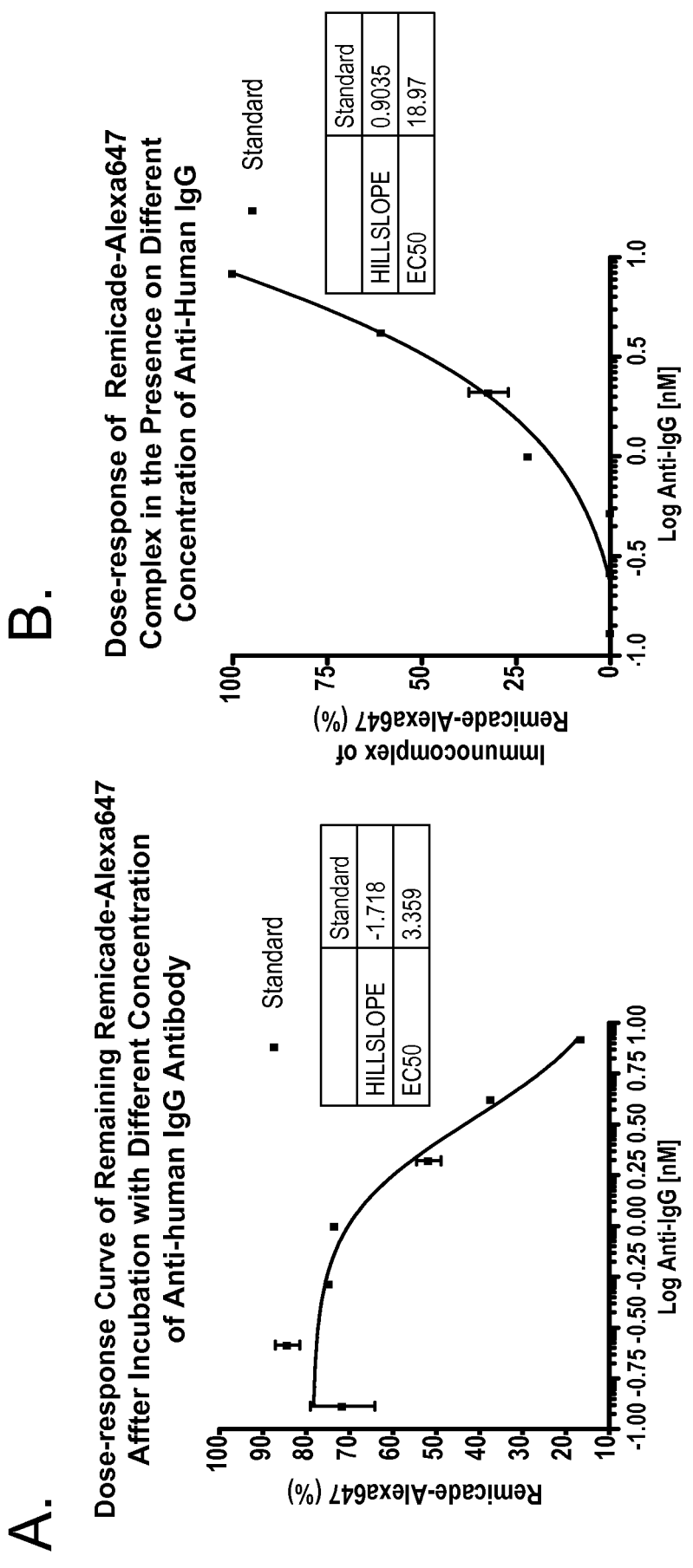

A.
Dose-response Curve of Remaining Remicade-Alexa647
Affter Incubation with Different Concentration
of Anti-human IgG Antibody
Remicade-Alexa647 (%)
Log Anti-IgG [nM]
Standard
HILLSLOPE -1.718
EC50 3.359
B.
Dose-response of Remicade-Alexa647
Complex in the Presence on Different
Concentration of Anti-Human IgG
Immunocomplex of
Remicade-Alexa647 (%)
Log Anti-IgG [nM]
Standard
HILLSLOPE 0.9035
EC50 18.97

FIG. 7

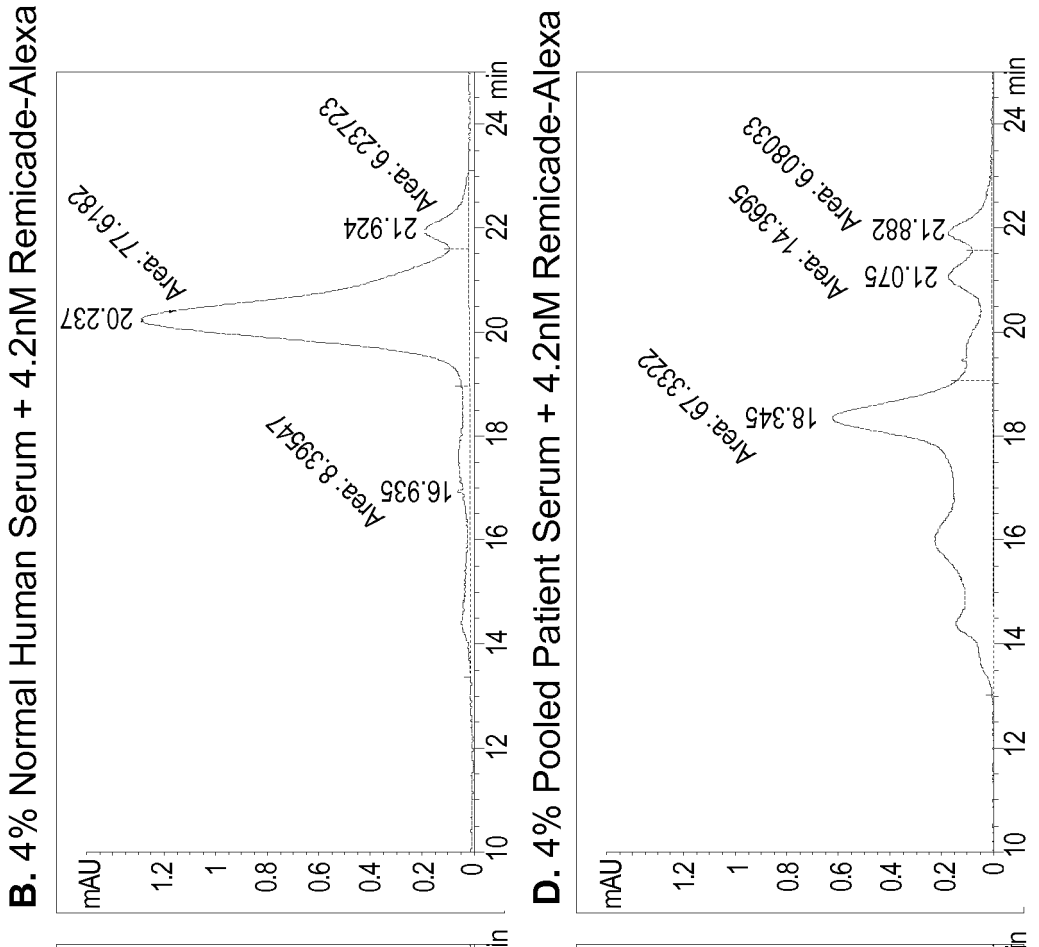

A. 4% Normal Human Serum
B. 4% Normal Human Serum + 4.2nM Remicade-Alexa
C. 4% Pooled Patient Serum
D. 4% Pooled Patient Serum + 4.2nM Remicade-Alexa
mAU
min
20.975 Area: 7.72633
21.882 Area: 6.14403
20.237 Area: 77.6182
16.935 Area: 8.39547
21.924 Area: 6.23723
14.265 Area: 9.24852
20.972 Area: 7.28035
21.845 Area: 5.03851
18.345 Area: 67.3322
21.075 Area: 14.3695
21.882 Area: 6.08033

FIG. 8

	Bridge ELISA		Mobility Shift Assay (4% Serum)		
Acession #	Quantitative Result	Qualitative Result	HACA Shift Assay	HACA Area/Remicade-647 Area	Remicade (nM)
SK07010477	22.26	Positive	Positive	0.78	3.33
SK07060083	1.41	Negative	Negative	**0.1**	4.06
SK07070083	1.41	Negative	Negative	**0.1**	8.81
SK07070305	1.41	Negative	Positive	0.46	7.34
SK07070595	1.41	Negative	Positive	0.25	8.35
SK07071213	2.48	Positive	Positive	0.16	5.30
SK07081127	22.07	Positive	Positive	0.28	3.00
SK07110035	1.41	Negative	Positive	0.18	**>66.7**
SK07141447	2.62	Positive	Positive	0.42	2.43
SK07171059	10.11	Positive	Positive	18.02	2.59
SK07171095	10.03	Positive	Positive	0.24	2.24
SK07210210	9.26	Positive	Positive	0.8	<0.67
SK07231216	25.58	Positive	Positive	Complete Shift	1.34
SK07310149	2.74	Positive	Positive	0.21	<0.67
SK08040168	22.21	Positive	Positive	Complete Shift	<0.67
SK08051035	9.72	Positive	Positive	8.7	1.89
SK08070307	2.49	Positive	Positive	0.23	3.14
SK08120222	9.2	Positive	Positive	0.25	<0.67
SK08260093	23.15	Positive	Positive	0.48	1.04
SK08260783	2.67	Positive	Positive	0.25	3.30
62.5ng Remicade - 647				**0.12**	
100ng TNF - 647					<0.67

FIG. 9

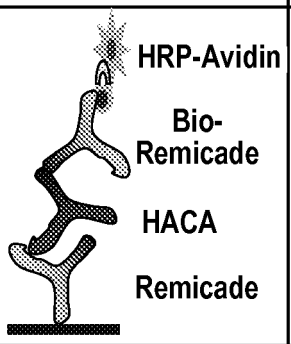
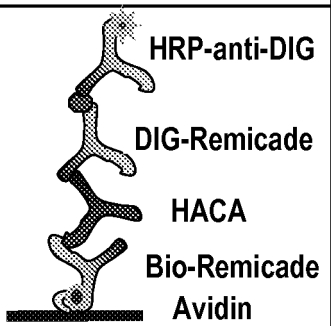
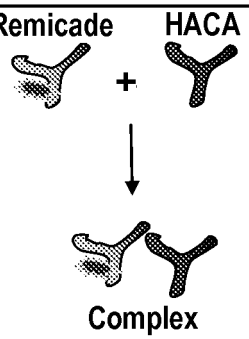
	Bridge HACA Assay	Biotin and DIG-Based Homogeneous Bridging ELISA	HACA Assay of the Present Invention
Assay Format			
Non-specific Background Interference	High	High	Low
Sensitivity	Low	Medium	High
Possibility of False-Positive and False-Negative	High	High	Low
IgG4 HACA Detection	No	No	Yes
Ig Isotype identification	No	No	Yes
Tolerance of Drug in the Sample	Poor	Poor	Good

FIG. 10

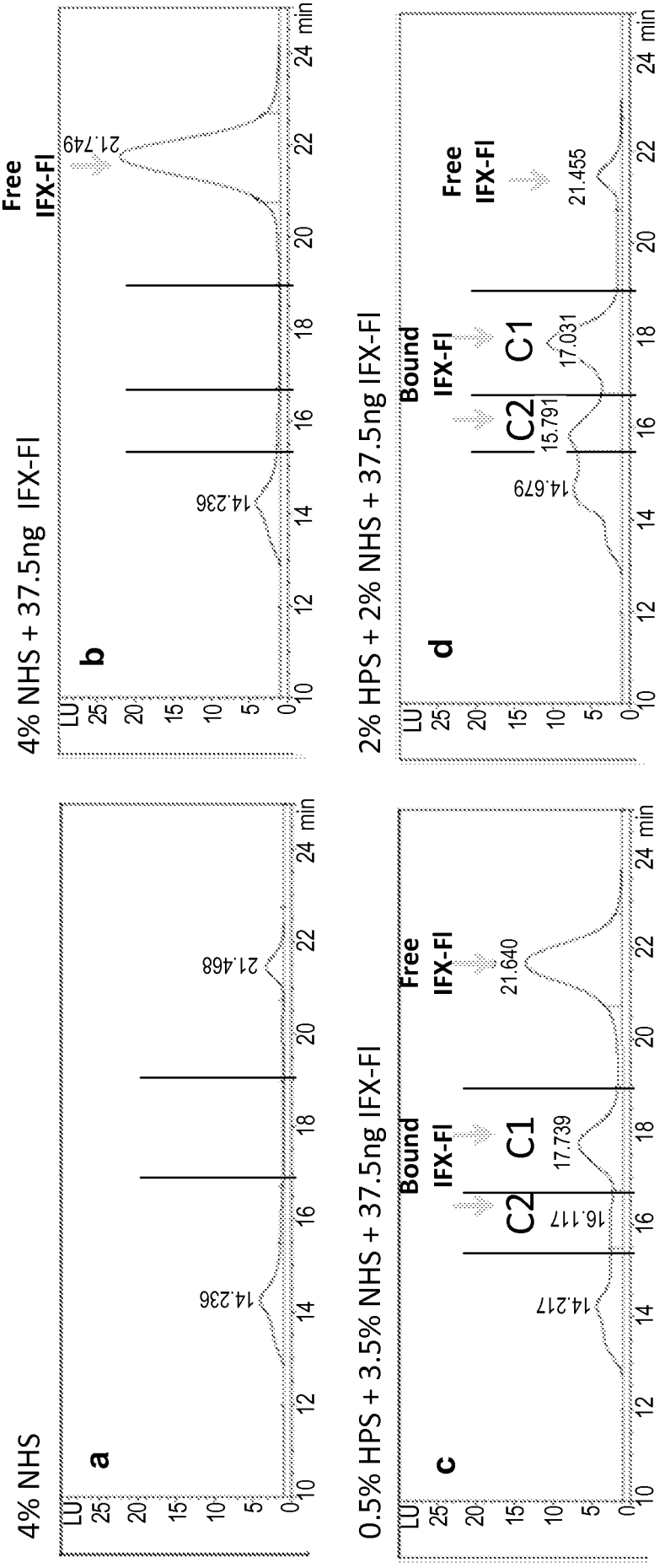

4% NHS
a
LU
25
20
15
10
5
0
10
12
14
16
18
20
22
24 min
14.236
21.468
4% NHS + 37.5ng IFX-FI
b
21.749
14.236
Free
IFX-FI
0.5% HPS + 3.5% NHS + 37.5ng IFX-FI
c
C2
C1
16.117
17.739
14.217
21.640
Bound
IFX-FI
Free
IFX-FI
2% HPS + 2% NHS + 37.5ng IFX-FI
d
C2
C1
15.791
17.031
14.679
21.455
Bound
IFX-FI
Free
IFX-FI

FIG. 11

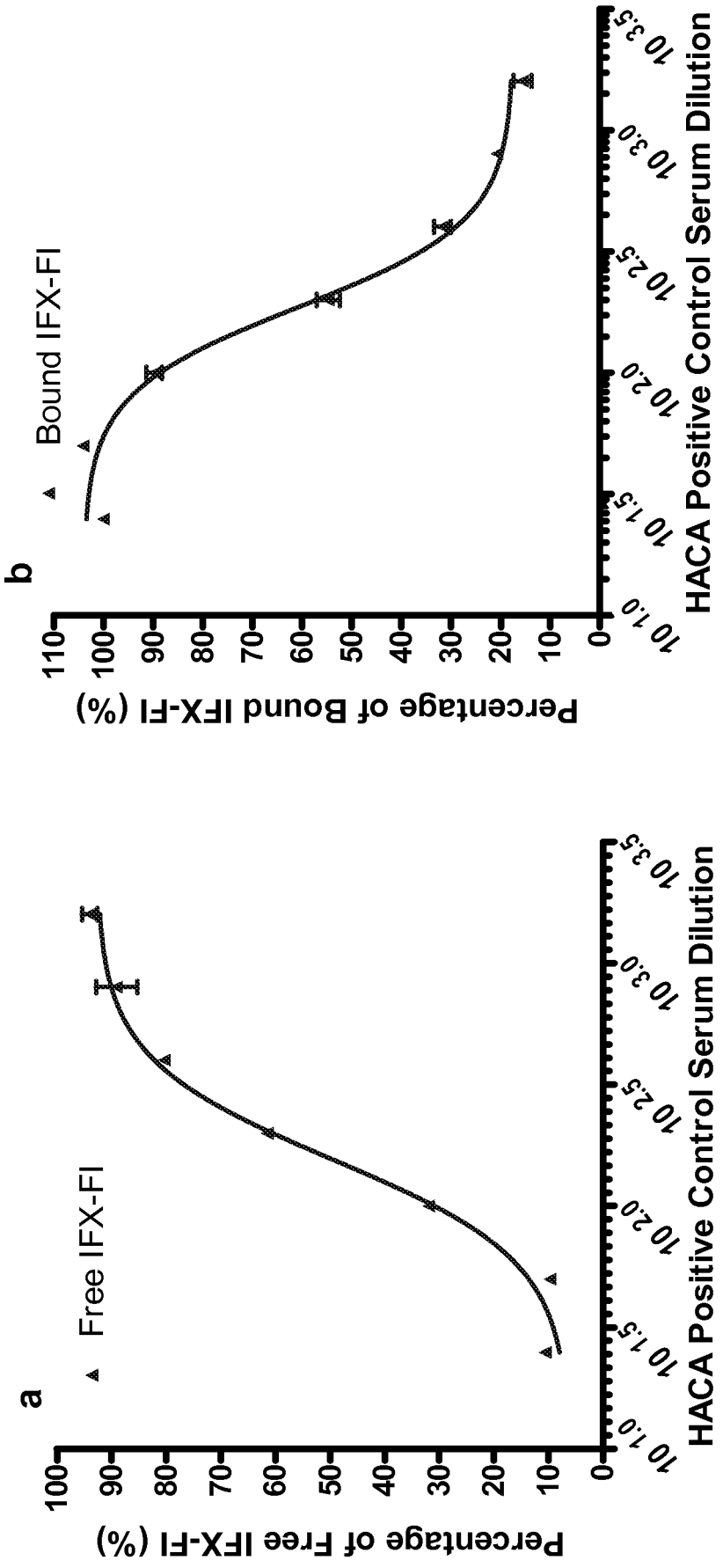

a
Free IFX-FI
Percentage of Free IFX-FI (%)
HACA Positive Control Serum Dilution
b
Bound IFX-FI
Percentage of Bound IFX-FI (%)
HACA Positive Control Serum Dilution

FIG. 12

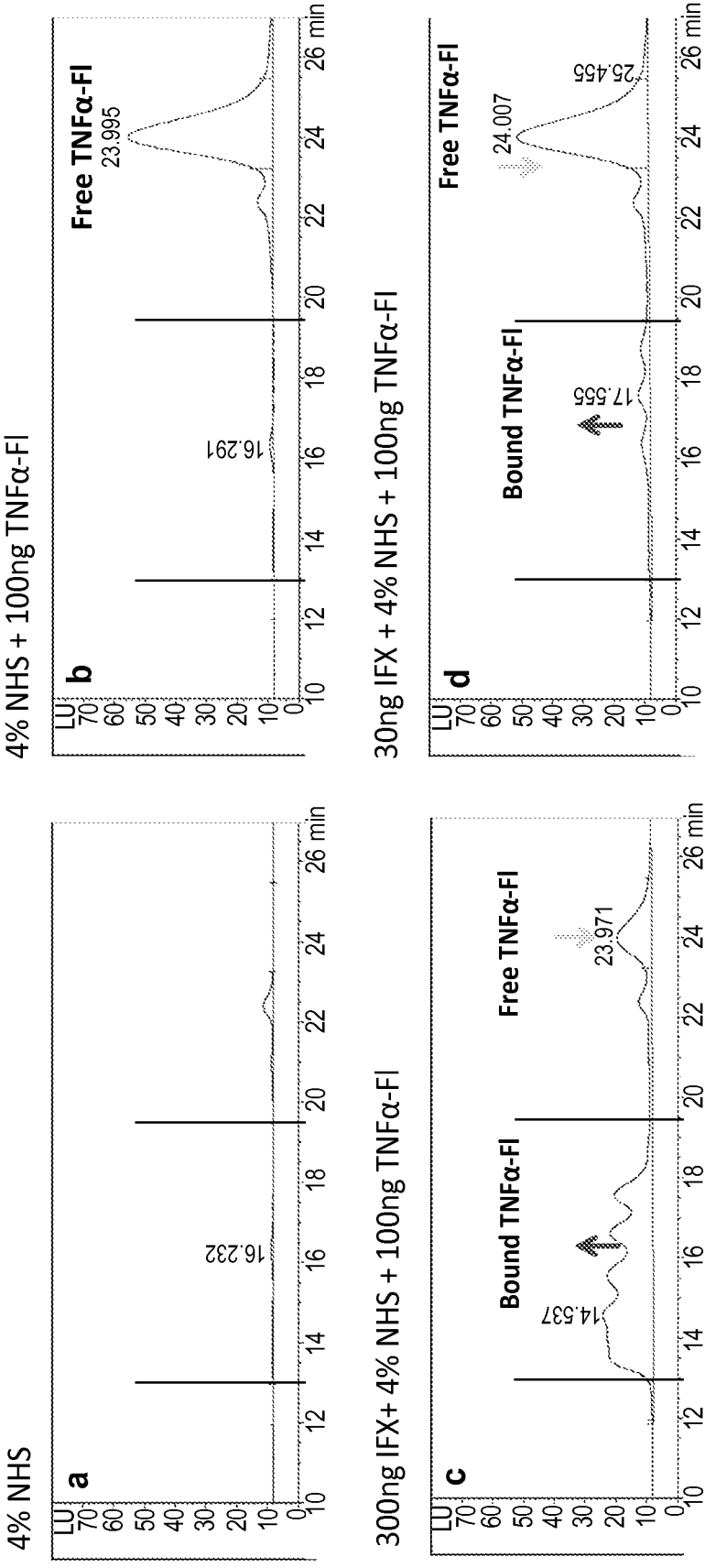

4% NHS
a
LU
16.232
4% NHS + 100ng TNFα-Fl
b
Free TNFα-Fl
23.995
16.291
300ng IFX+ 4% NHS + 100ng TNFα-Fl
c
Bound TNFα-Fl
14.537
Free TNFα-Fl
23.971
30ng IFX + 4% NHS + 100ng TNFα-Fl
d
Free TNFα-Fl
24.007
25.455
Bound TNFα-Fl
17.555
min

FIG. 13

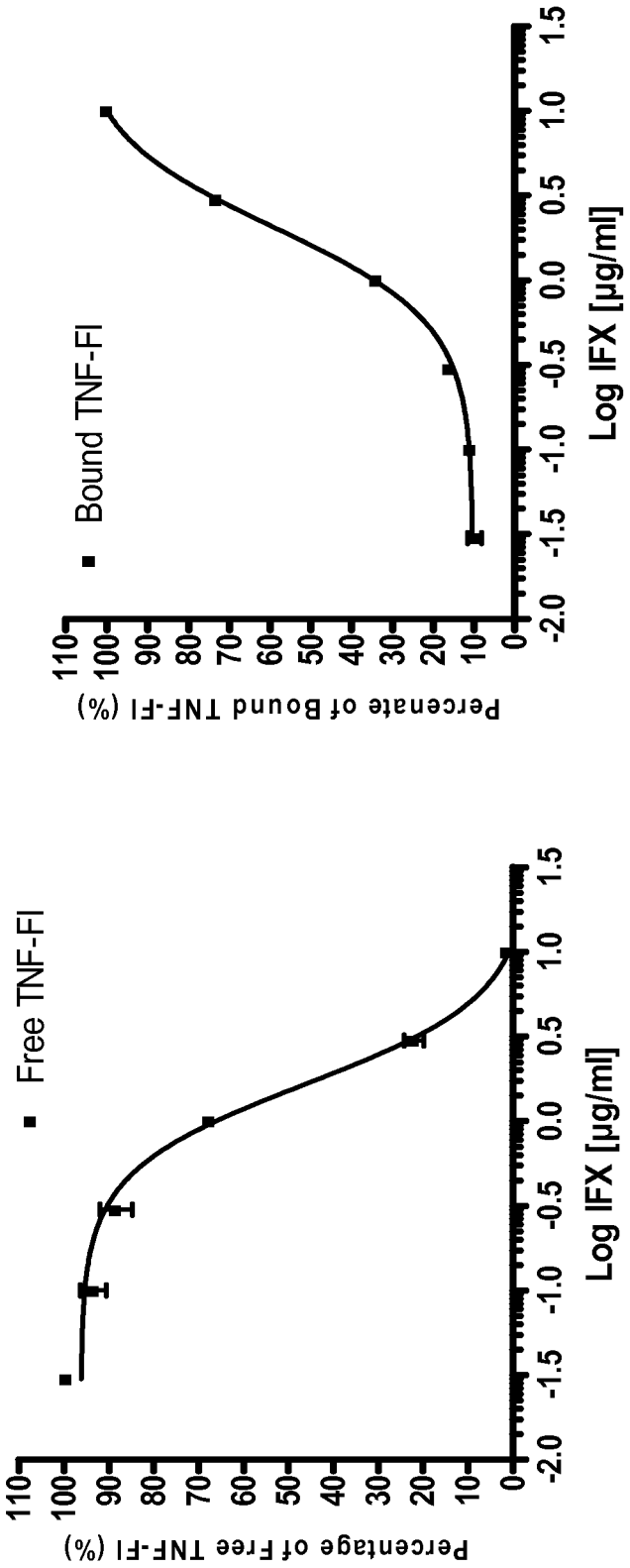

Percentage of Free TNF-FI (%)
110
100
90
80
70
60
50
40
30
20
10
0
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
Log IFX [µg/ml]
Free TNF-FI
Percentage of Bound TNF-FI (%)
Log IFX [µg/ml]
Bound TNF-FI

FIG. 14

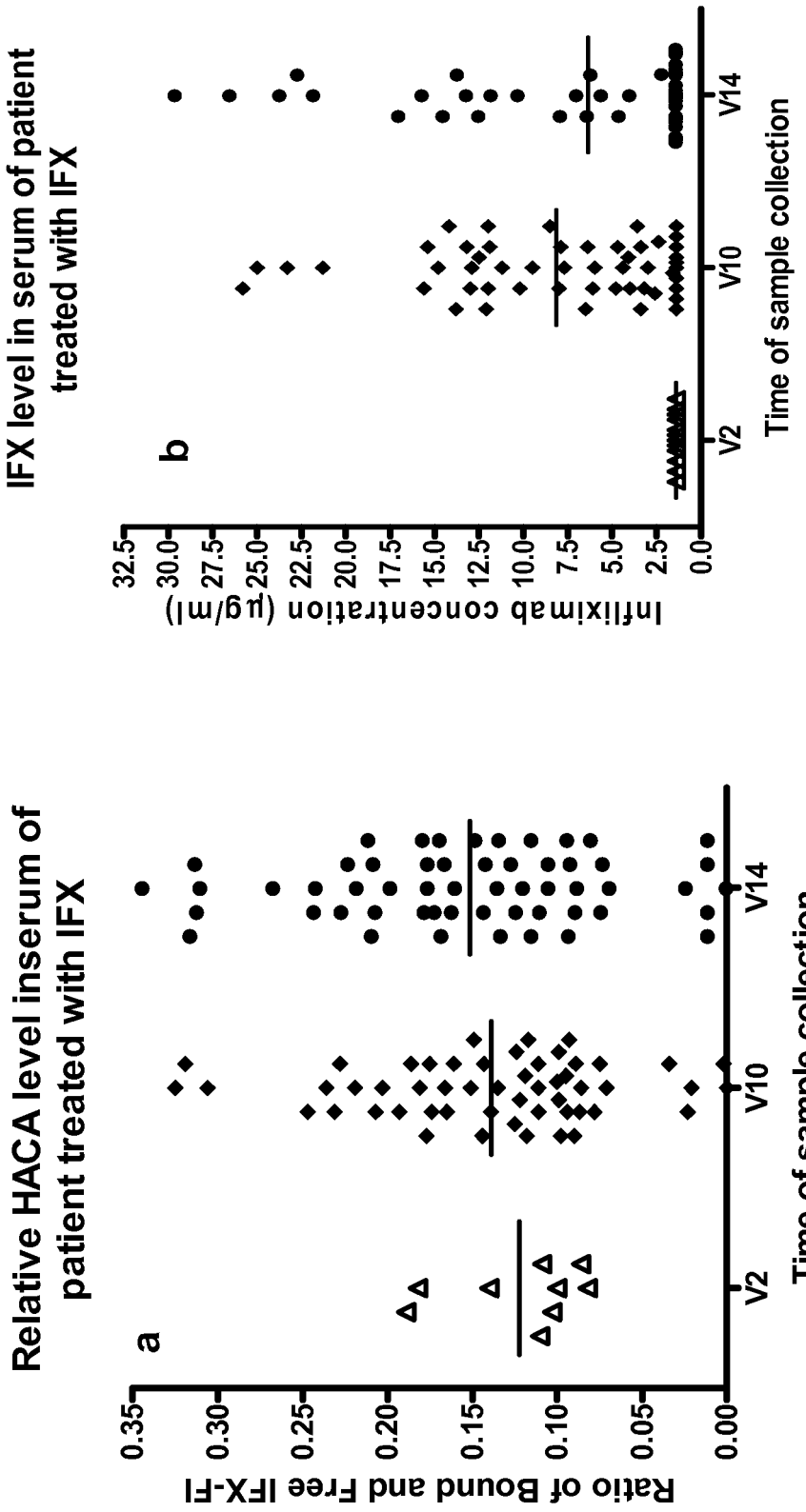

Relative HACA level inserum of patient treated with IFX
a
Ratio of Bound and Free IFX-FI
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
V2
V10
V14
Time of sample collection
IFX level in serum of patient treated with IFX
b
Infliximab concentration (µg/ml)
32.5
30.0
27.5
25.0
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
V2
V10
V14
Time of sample collection

FIG. 15

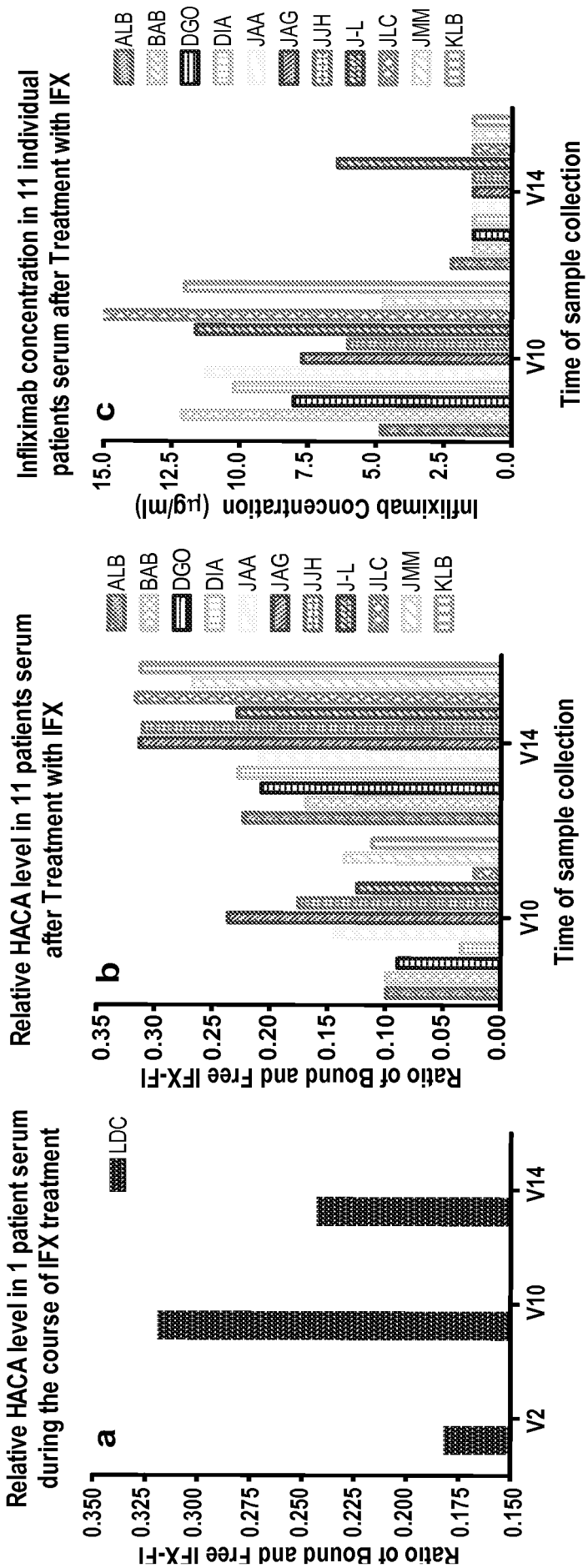
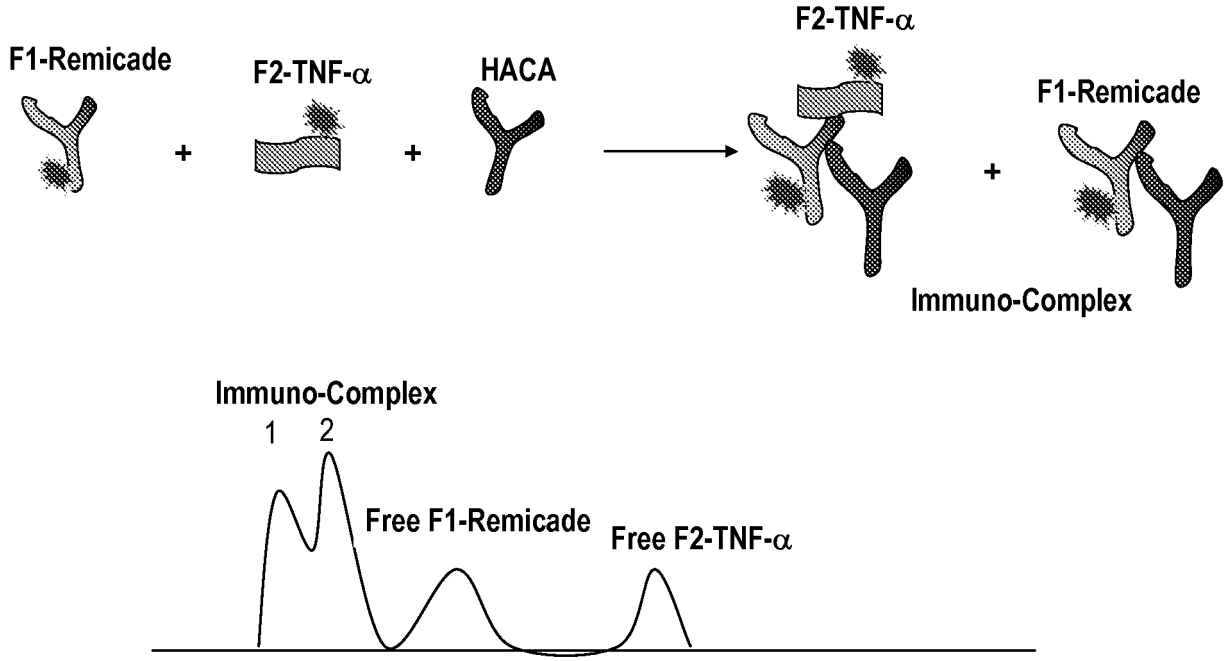

Relative HACA level in 1 patient serum during the course of IFX treatment
a
LDC
Ratio of Bound and Free IFX-Fl
0.350
0.325
0.300
0.275
0.250
0.225
0.200
0.175
0.150
V2
V10
V14
Relative HACA level in 11 patients serum after Treatment with IFX
b
ALB
BAB
DGO
DIA
JAA
JAG
JJH
J-L
JLC
JMM
KLB
Ratio of Bound and Free IFX-Fl
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
V10
V14
Time of sample collection
Infliximab concentration in 11 individual patients serum after Treatment with IFX
c
Infliximab Concentration (µg/ml)
15.0
12.5
10.0
7.5
5.0
2.5
0.0
V10
V14
Time of sample collection

FIG. 16

A. Non-neutralizing HACA Assay



B. Neutralizing HACA Assay

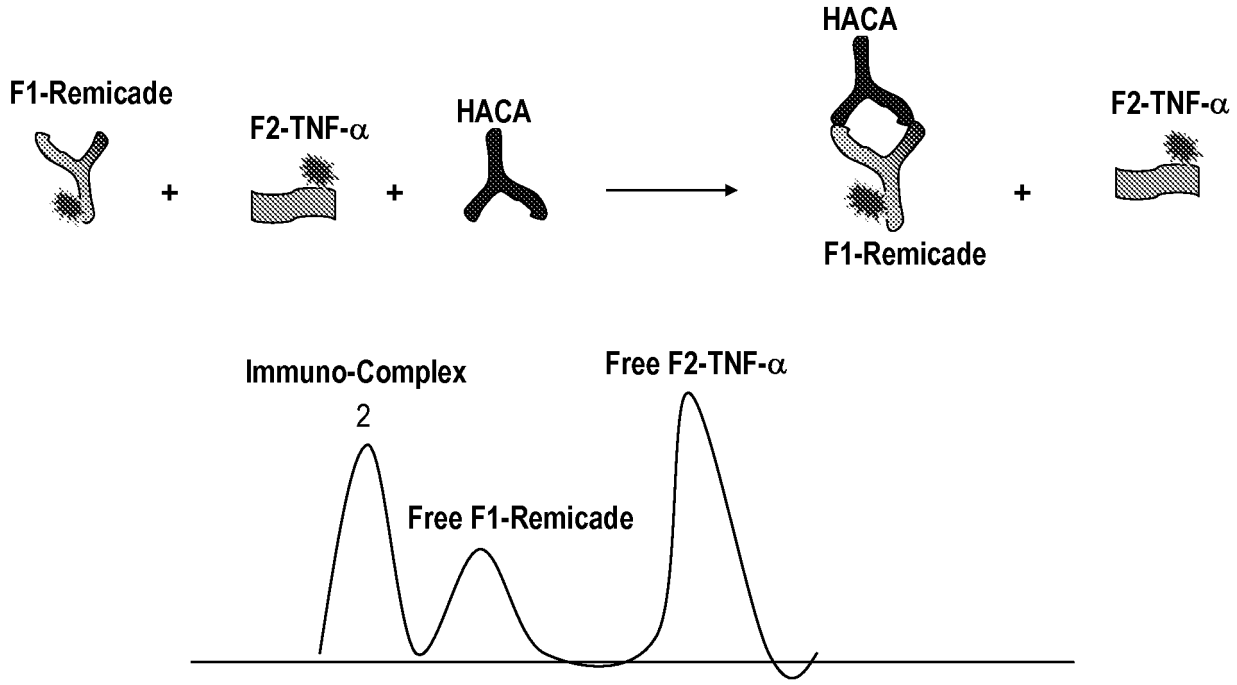


FIG. 17

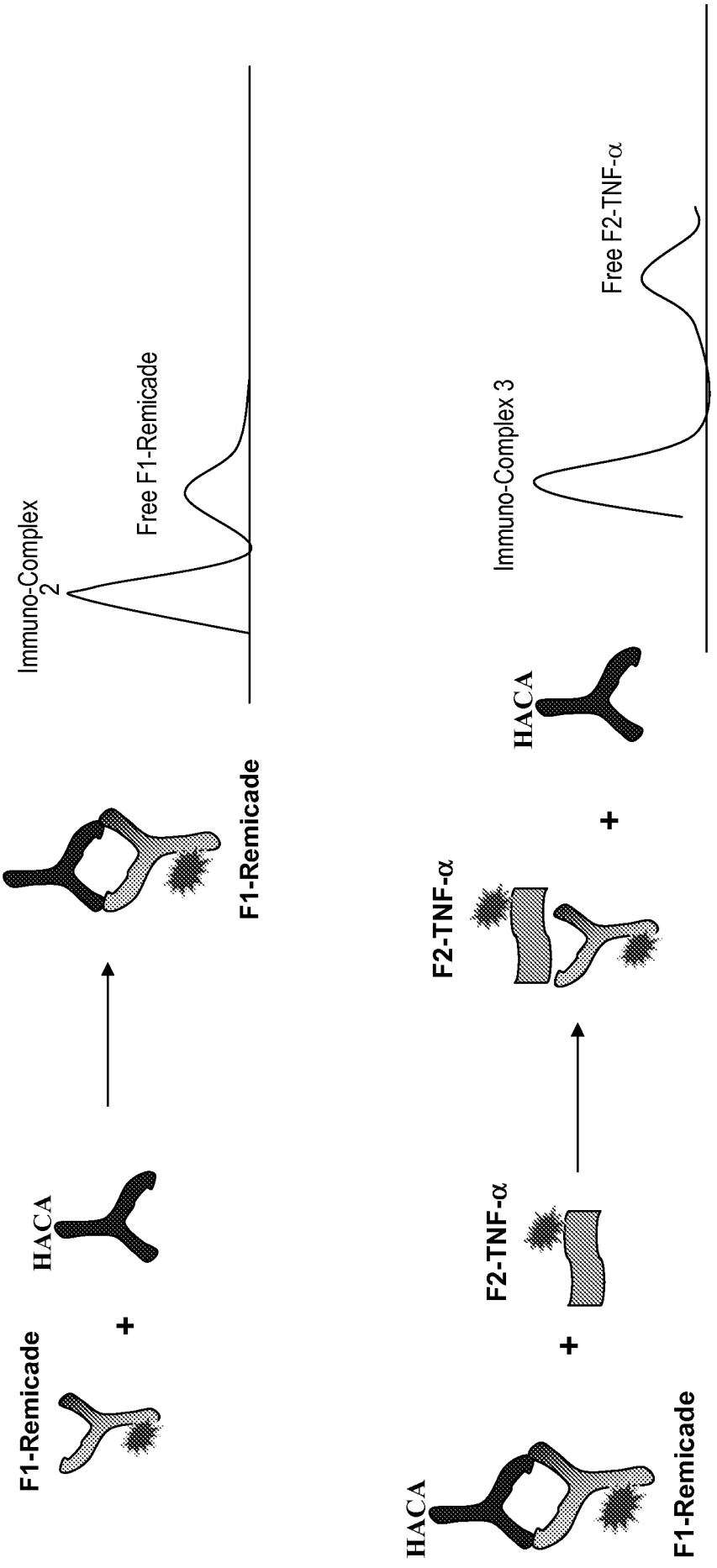

F1-Remicade
+
HACA
HACA
F1-Remicade
Immuno-Complex 2
Free F1-Remicade
F2-TNF-α
+
HACA
F2-TNF-α
+
HACA
F1-Remicade
Immuno-Complex 3
Free F2-TNF-α

FIG. 18

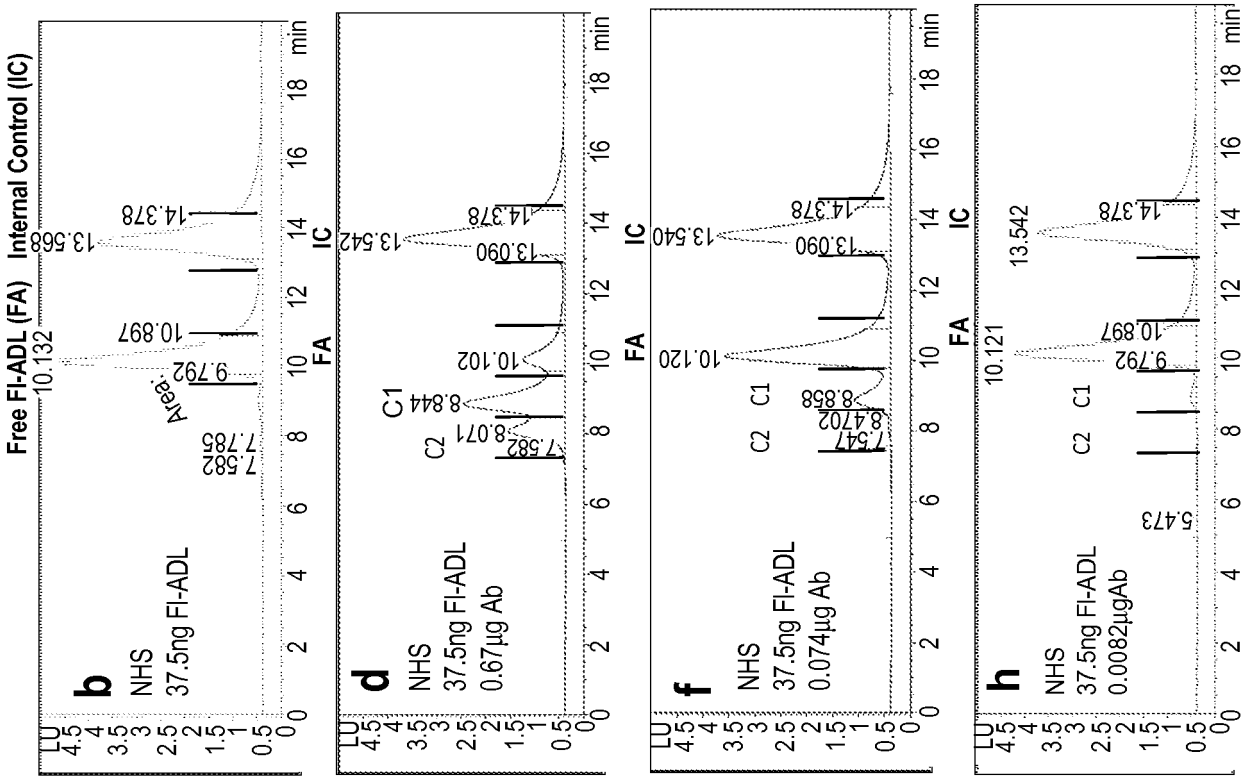
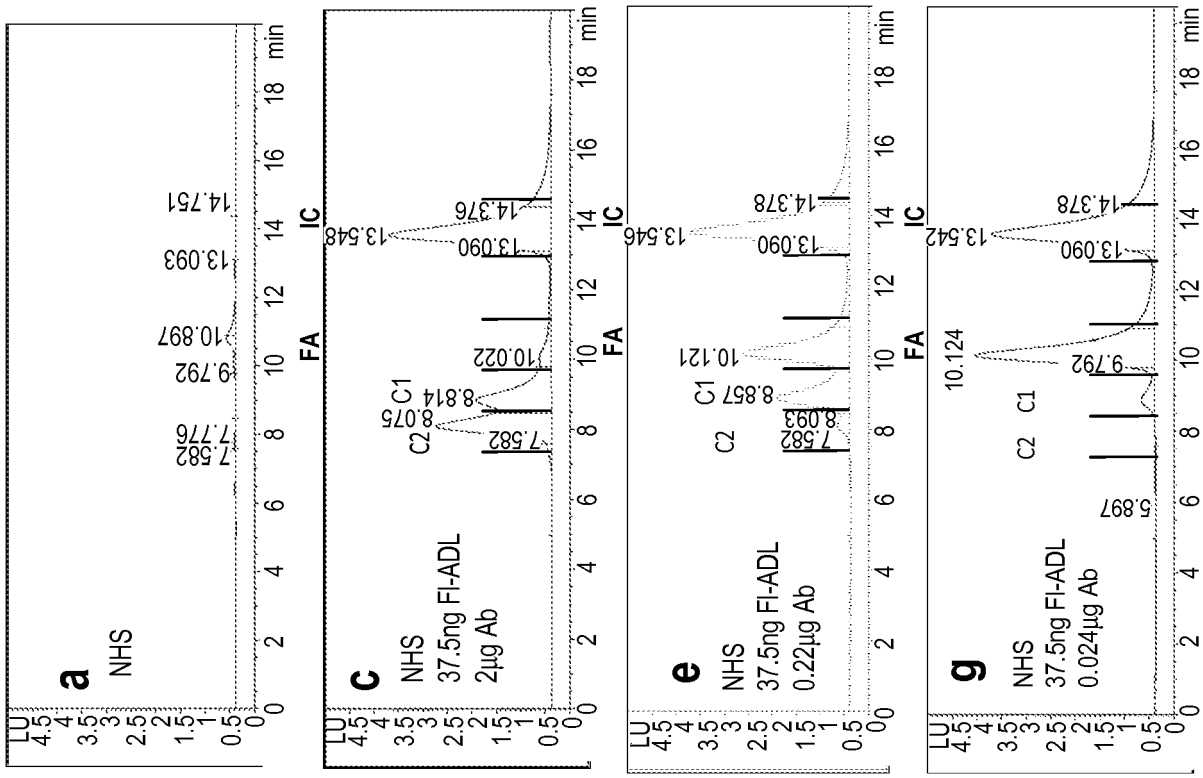

Free FI-ADL (FA) Internal Control (IC)
b NHS 37.5ng FI-ADL
d NHS 37.5ng FI-ADL 0.67µg Ab
f NHS 37.5ng FI-ADL 0.074µg Ab
h NHS 37.5ng FI-ADL 0.0082µgAb
LU
min
FA
IC
Area
C1
C2

FIG. 19


a NHS
c NHS 37.5ng FI-ADL 2µg Ab
e NHS 37.5ng FI-ADL 0.22µg Ab
g NHS 37.5ng FI-ADL 0.024µg Ab
LU
min
FA
IC
C1
C2

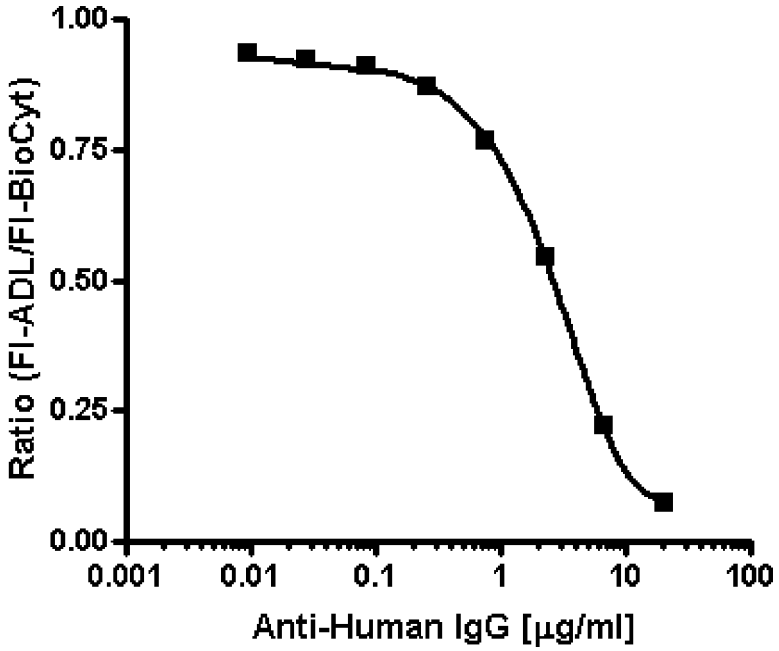

1.00
0.75
0.50
0.25
0.00
Ratio (FI-ADL/FI-BioCyt)
0.001
0.01
0.1
1
10
100
Anti-Human IgG [µg/ml]

FIG. 20

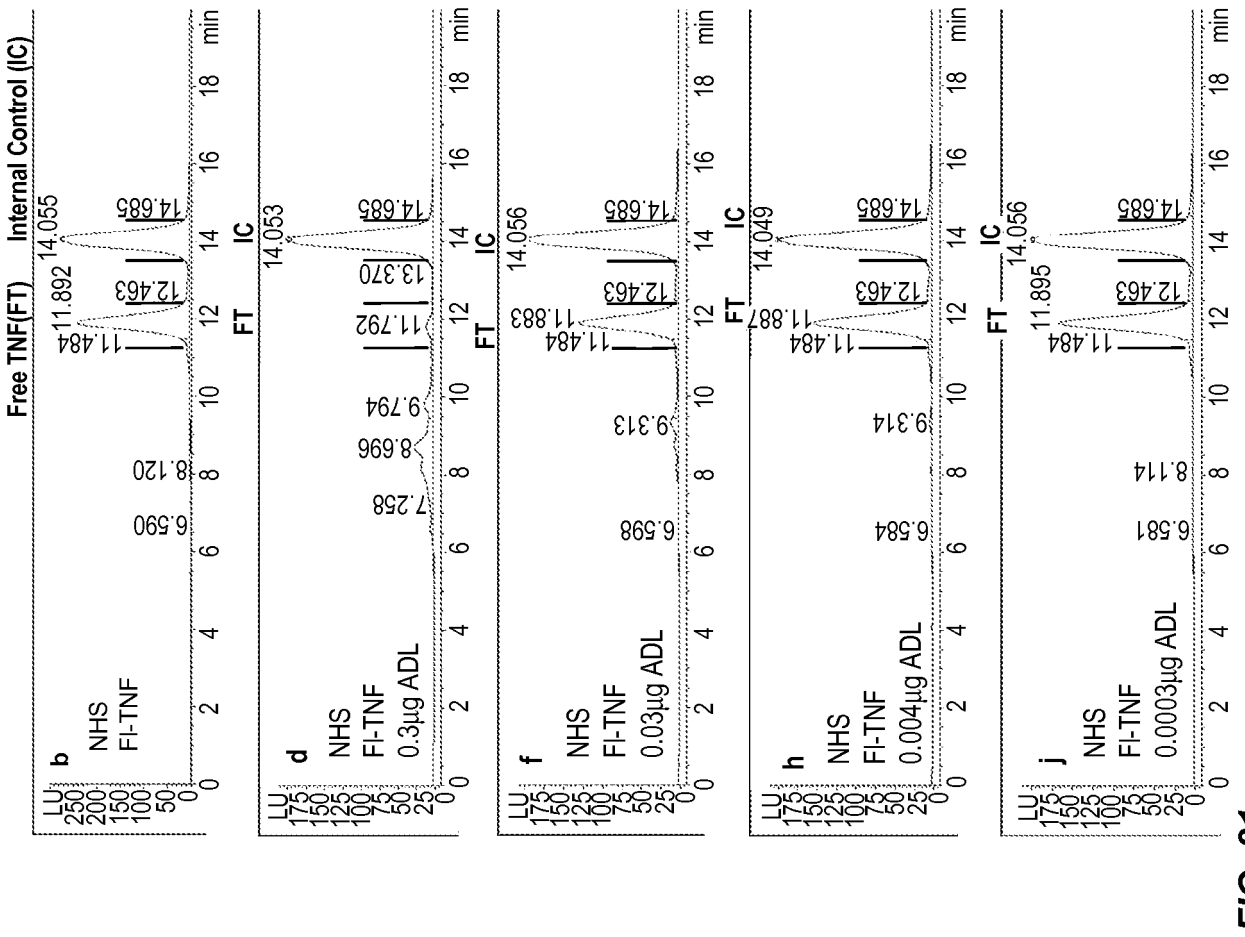
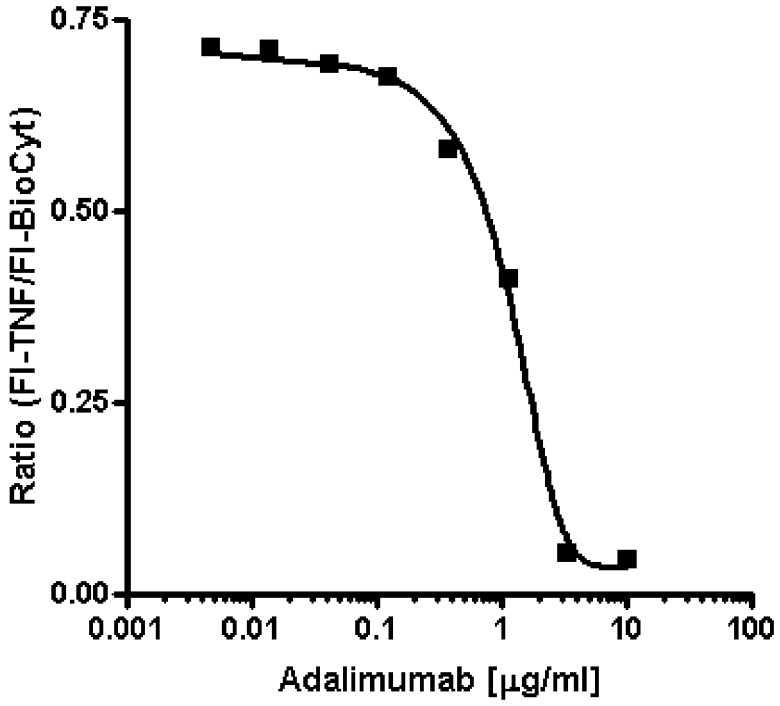

Free TNF(FT)
Internal Control (IC)
a NHS
b NHS FI-TNF
c NHS FI-TNF 1µg ADL
d NHS FI-TNF 0.3µg ADL
e NHS FI-TNF 0.1µg ADL
f NHS FI-TNF 0.03µg ADL
g NHS FI-TNF 0.012µg ADL
h NHS FI-TNF 0.004µg ADL
i NHS FI-TNF 0.001µg ADL
j NHS FI-TNF 0.0003µg ADL
FT
IC
LU
min

FIG. 21



HILLSLOPE	-1.813
EC50	1.213

FIG. 22

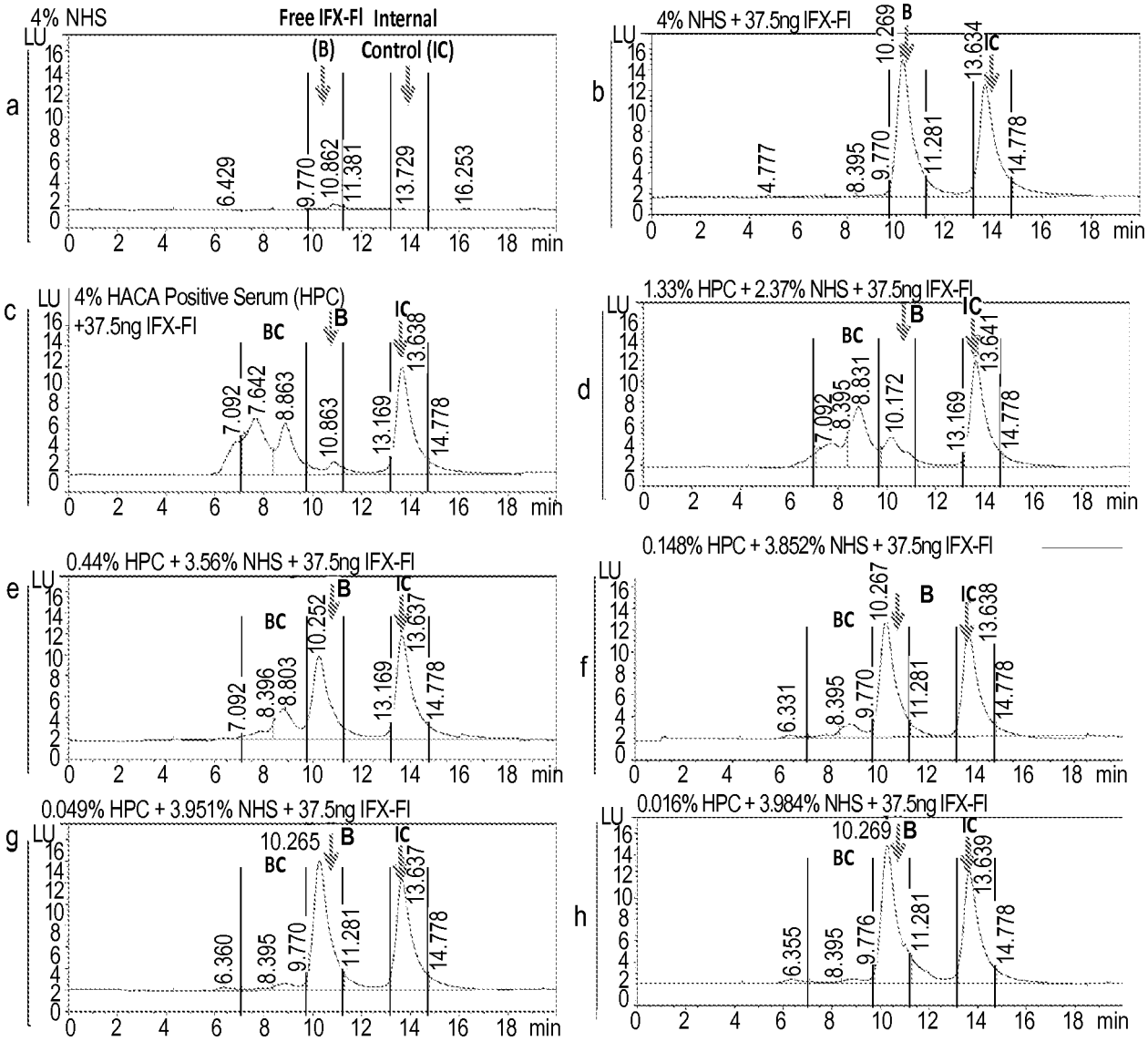

a
4% NHS
Free IFX-FI Internal
(B) Control (IC)
6.429
9.770
10.862
11.381
13.729
16.253
b
4% NHS + 37.5ng IFX-FI
4.777
8.395
9.770
10.269
11.281
13.634
14.778
c
4% HACA Positive Serum (HPC)
+37.5ng IFX-FI
BC
B
IC
7.092
7.642
8.863
10.863
13.169
13.638
14.778
d
1.33% HPC + 2.37% NHS + 37.5ng IFX-FI
7.092
8.395
8.831
10.172
13.169
13.641
14.778
e
0.44% HPC + 3.56% NHS + 37.5ng IFX-FI
7.092
8.396
8.803
10.252
13.169
13.637
14.778
f
0.148% HPC + 3.852% NHS + 37.5ng IFX-FI
6.331
8.395
9.770
10.267
11.281
13.638
14.778
g
0.049% HPC + 3.951% NHS + 37.5ng IFX-FI
6.360
8.395
9.770
10.265
11.281
13.637
14.778
h
0.016% HPC + 3.984% NHS + 37.5ng IFX-FI
6.355
8.395
9.776
10.269
11.281
13.639
14.778
LU
min

FIG. 23

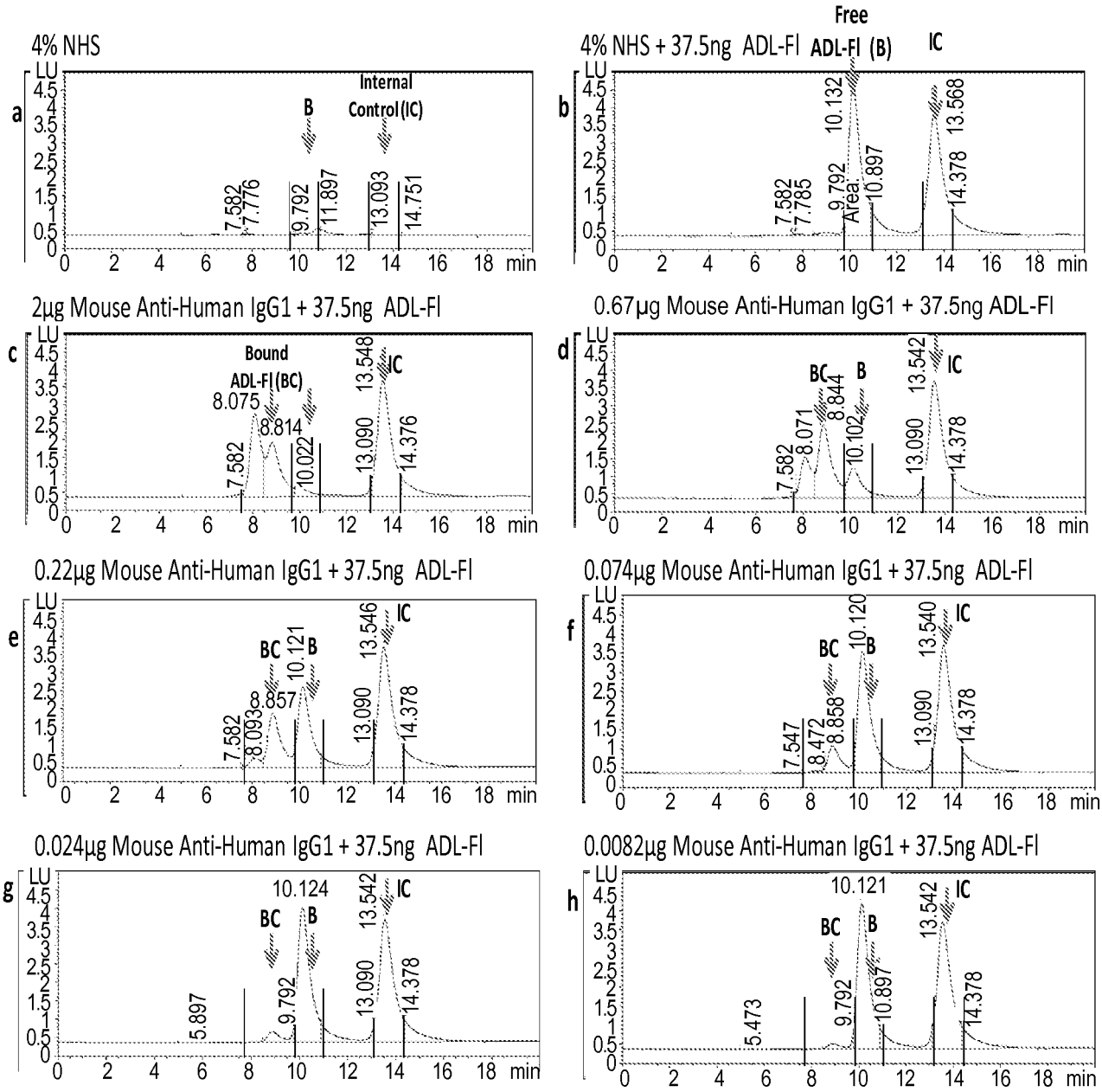

a
4% NHS
Internal Control (IC)
B
7.582
7.776
9.792
11.897
13.093
14.751
b
4% NHS + 37.5ng ADL-Fl
Free ADL-Fl (B)
IC
7.582
7.785
9.792
Area
10.132
10.897
13.568
14.378
c
2µg Mouse Anti-Human IgG1 + 37.5ng ADL-Fl
Bound ADL-Fl (BC)
IC
7.582
8.075
8.814
10.022
13.090
13.548
14.376
d
0.67µg Mouse Anti-Human IgG1 + 37.5ng ADL-Fl
BC
B
IC
7.582
8.071
8.844
10.102
13.090
13.542
14.378
e
0.22µg Mouse Anti-Human IgG1 + 37.5ng ADL-Fl
BC
B
IC
7.582
8.093
8.857
10.121
13.090
13.546
14.378
f
0.074µg Mouse Anti-Human IgG1 + 37.5ng ADL-Fl
BC
B
IC
7.547
8.472
8.858
10.120
13.090
13.540
14.378
g
0.024µg Mouse Anti-Human IgG1 + 37.5ng ADL-Fl
BC
B
IC
5.897
9.792
10.124
13.090
13.542
14.378
h
0.0082µg Mouse Anti-Human IgG1 + 37.5ng ADL-Fl
BC
B
IC
5.473
9.792
10.121
10.897
13.542
14.378
LU
min

FIG. 24

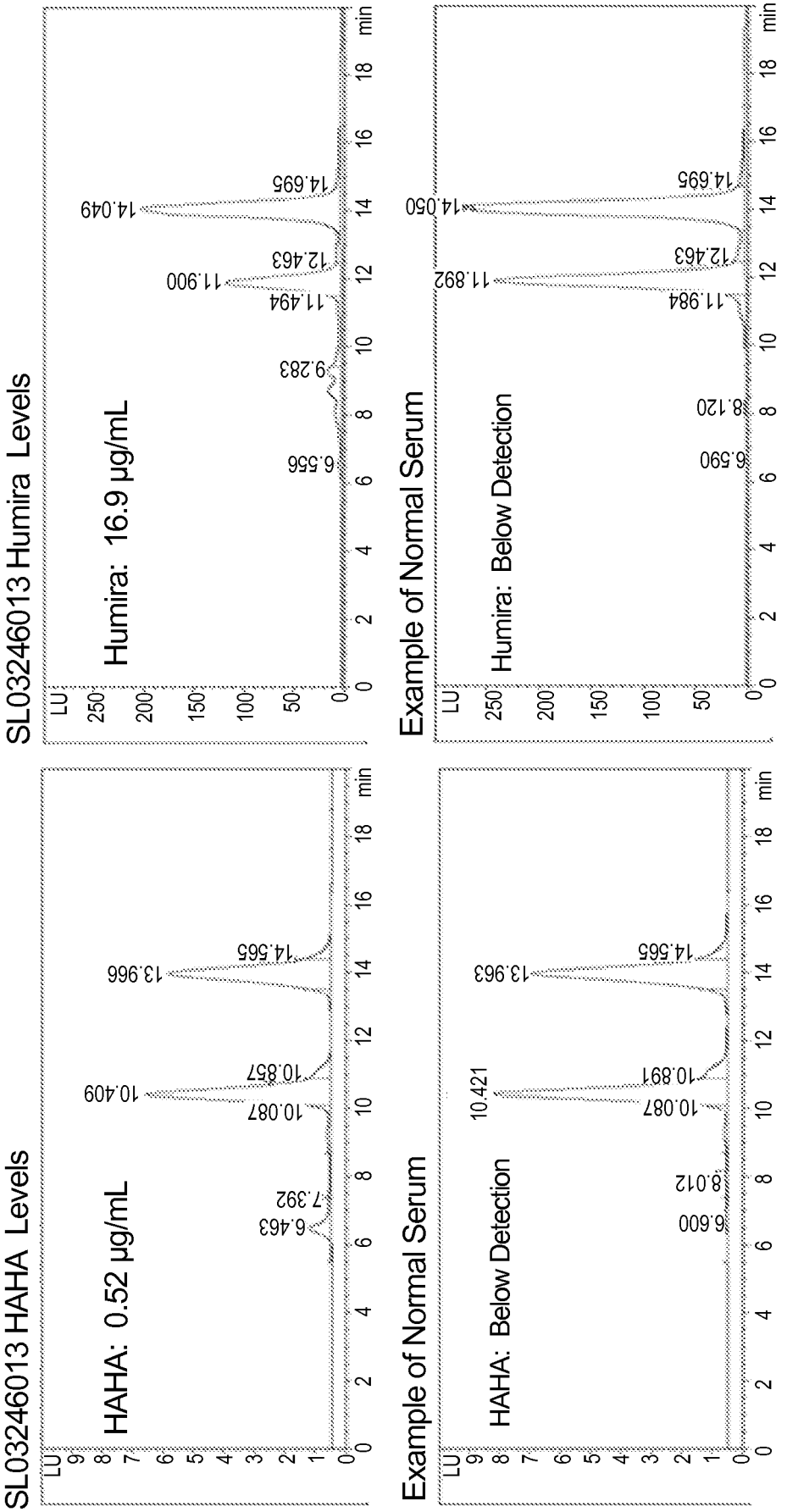

SL03246013 HAHA Levels
HAHA: 0.52 µg/mL
SL03246013 Humira Levels
Humira: 16.9 µg/mL
Example of Normal Serum
HAHA: Below Detection
Example of Normal Serum
Humira: Below Detection
LU
min

FIG. 25