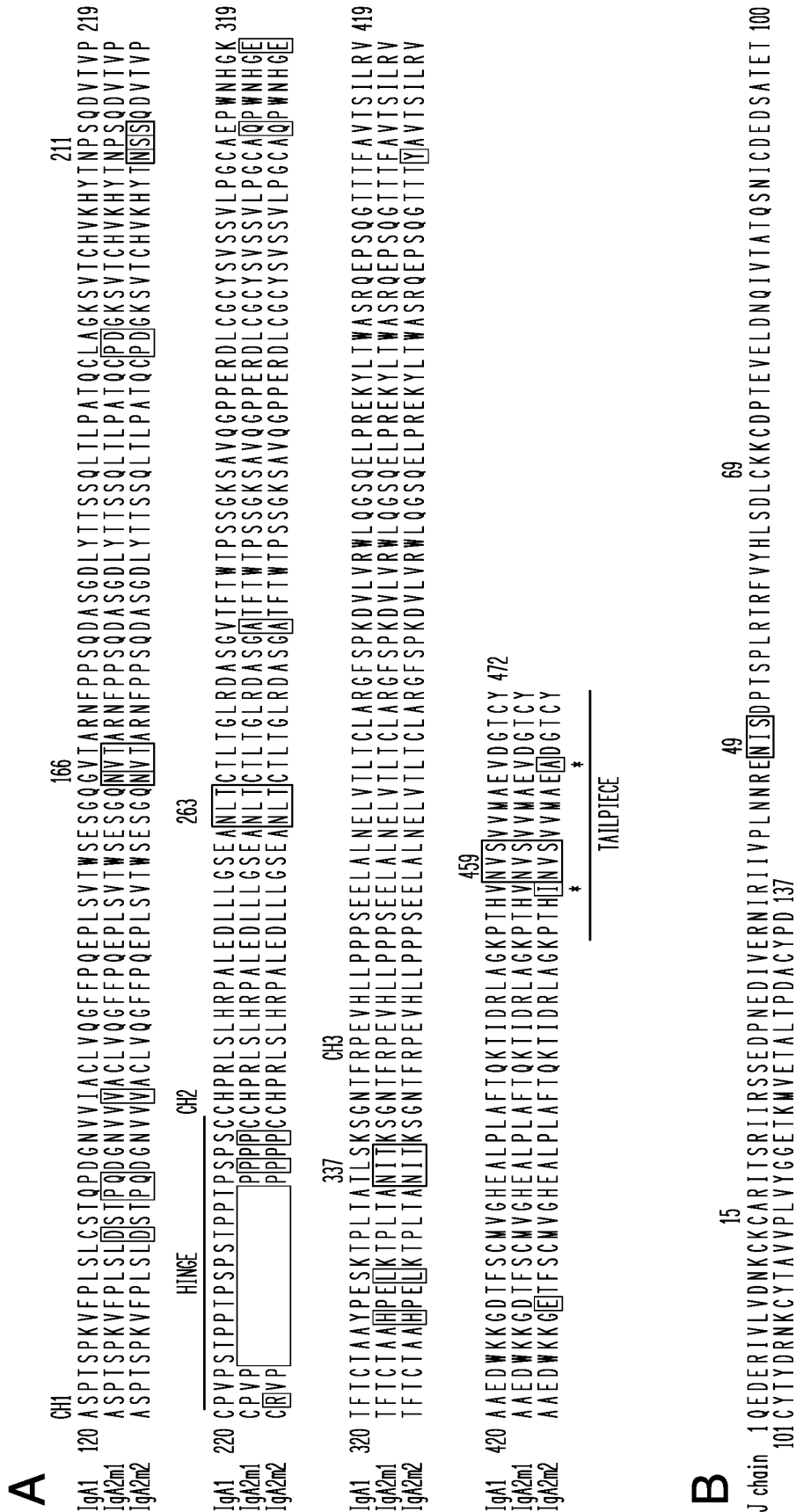




IgA2_{mn} constant region

120 ASPTSPKVFPLSLDSTPQDGNVAVCLVQFFFPQEPLSVTWSESGQNV¹TARNFPPSQDAS
GDLYTTSSQLTLPATQCPDGKSVTCHVKHY²TNSSQDVTVPCRVP³PPPCCHPRLSLHRPA
LEDLLGSEANLTCTLTGLRDASGATFTWTPSSGKSAVQGP⁴PERDLGGCYSVSSVLPCCA
QPWNHGETFTCTAAHPELKTPLTANITKSGNTFRPEVHLLPP⁵PSSEELNELVTLTCLAR
GFSPKDVLRWLQGSQELPREKYL⁶TWASRQEPSQGT⁷TTFAVTSILRVA⁸AEDWKKGD⁹TFSC
MVGHEALPLAFTQKTIDRLAGKPTHVNVSVMAEVDGTCY 472

Figure 1C

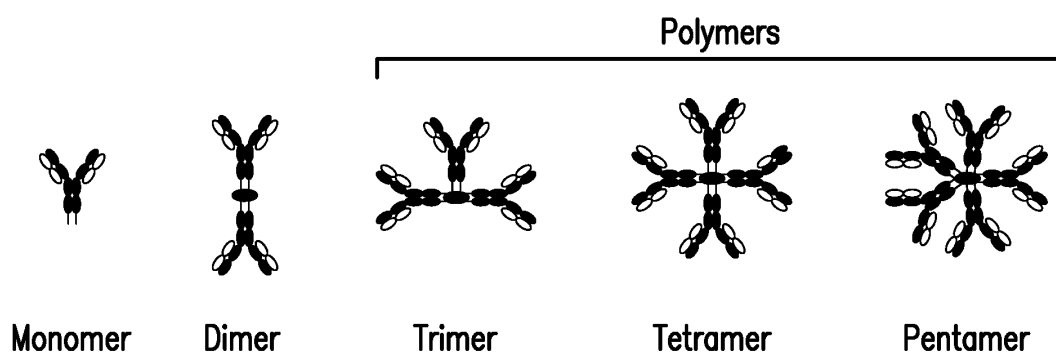


Figure 1D

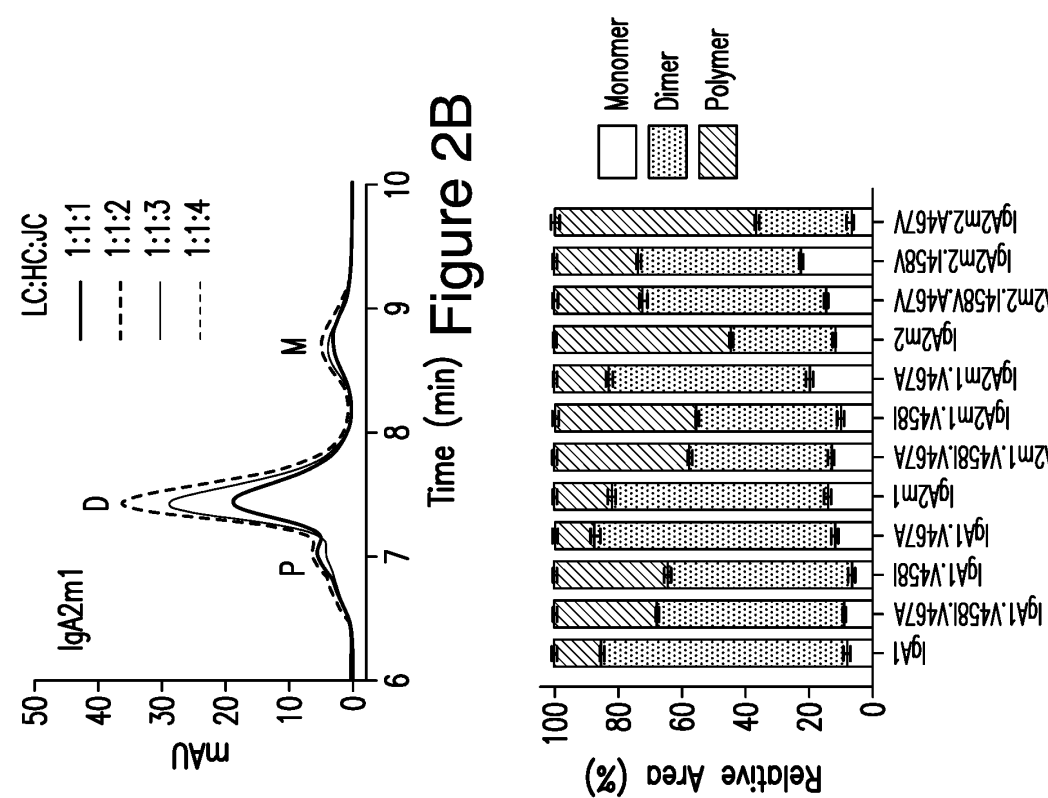
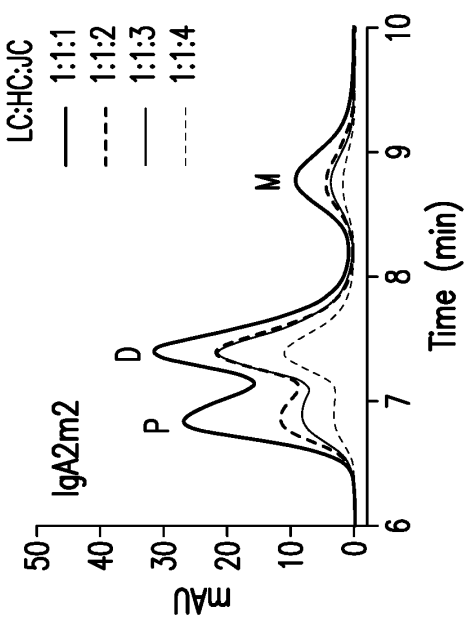
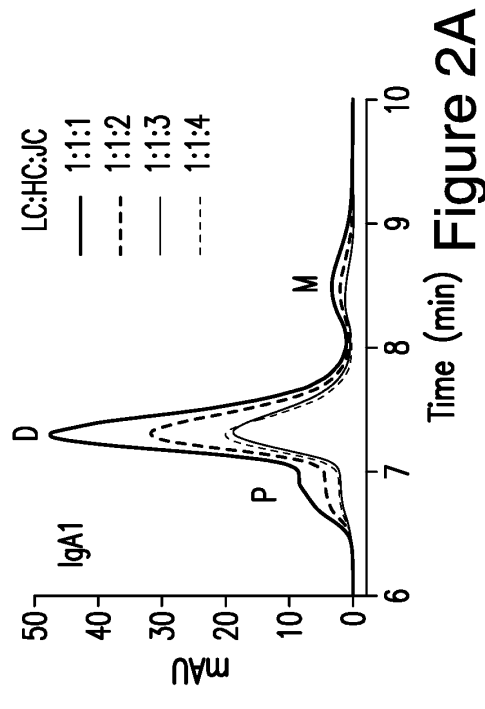


Figure 2A-2D

Figure 2D

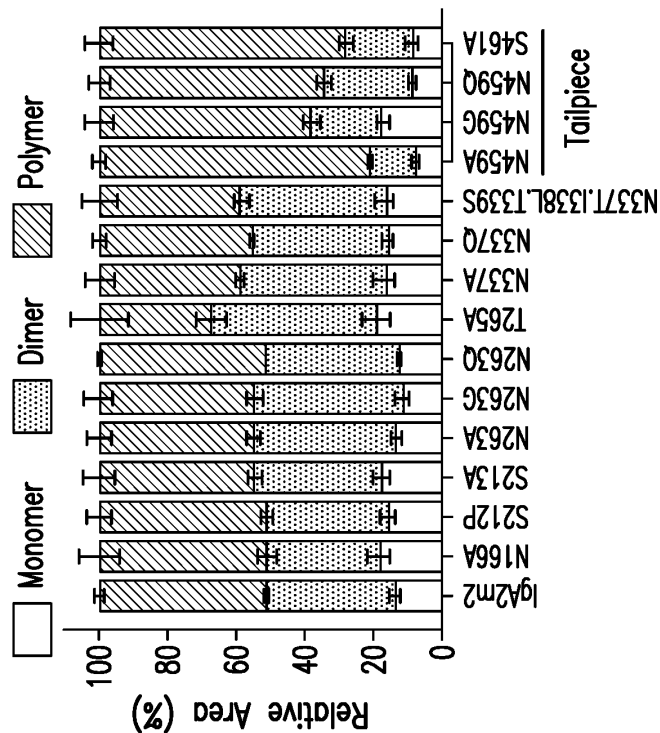


Figure 2F

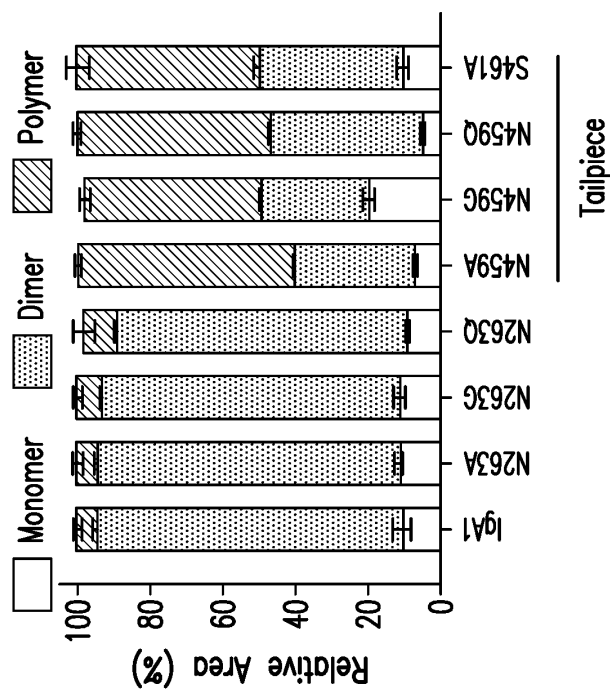


Figure 2E

Figure 2E-2F

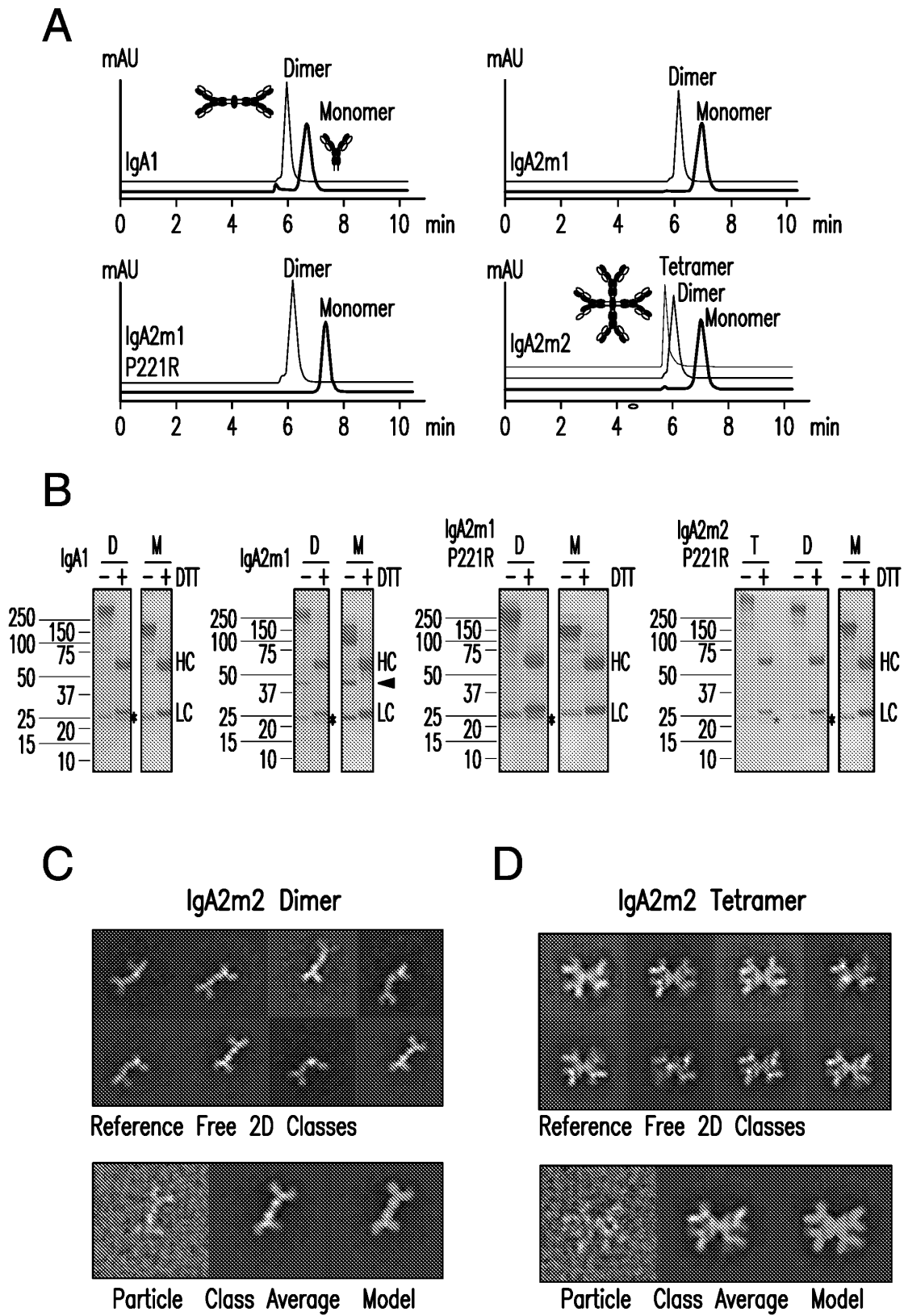


Figure 3A-3D

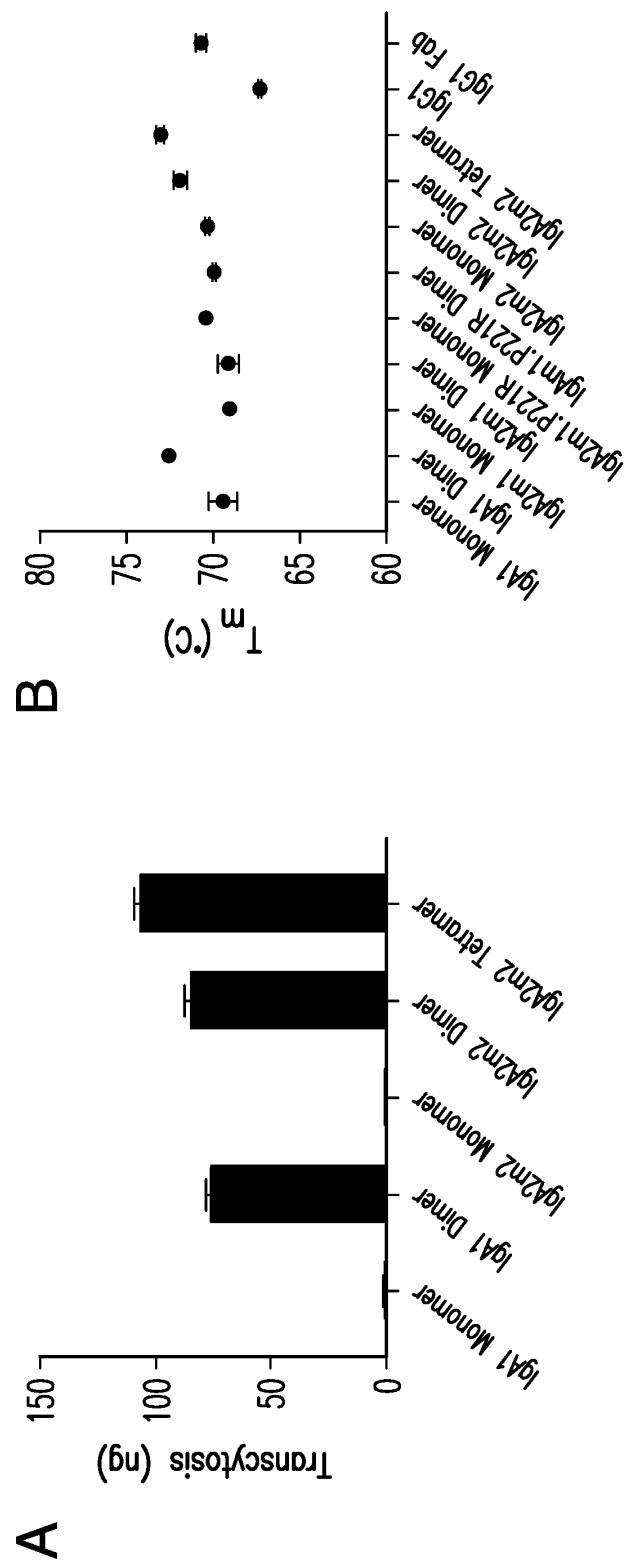
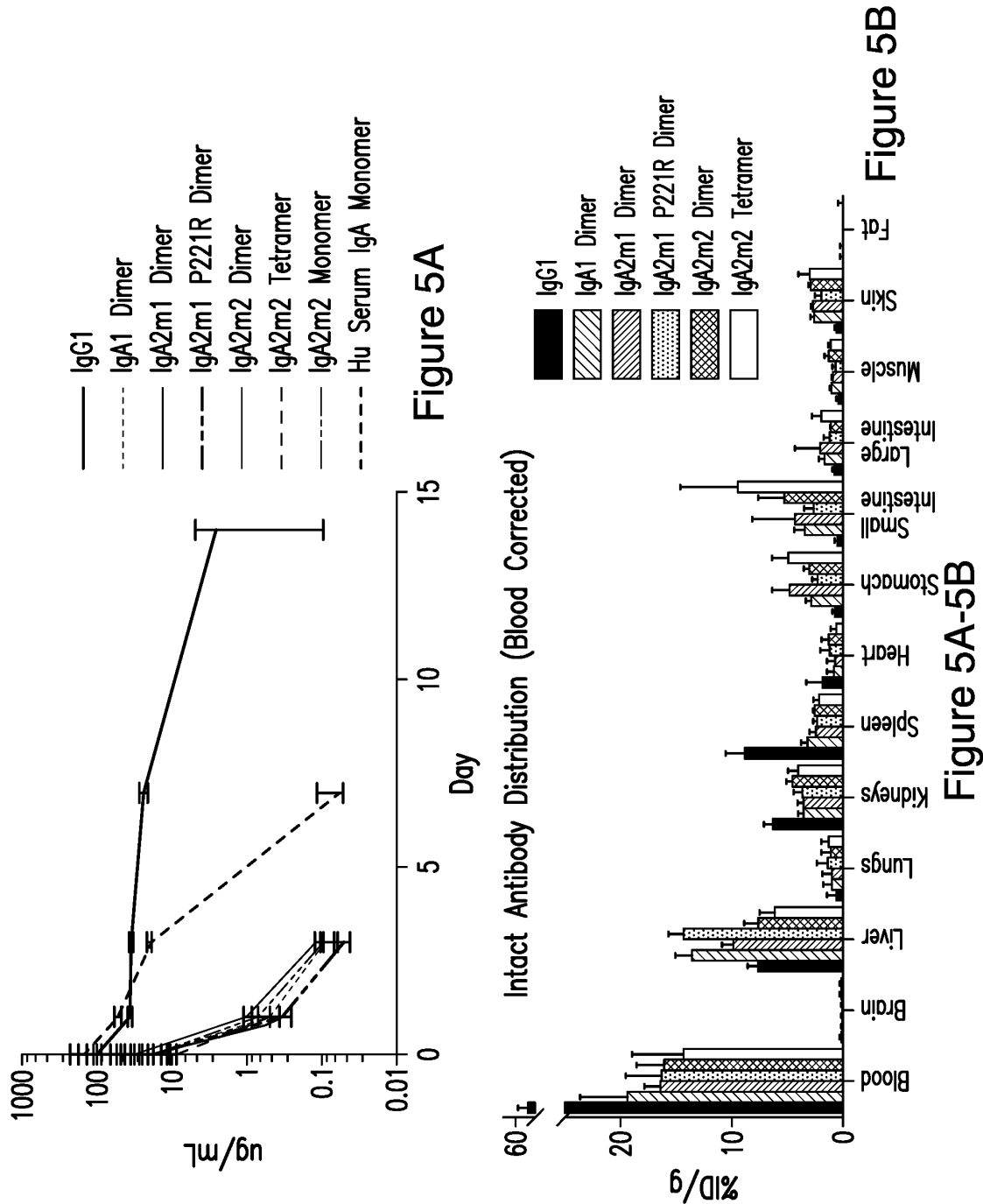


Figure 4A-4B



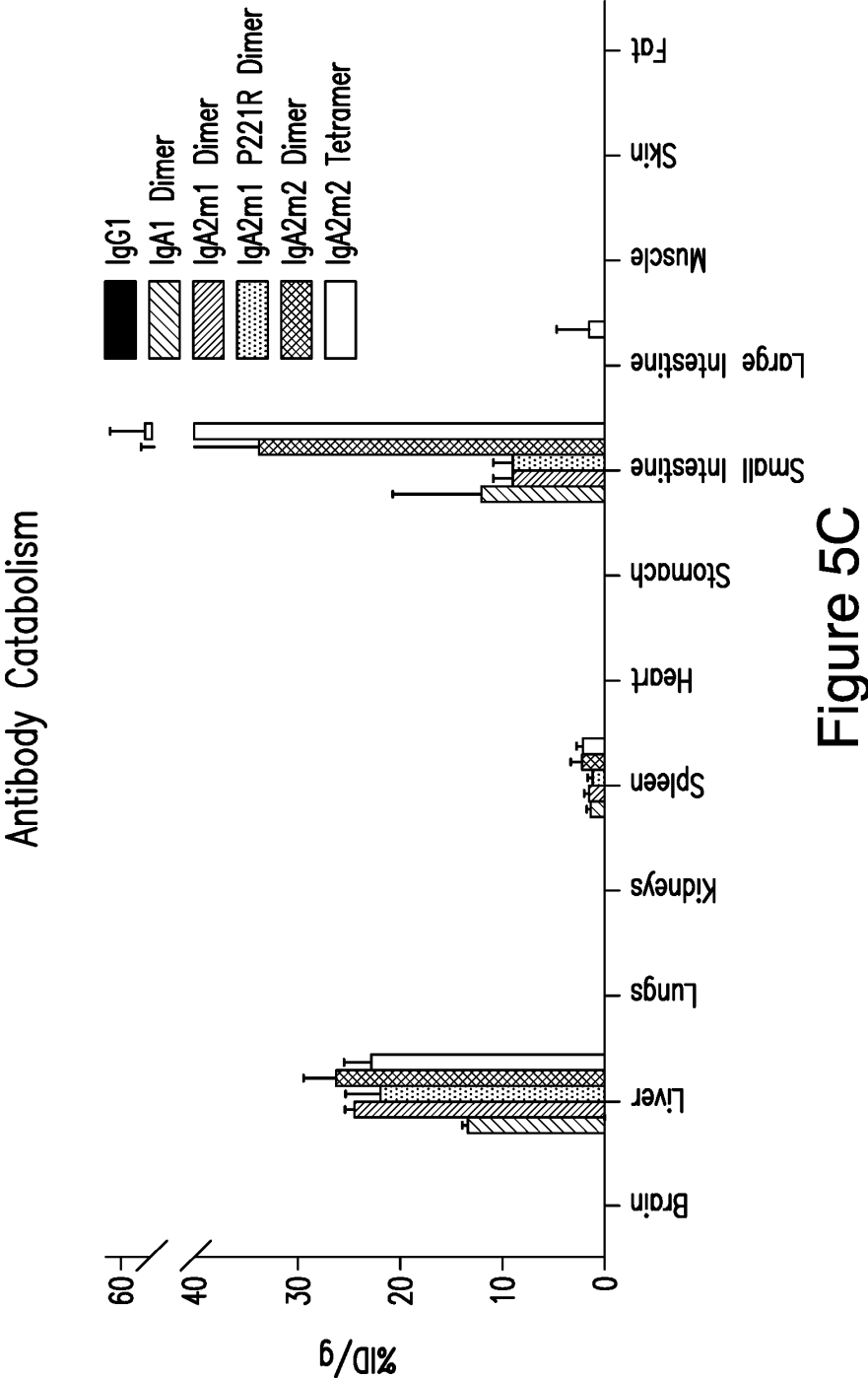


Figure 5C

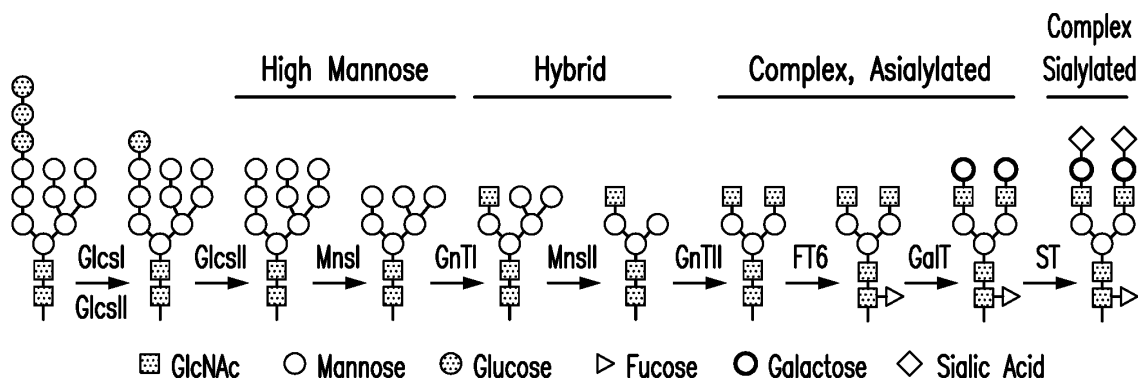


Figure 6A

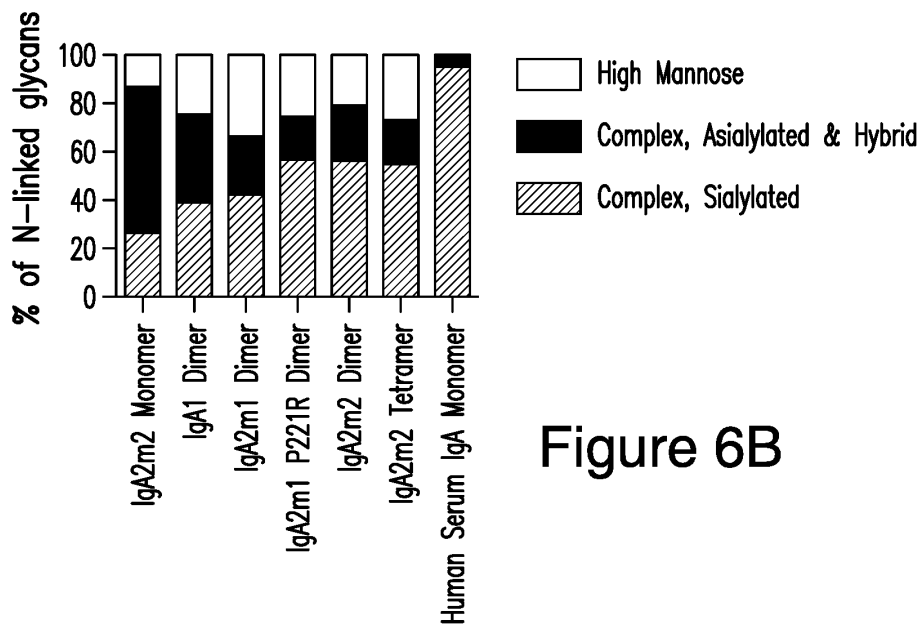


Figure 6B

Figure 6A-6B

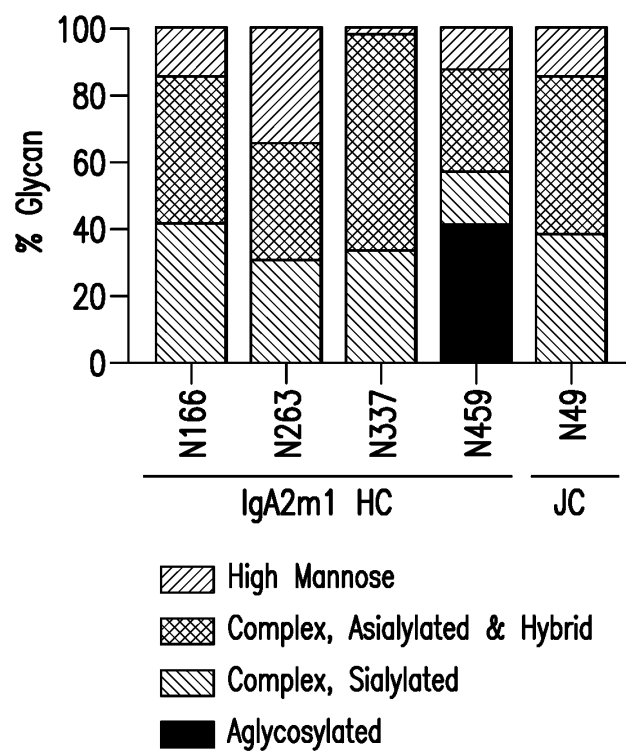


Figure 6C

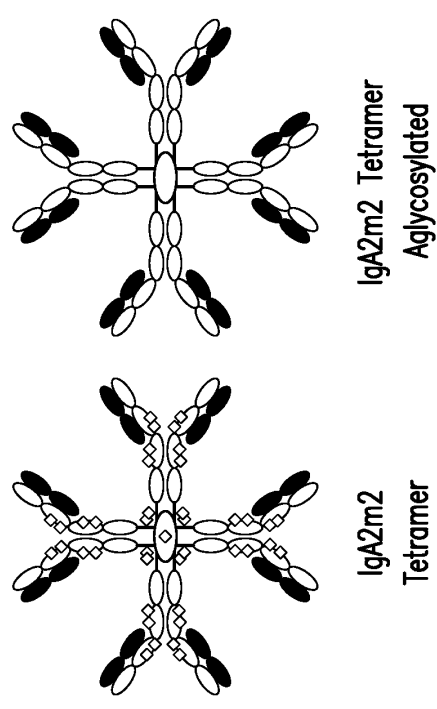
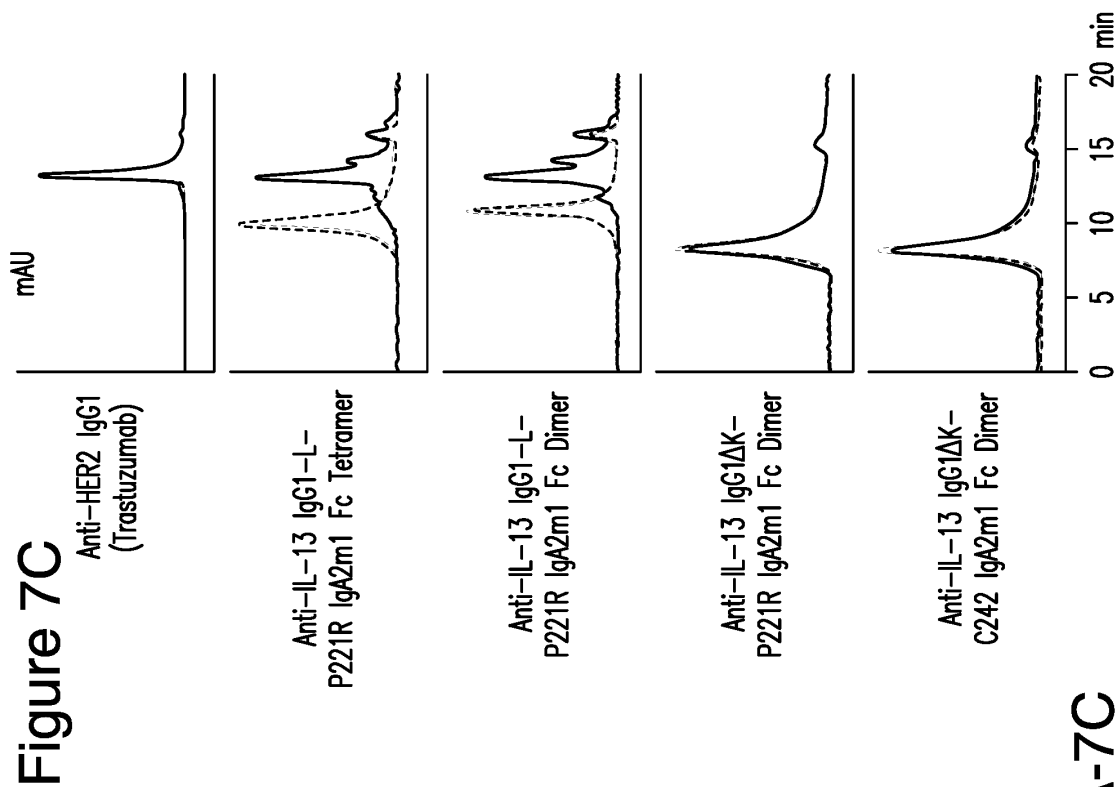


Figure 7A

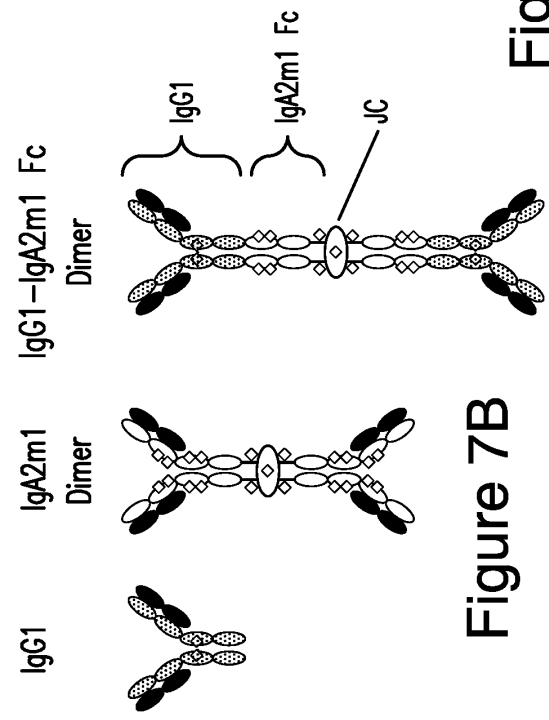


Figure 7B

Figure 7A-7C

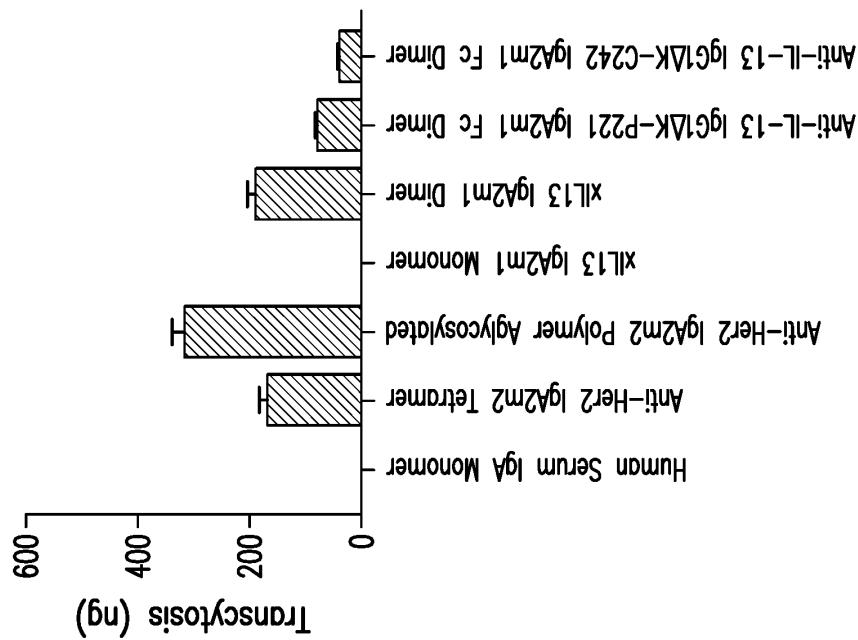


Figure 7E

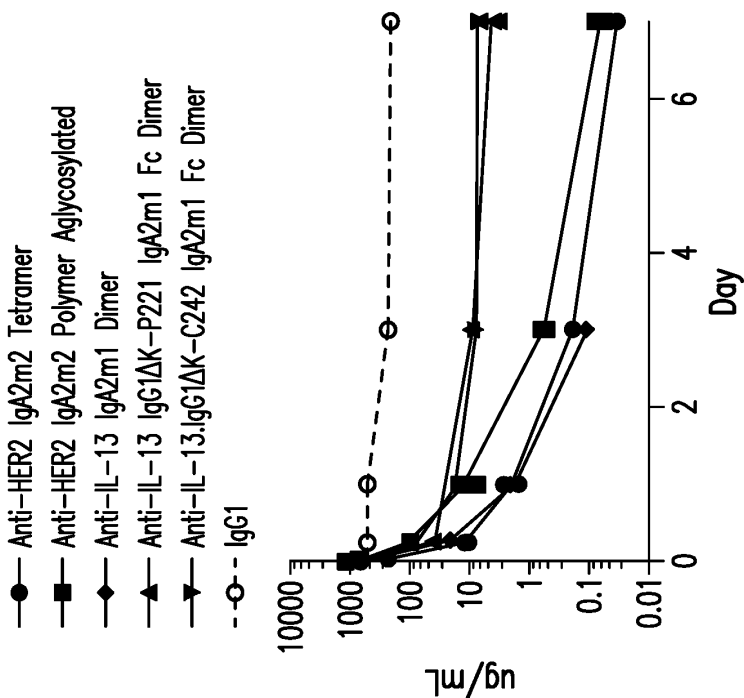


Figure 7D

Figure 7D-7E

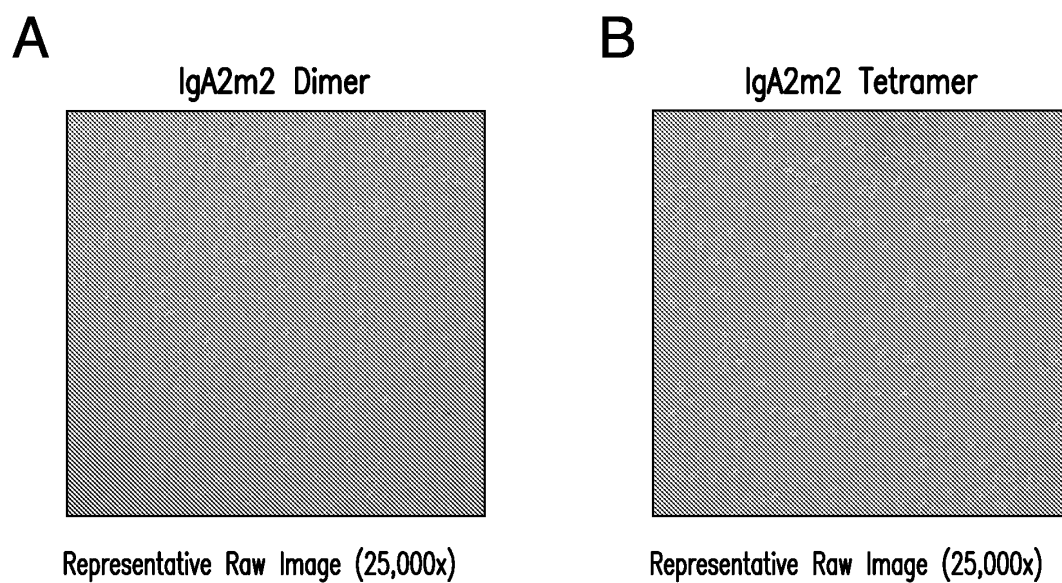


Figure 8A-8B

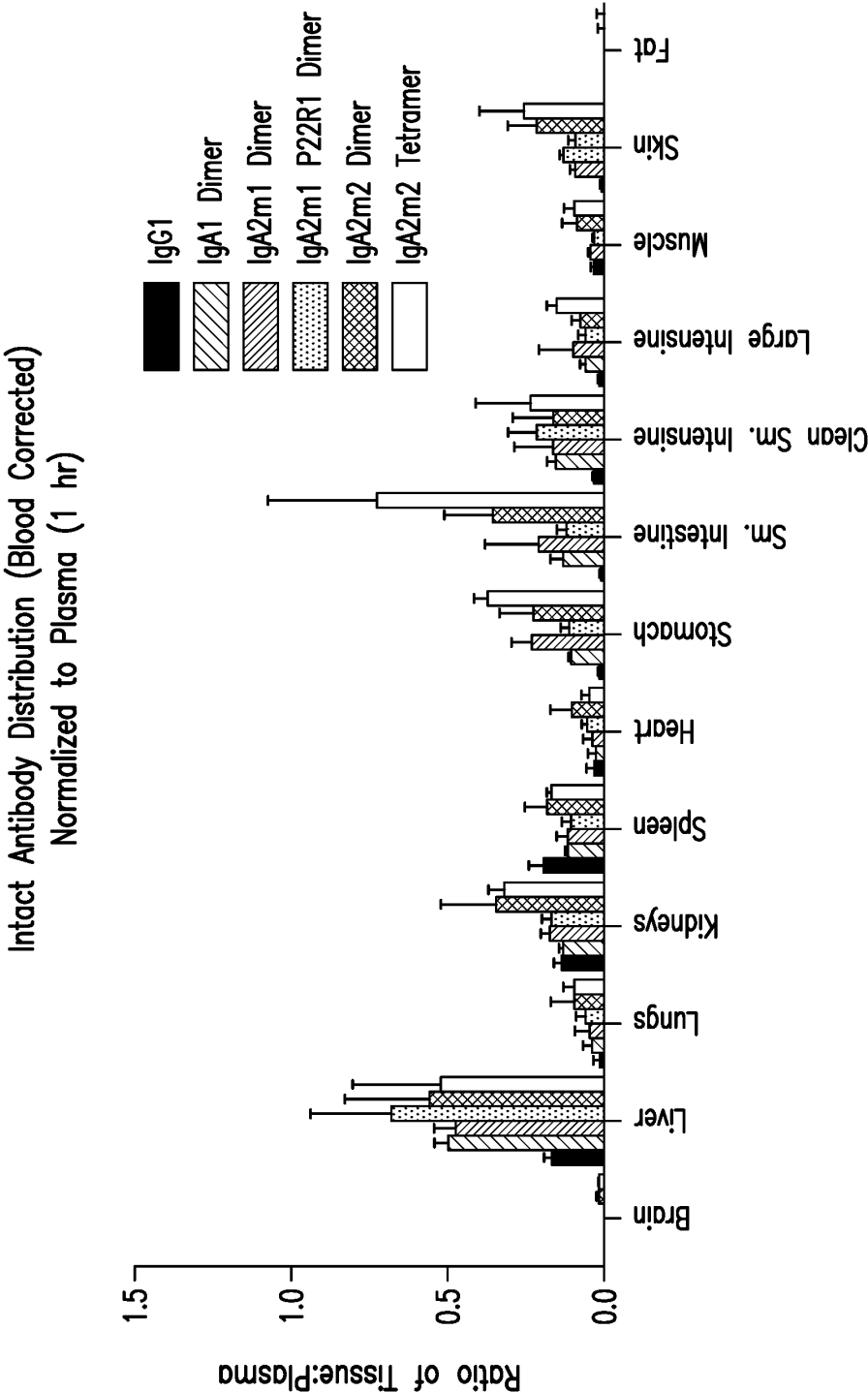


Figure 9

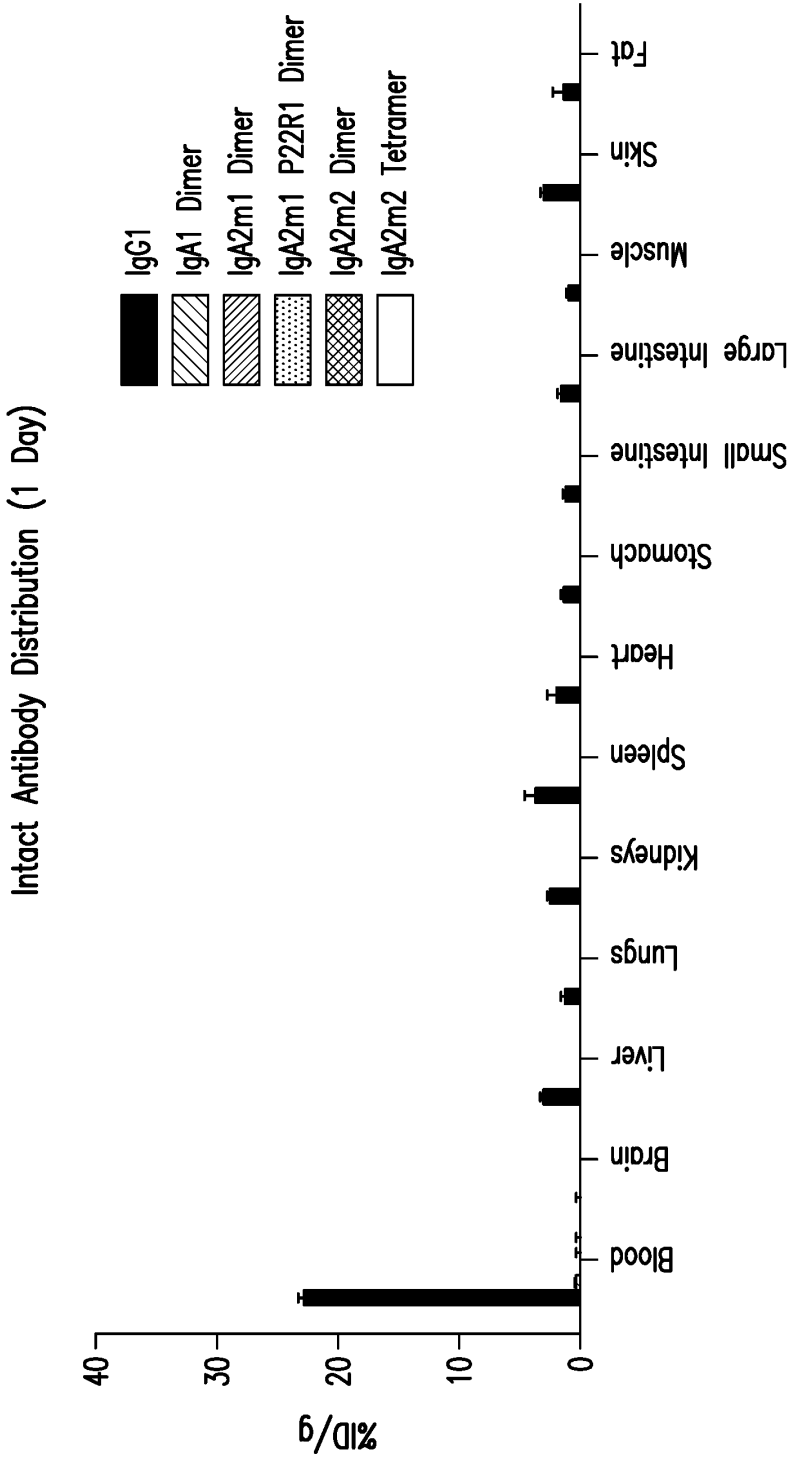


Figure 10

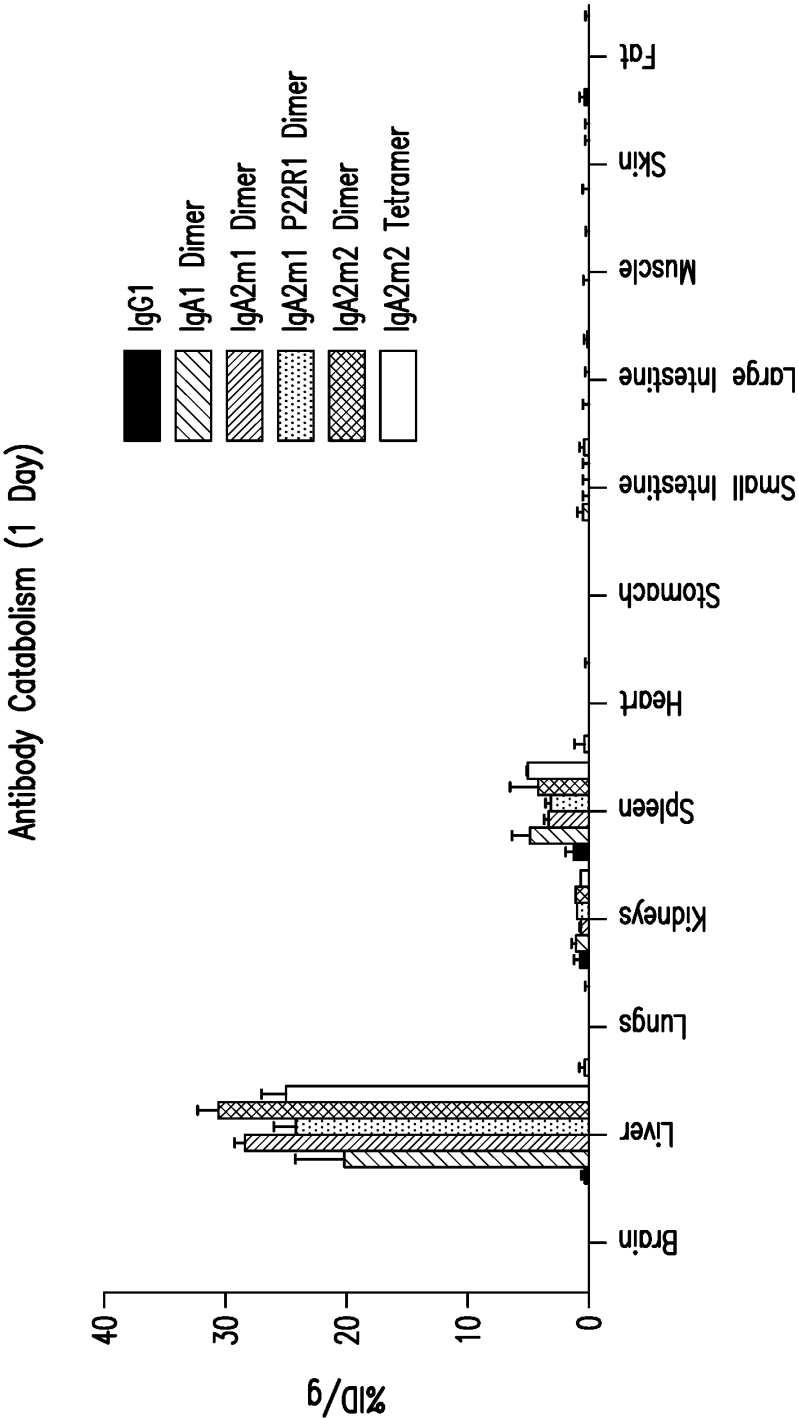


Figure 11

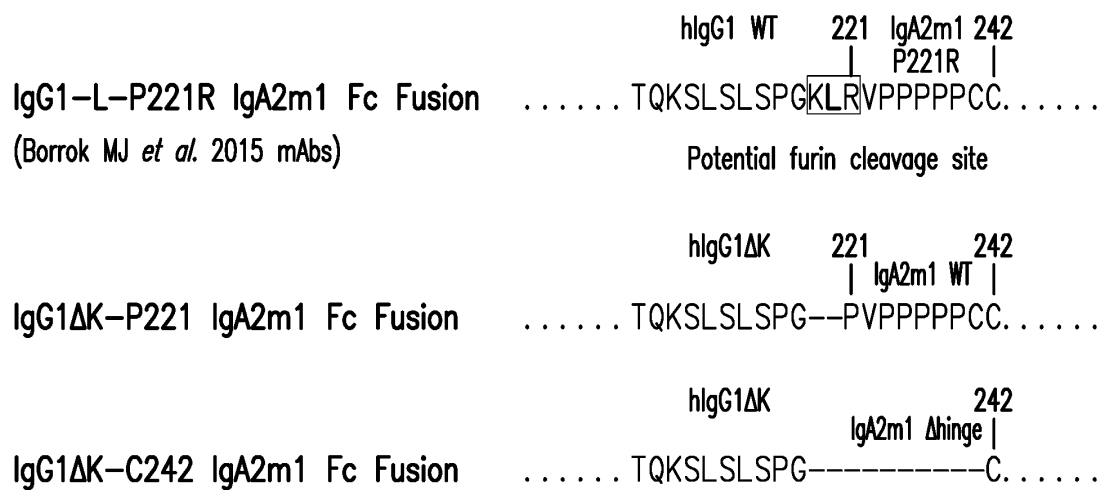


Figure 12

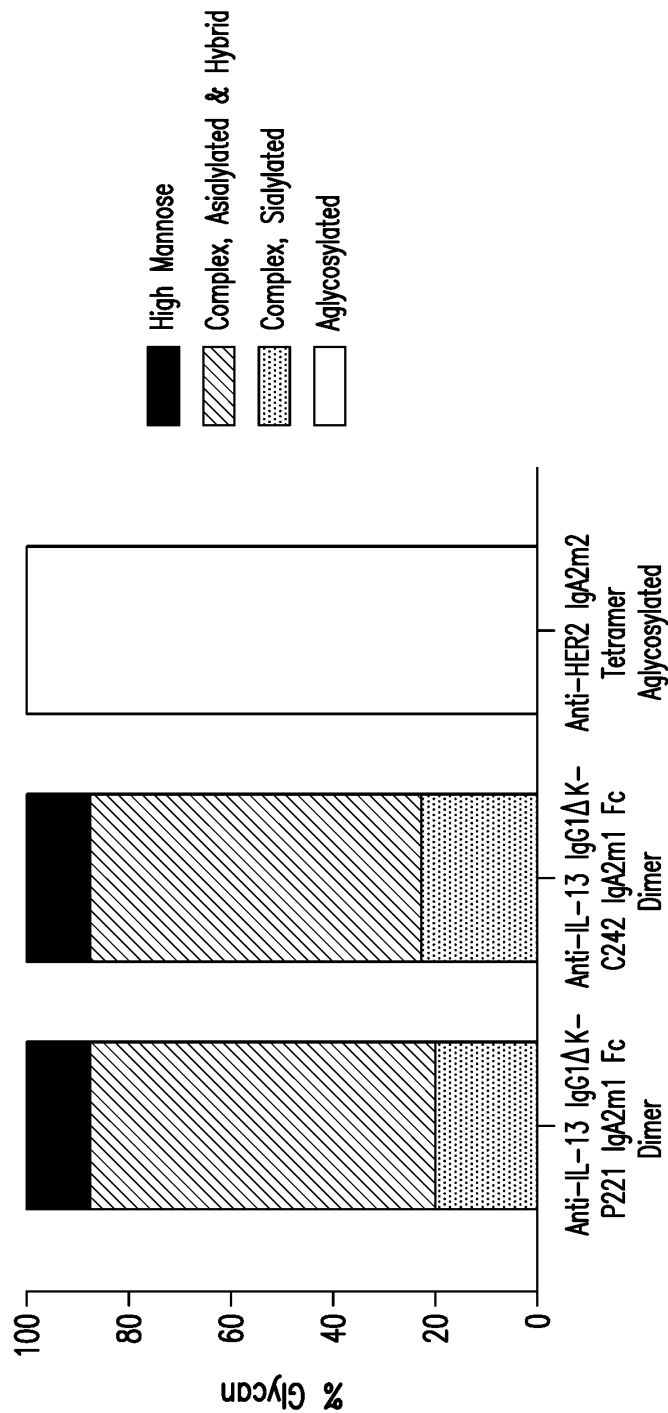


Figure 13

Mouse
Rat
Rabbit
Pig
Gorilla
Hulga1
Hulga2m1
Hulga2m2

Mouse
Rat
Rabbit
Pig
Gorilla
HuIgA1
HuIgA2m1
HuIgA2m2

Mouse
Rat
Rabbit
Pig
Gorilla
HuIgA1
HuIgA2m1
HuIgA2m2

Mouse
Rat
Rabbit
Pig
Gorilla
HuIgA1
HuIgA2m1
HuIgA2m2

Figure 14

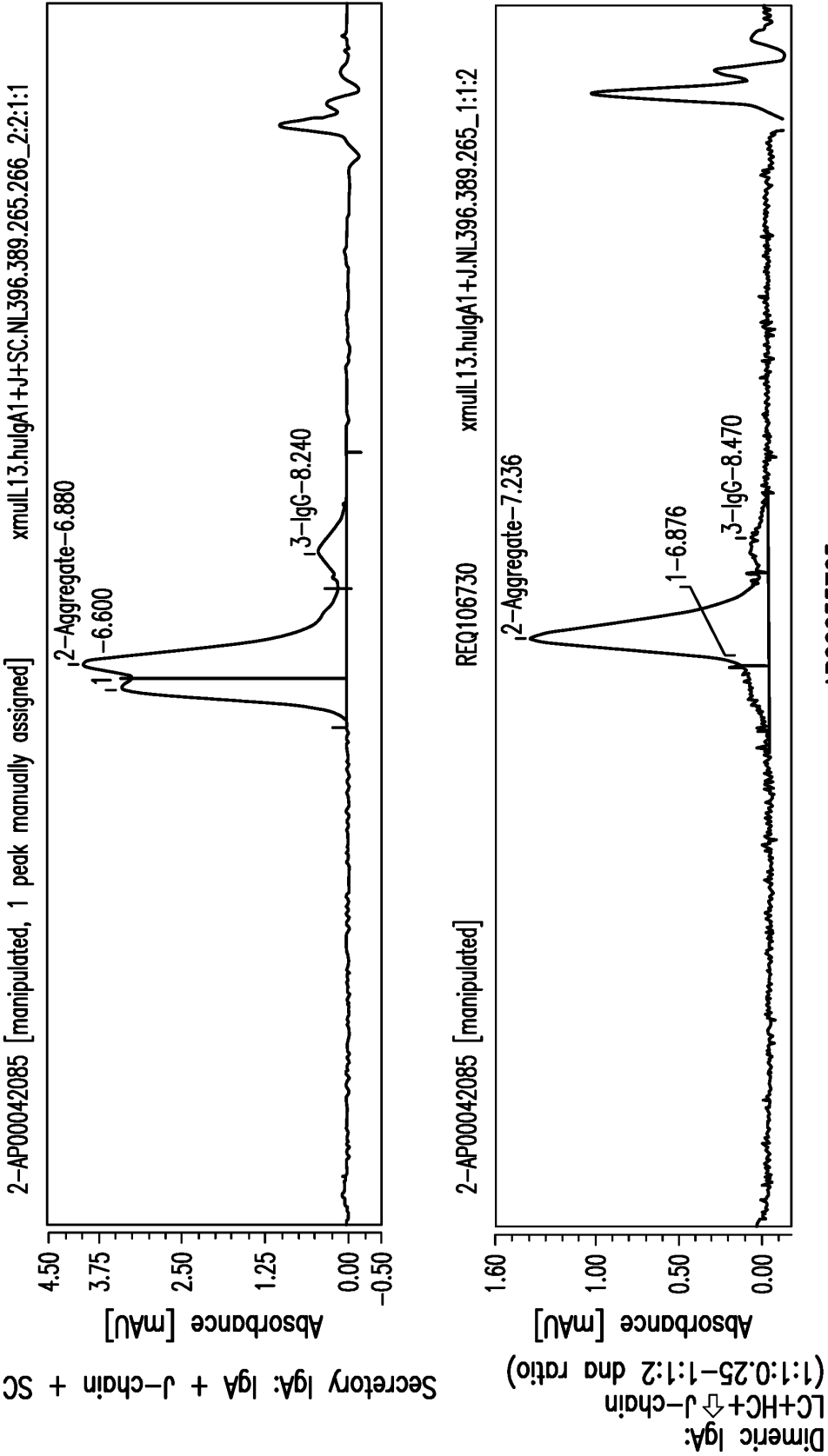
Mouse
Rat
Rabbit
Pig
Gorilla
Hulga1
Hulga2m1
Hulga2m2

Mouse
Rat
Rabbit
Pig
Gorilla
Hulga1
Hulga2m1
Hulga2m2

Mouse
Rat
Rabbit
Pig
Gorilla
Hulga1
Hulga2m1
Hulga2m2

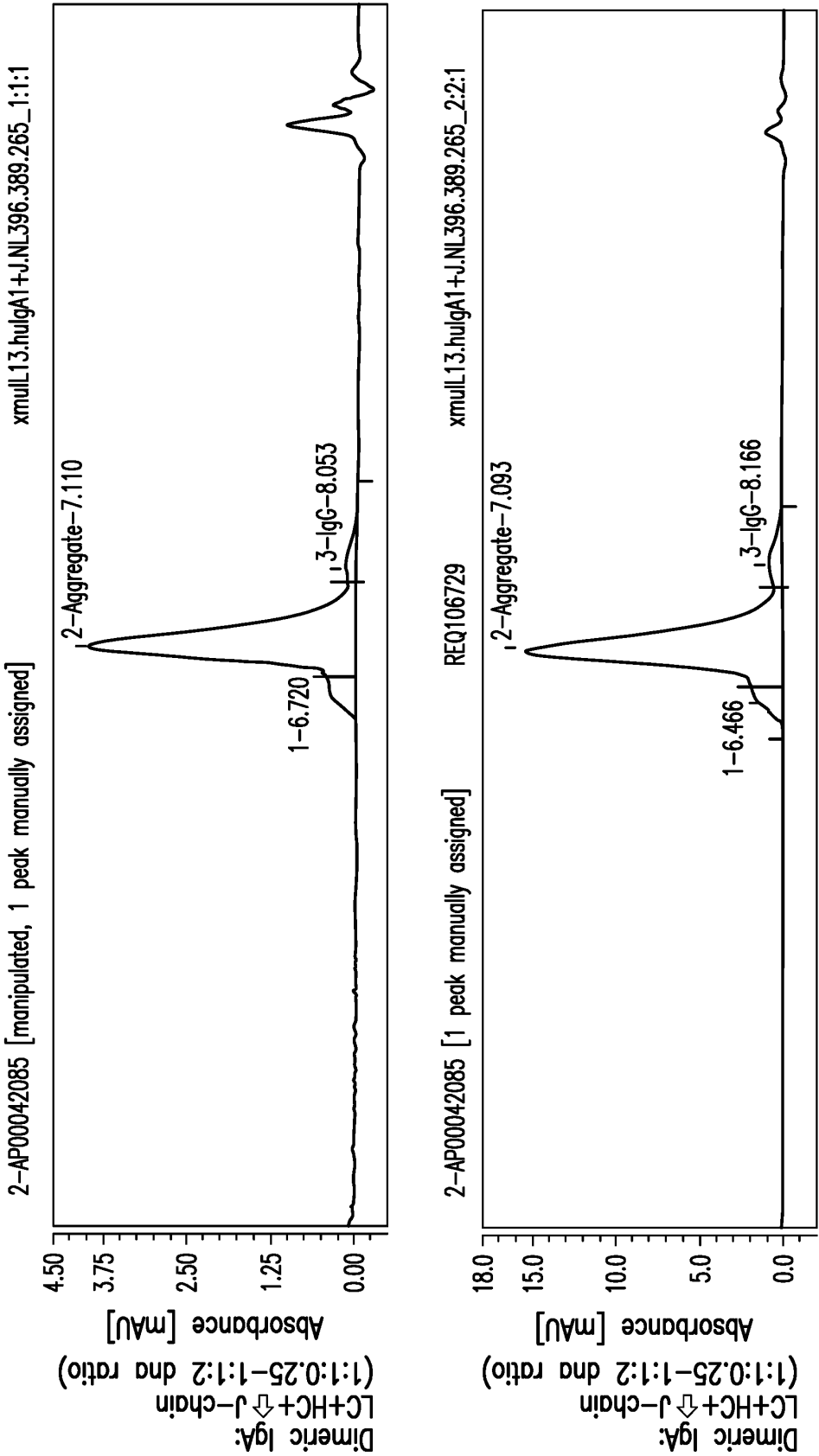
Mouse
Rat
Rabbit
Pig
Gorilla
HuIgA1
HuIgA2m1
HuIgA2m2

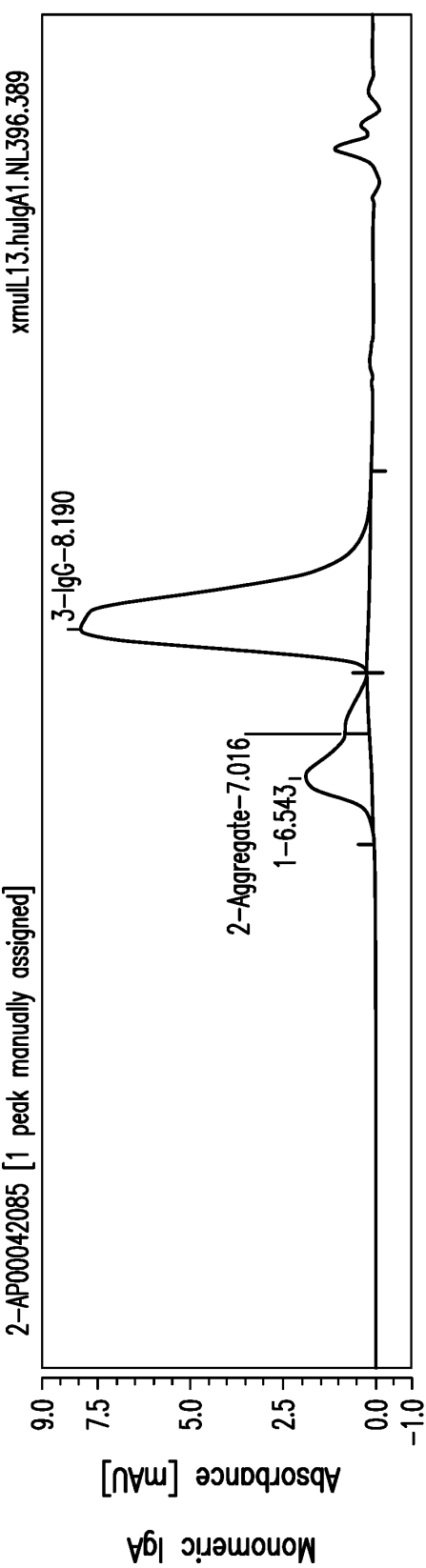
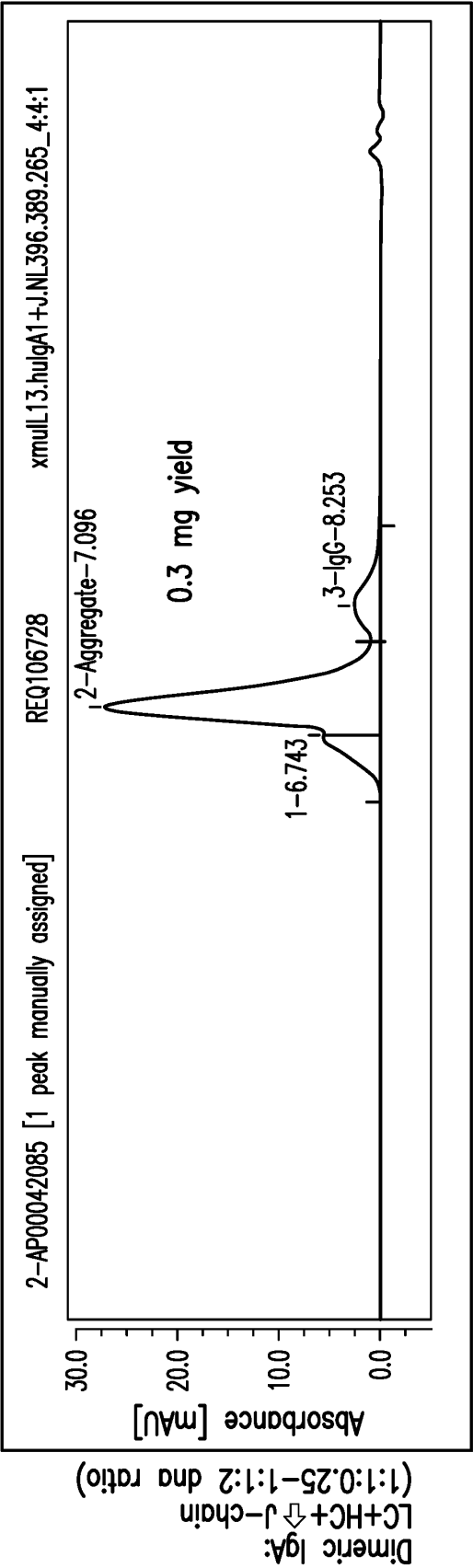
Figure 14 continued



AP00055705

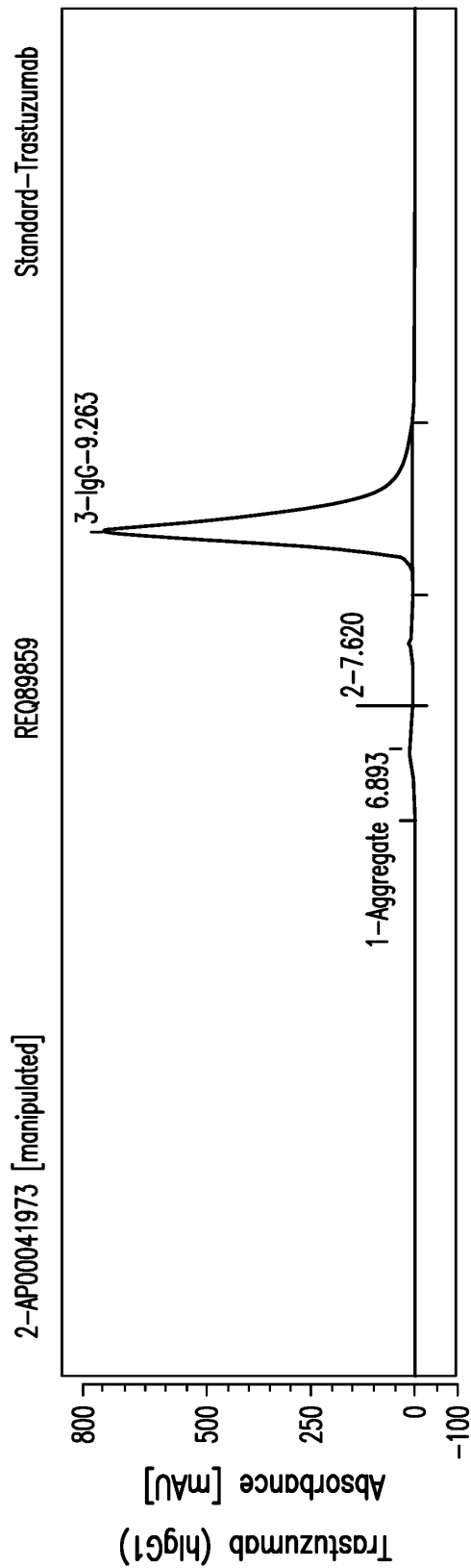
Figure 15A





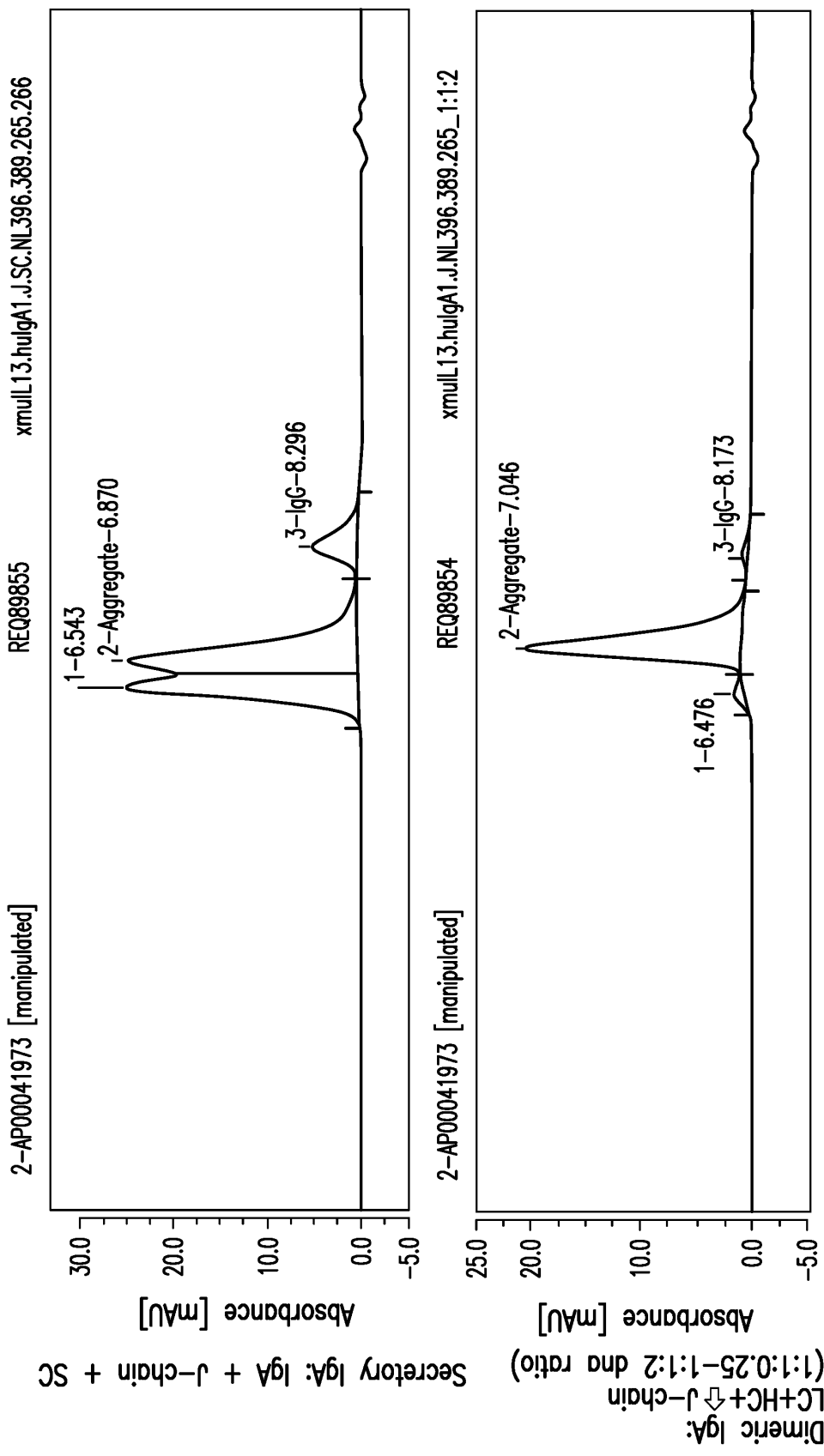
AP00055705

Figure 15A continued



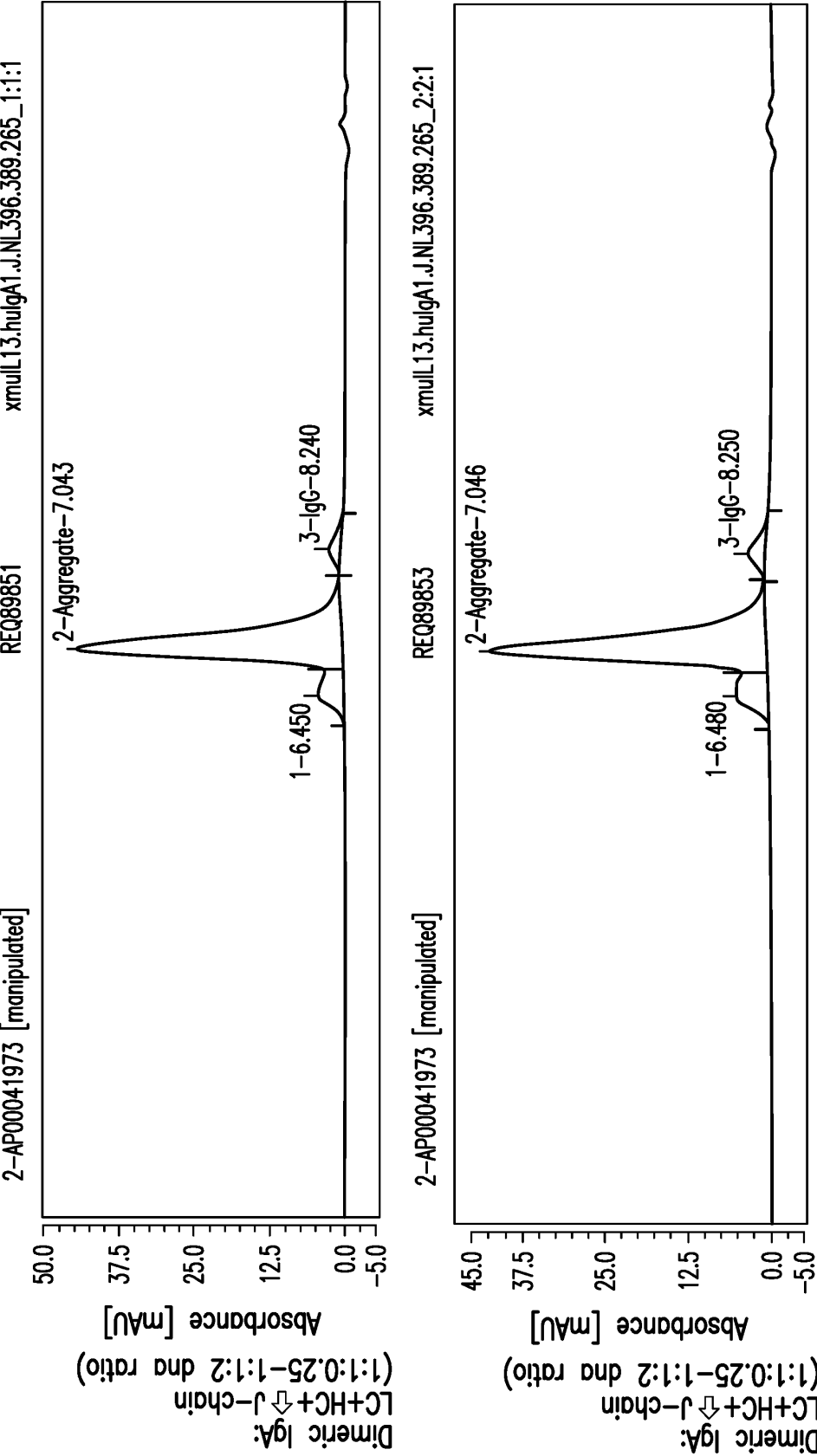
AP00055705

Figure 15A continued



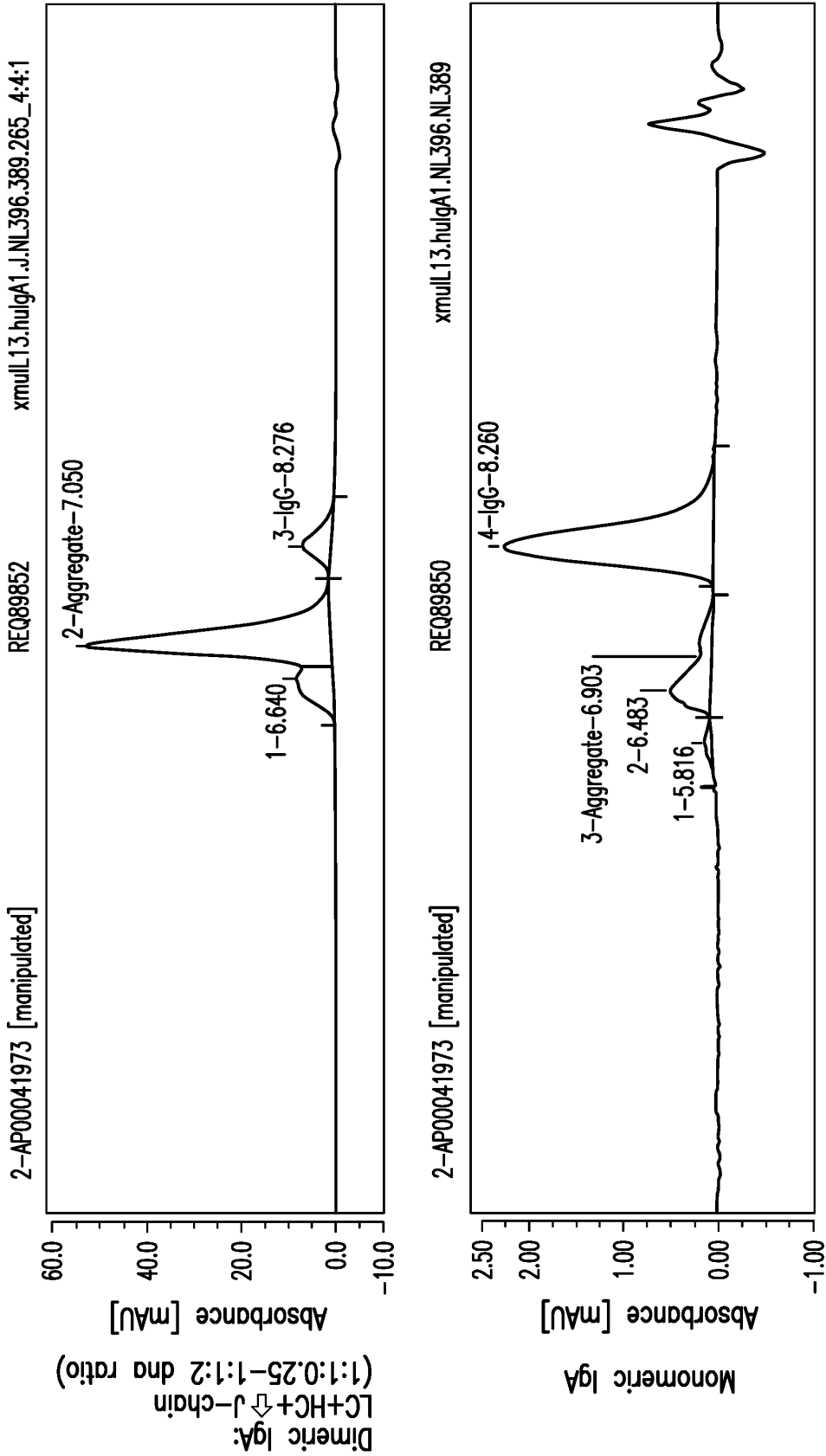
AP00055587

Figure 15A continued



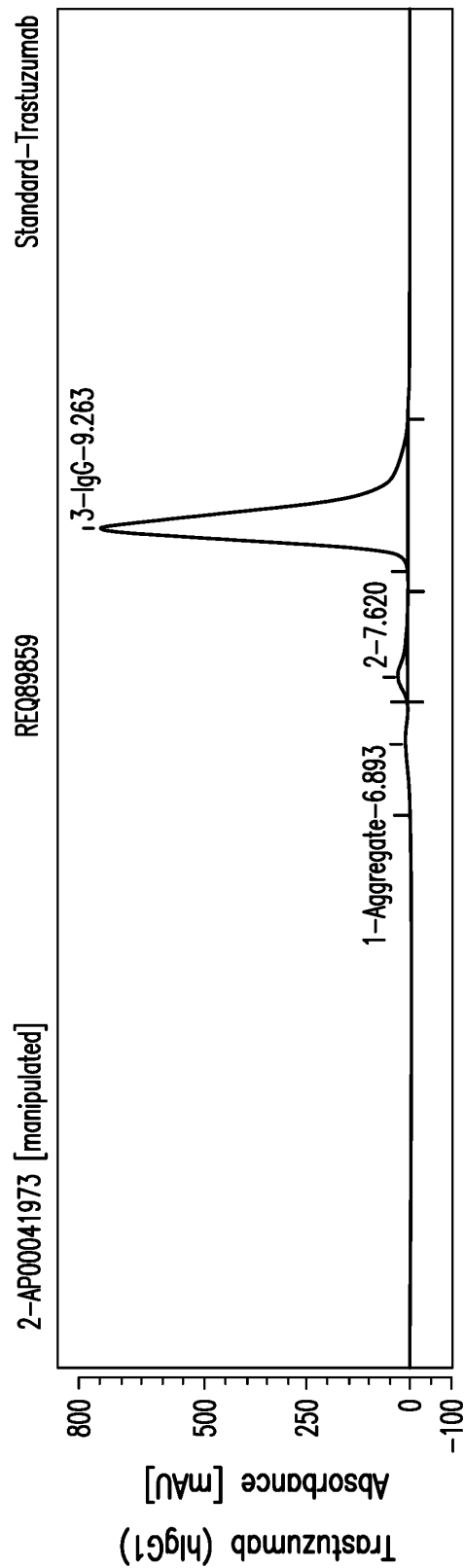
AP00055587

Figure 15A continued



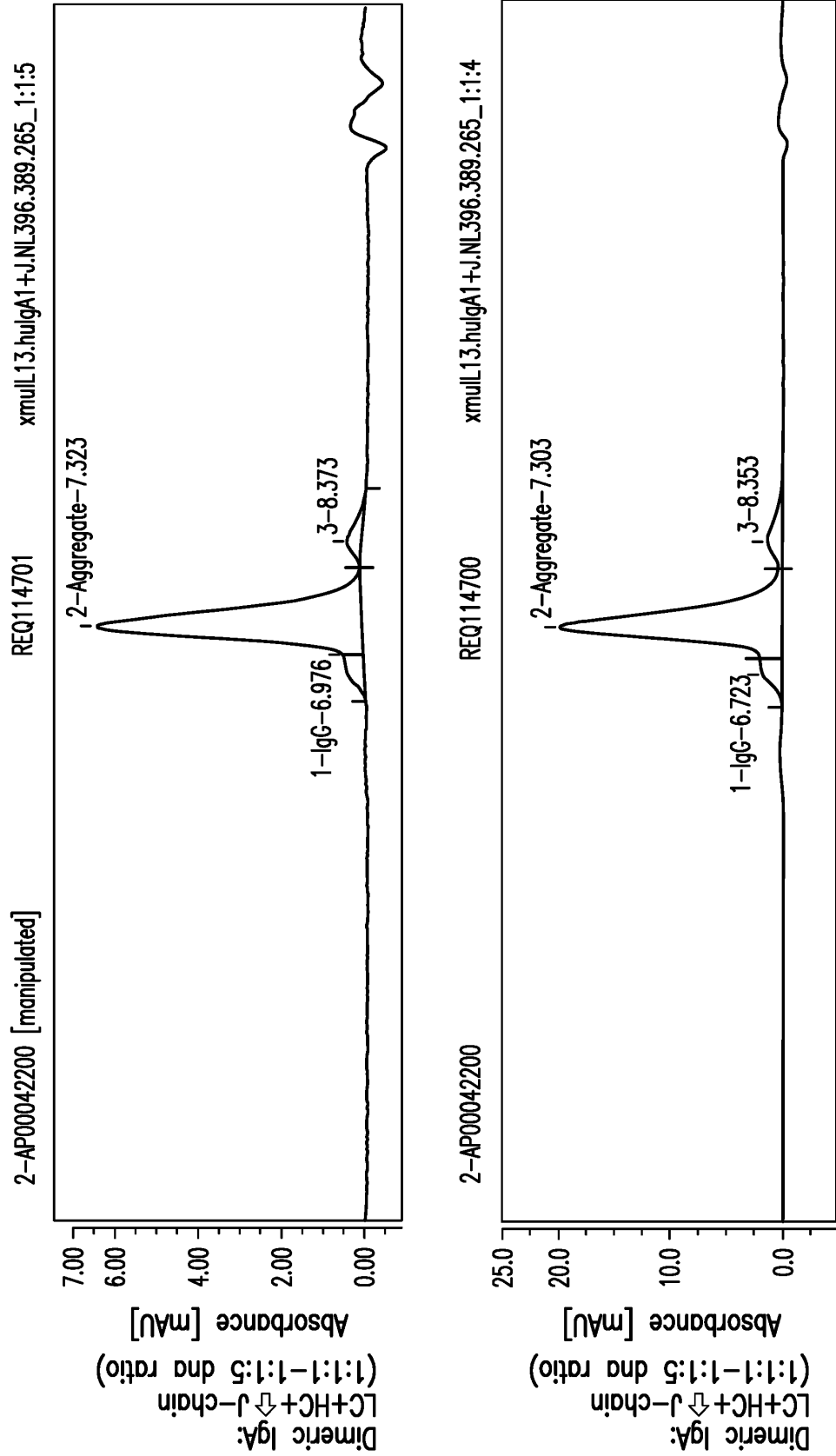
AP00055587

Figure 15A continued



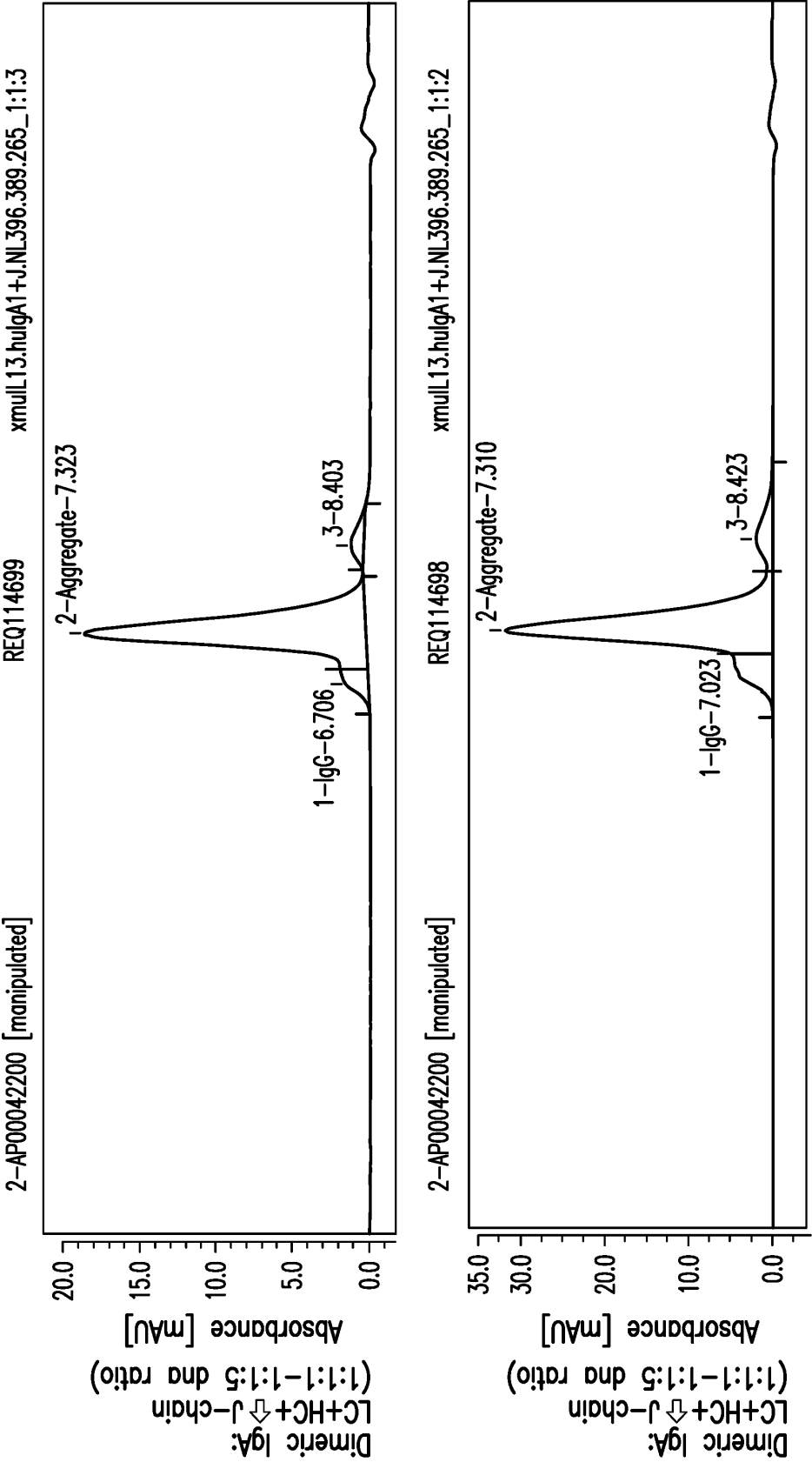
AP00055587

Figure 15A continued



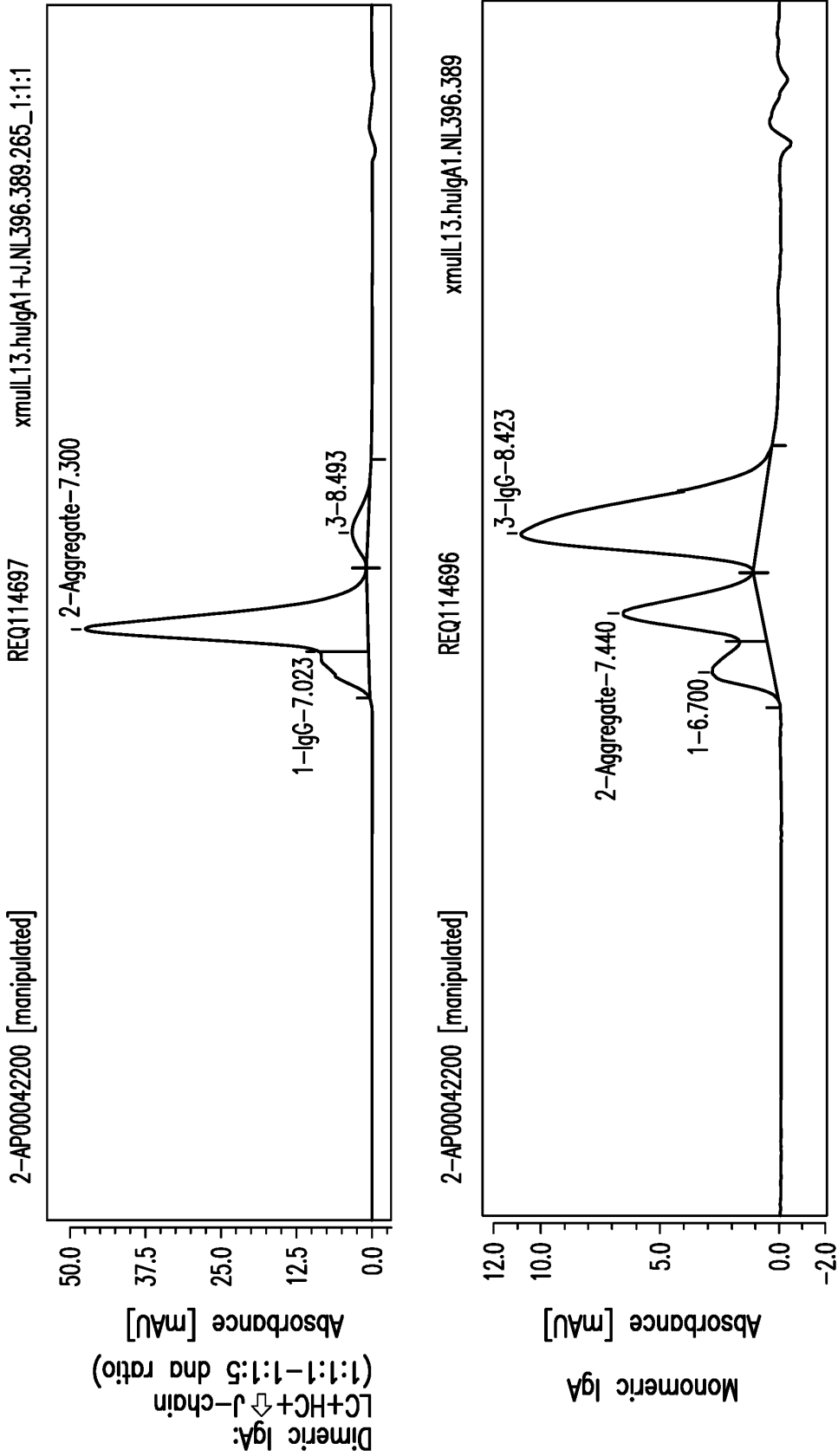
AP00055699

Figure 15B



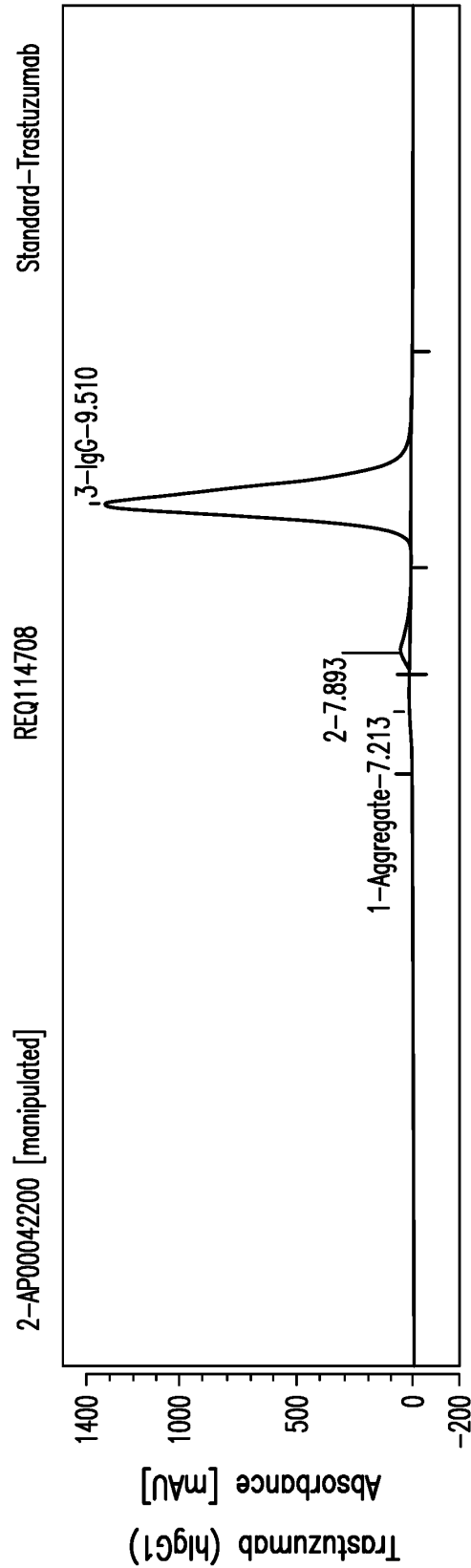
AP00055699

Figure 15B continued



AP00055699

Figure 15B continued

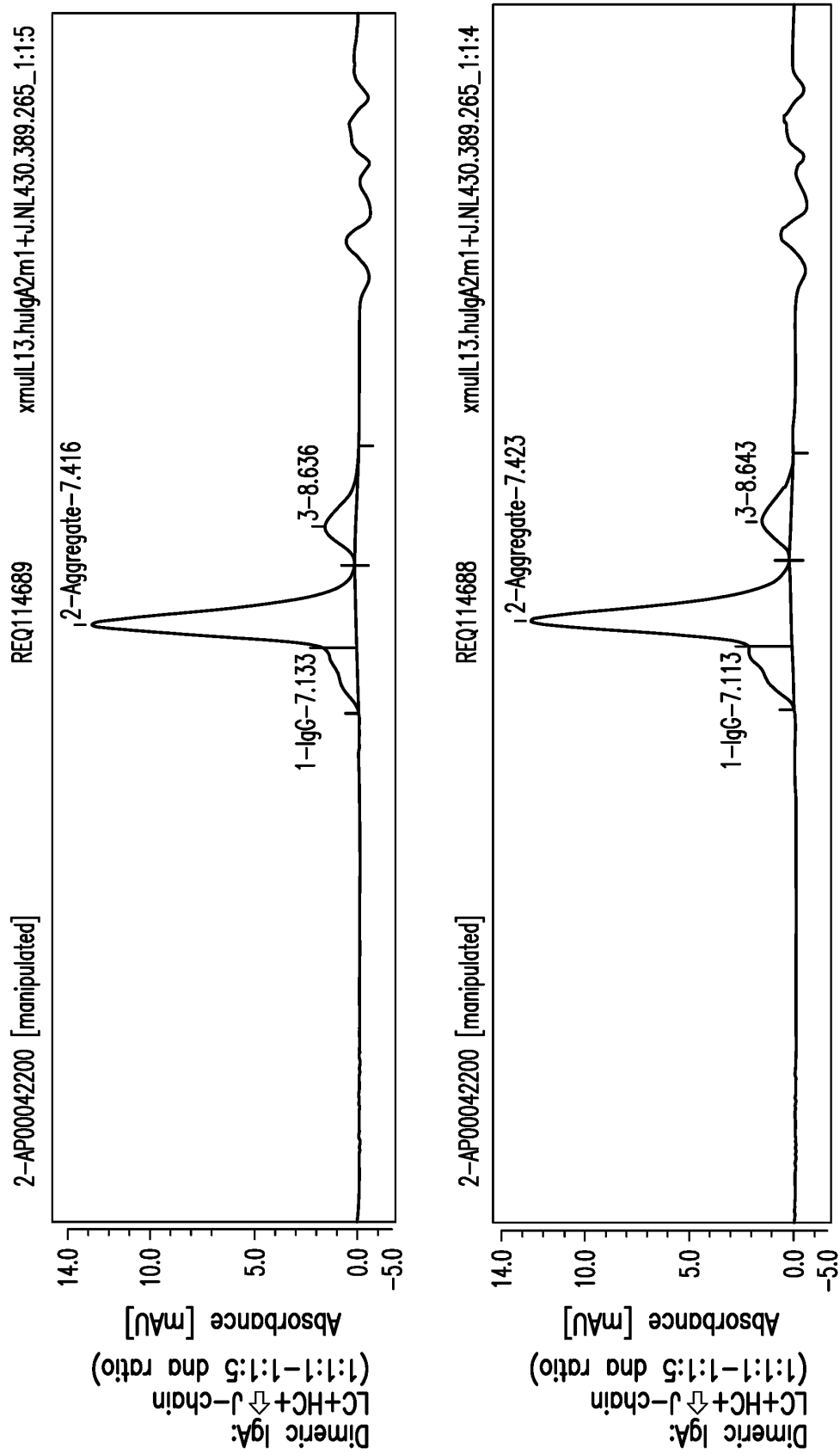


AP00055699

Figure 15B continued

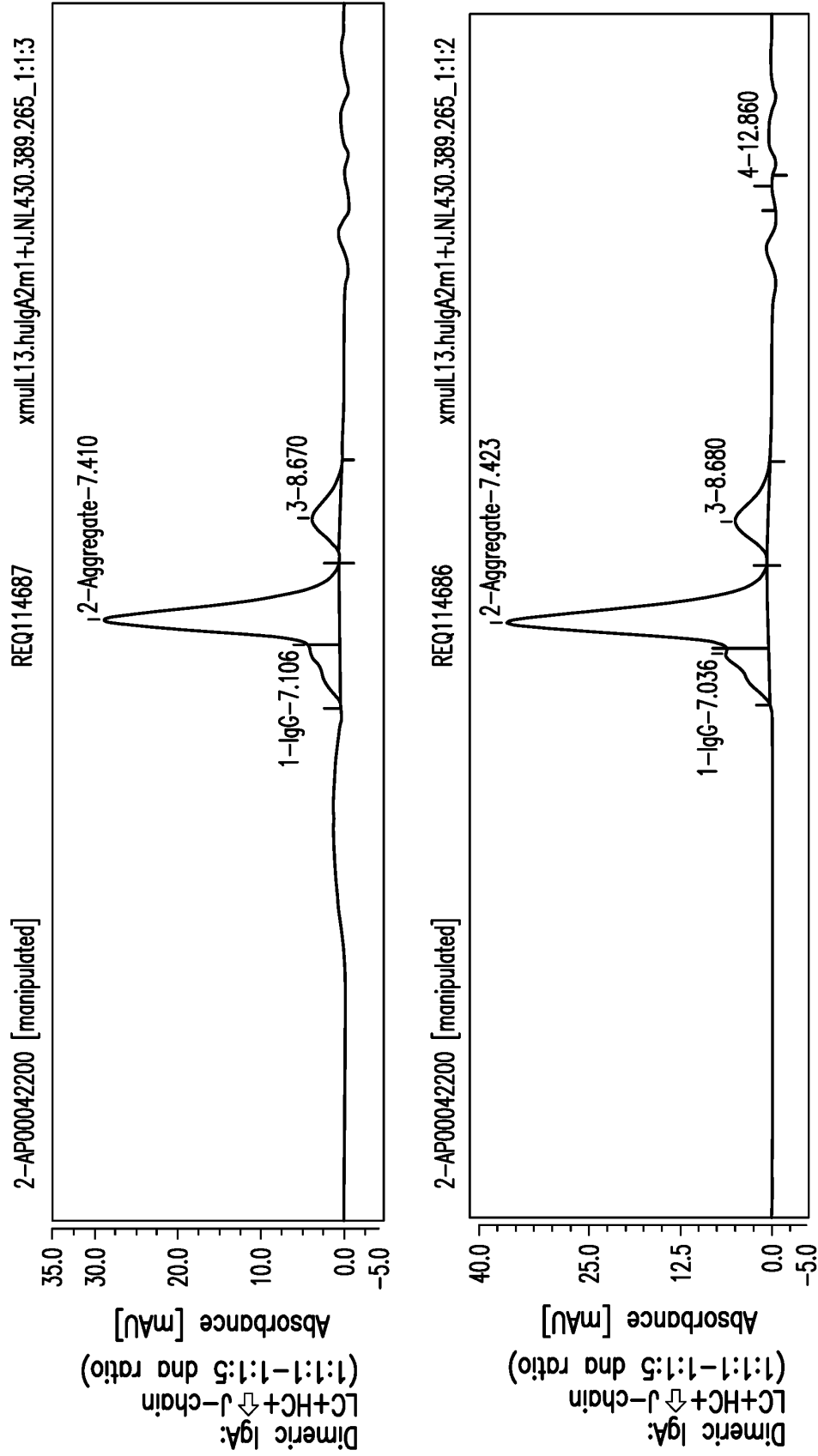
xmull13.IgA1 LC:HC:J-chain Ratio	Dimer Rel Area %	Dimer Area mAU/min	Tetramer Rel Area %	Tetramer Area mAU/min	Total Yield (mg)
1:1:0	24	2.487	11.3	1.171	0.31
1:1:1	81.06	17.925	12.52	2.769	0.52
1:1:2	80.73	12.699	11.5	1.809	0.28
1:1:3	89.66	7.471	6.02	0.502	0.14
1:1:4	85.52	7.982	6.53	0.609	0.16
1:1:5	88.98	2.56	5.91	0.17	0.11

Figure 15C



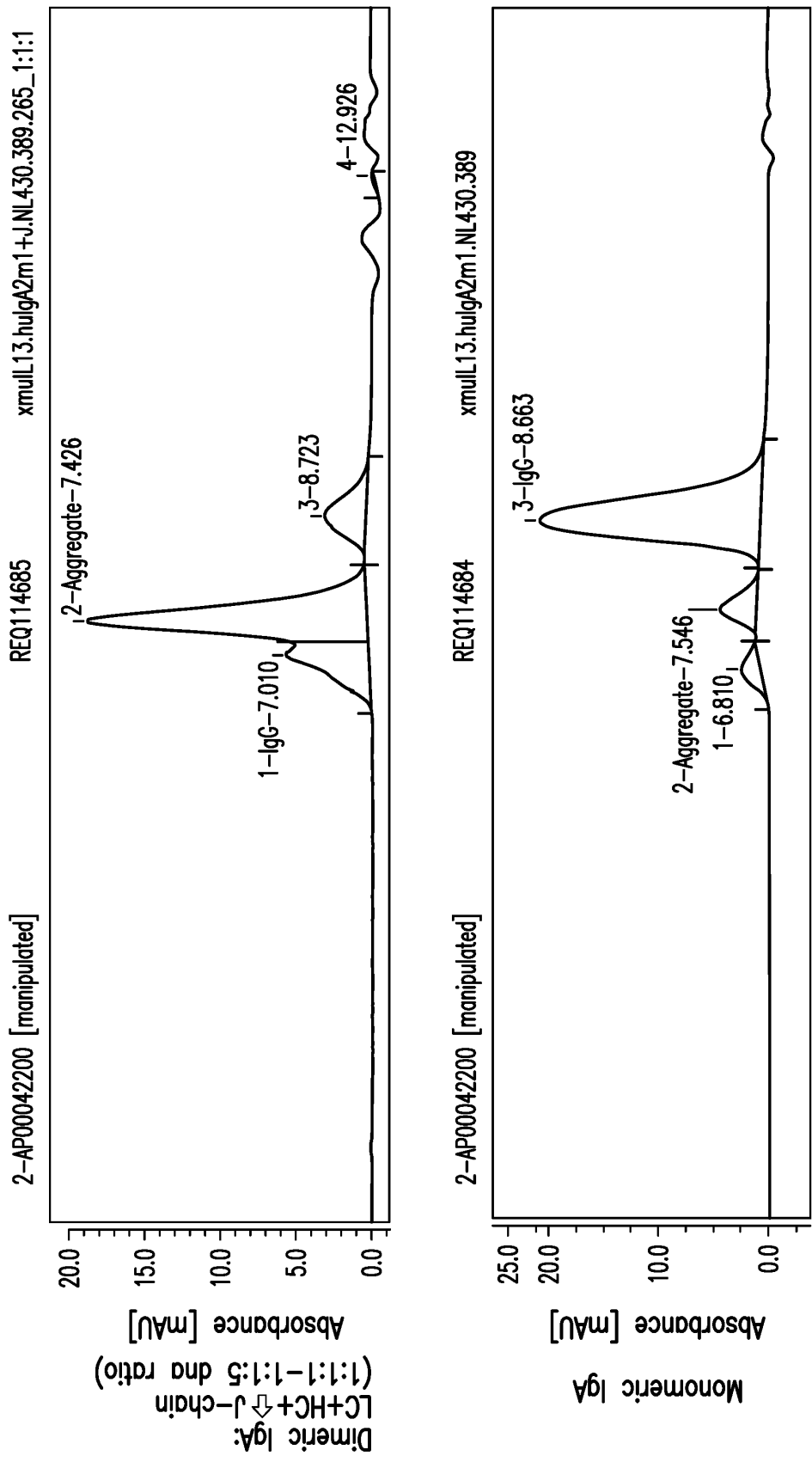
AP00055699

Figure 16A



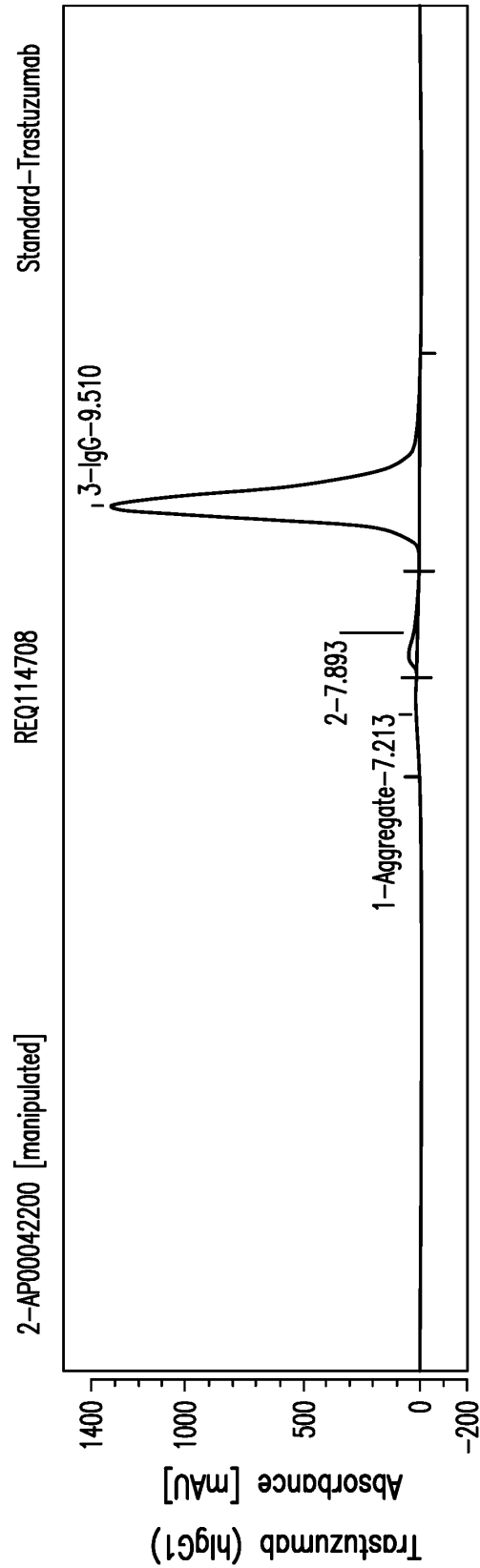
AP00055699

Figure 16A continued



AP00055699

Figure 16A continued

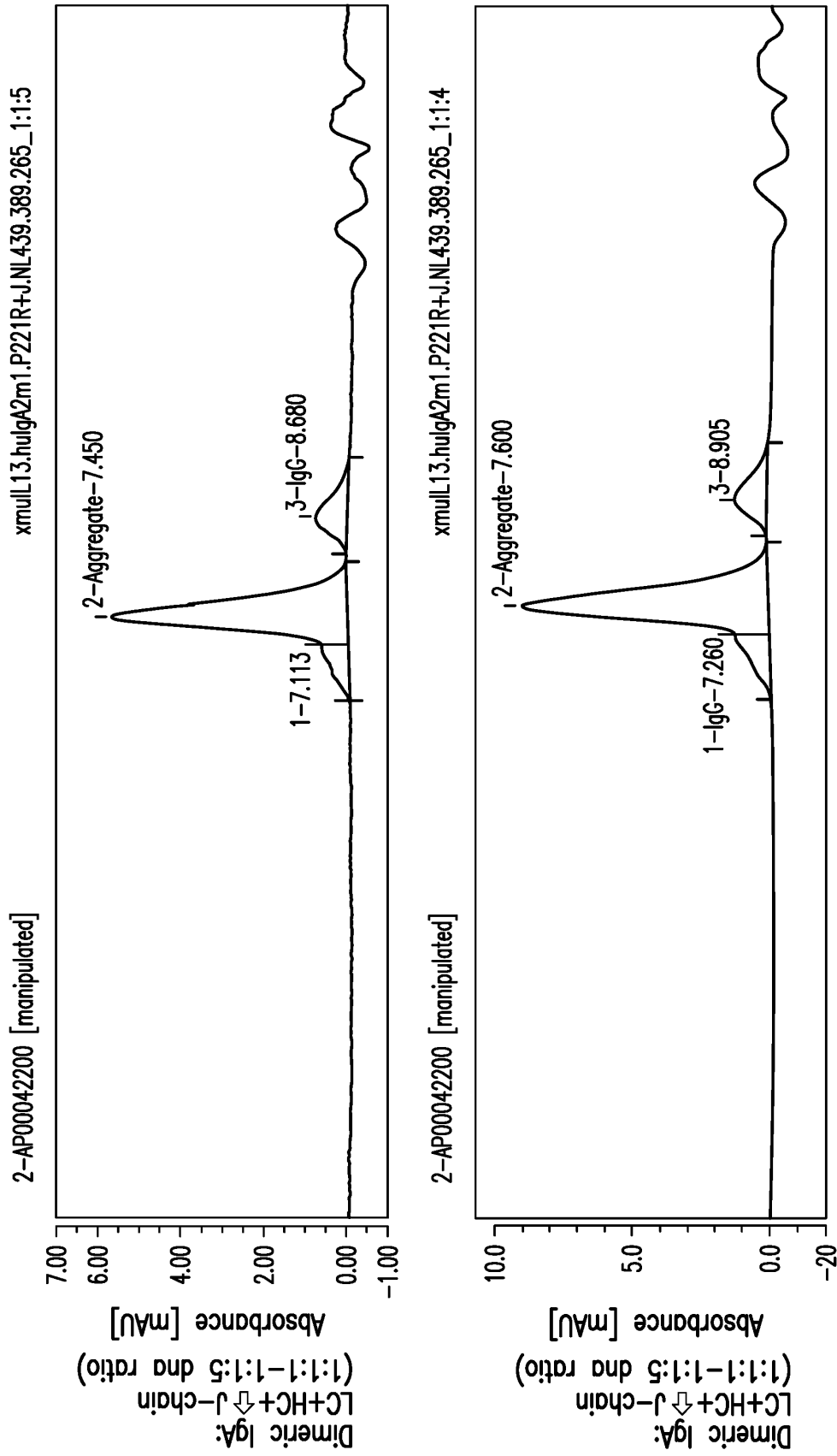


AP00055699

Figure 16A continued

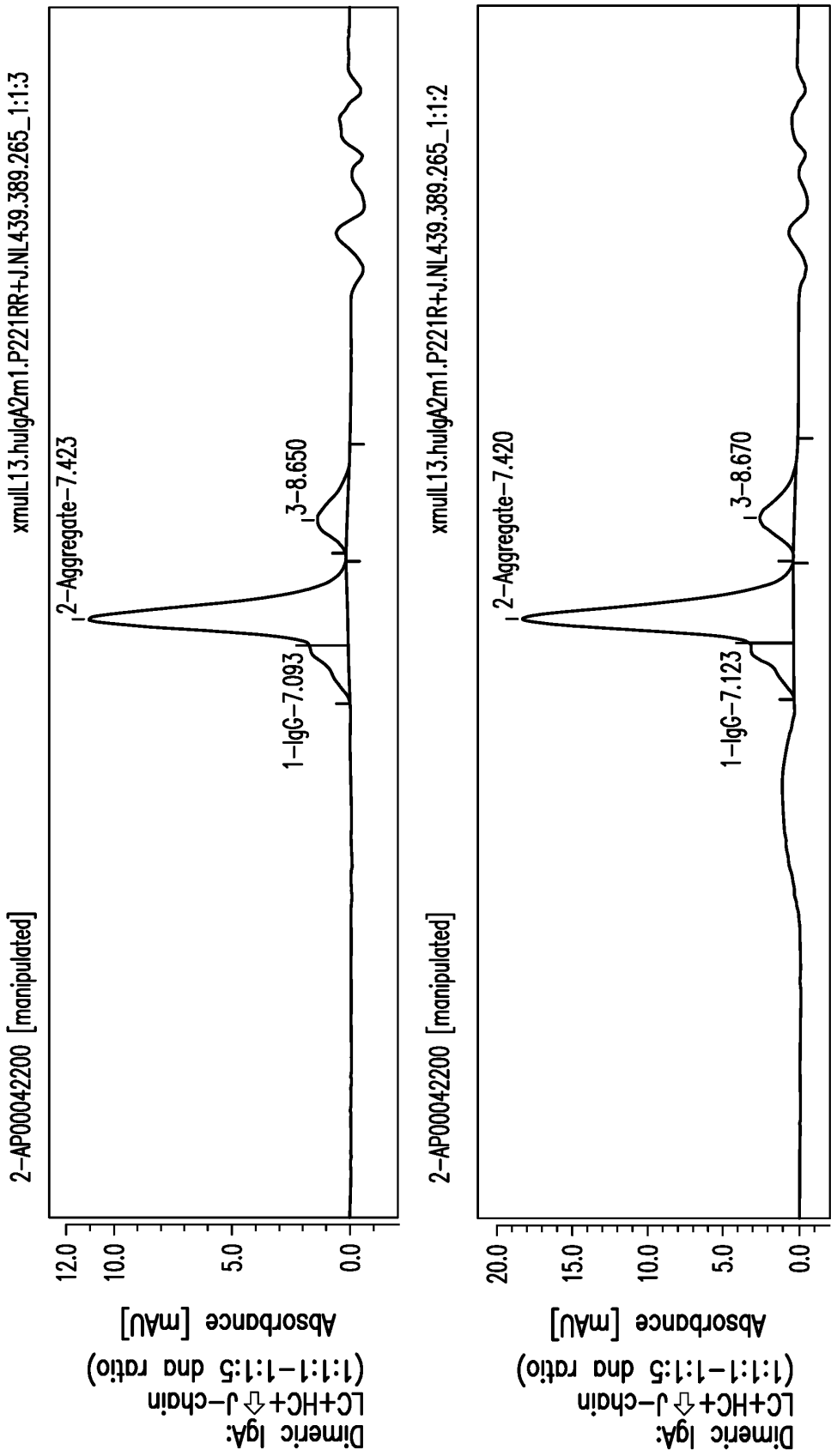
xmull13.IgA1 LC:HC:J-chain Ratio	Dimer Rel Area %	Dimer Area mAU/min	Tetramer Rel Area %	Tetramer Area mAU/min	Total Yield (mg)
1:1:0	8.75	1.207	5.03	0.694	0.54
1:1:1	65.44	7.325	21.01	2.352	0.33
1:1:2	74.42	13.785	12.54	2.322	0.33
1:1:3	76.56	10.756	11.04	1.55	0.27
1:1:4	74.85	4.913	13.93	0.914	0.17
1:1:5	77.04	4.772	10.52	0.652	0.11

Figure 16B



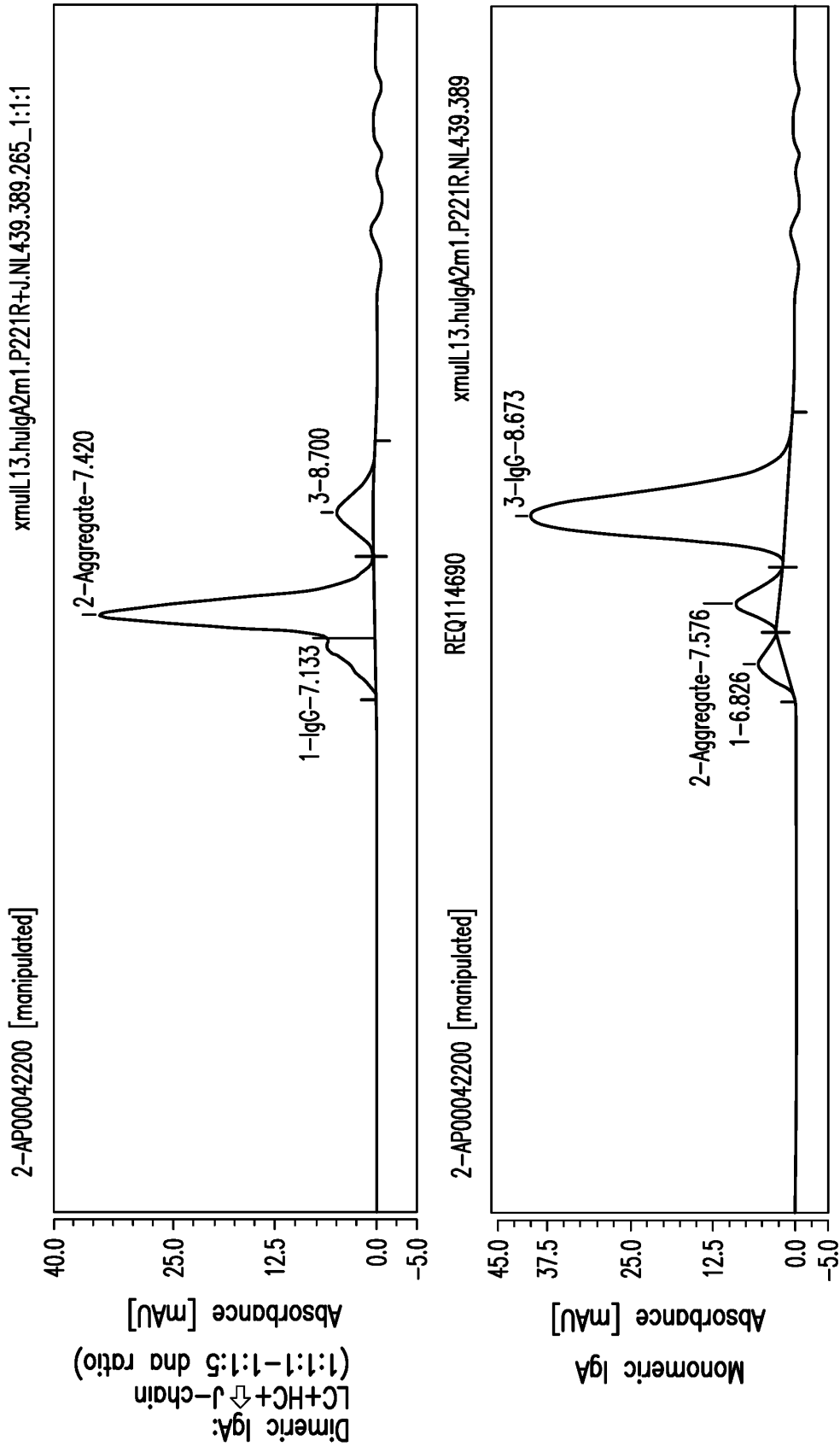
AP00055699

Figure 17A



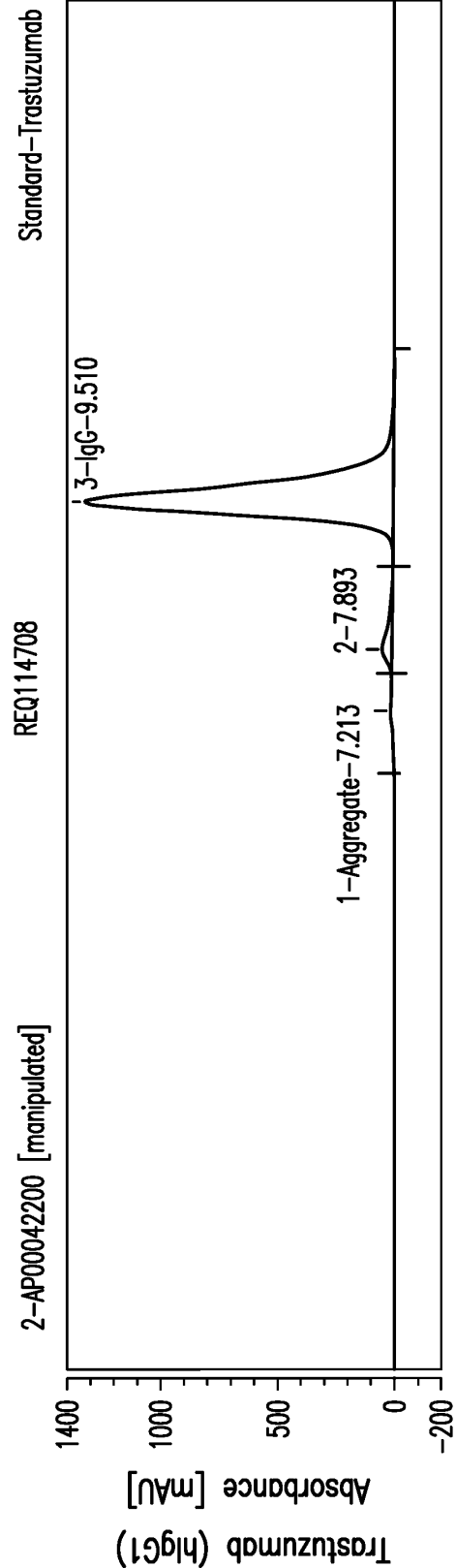
AP00055699

Figure 17A continued



AP00055699

Figure 17A continued

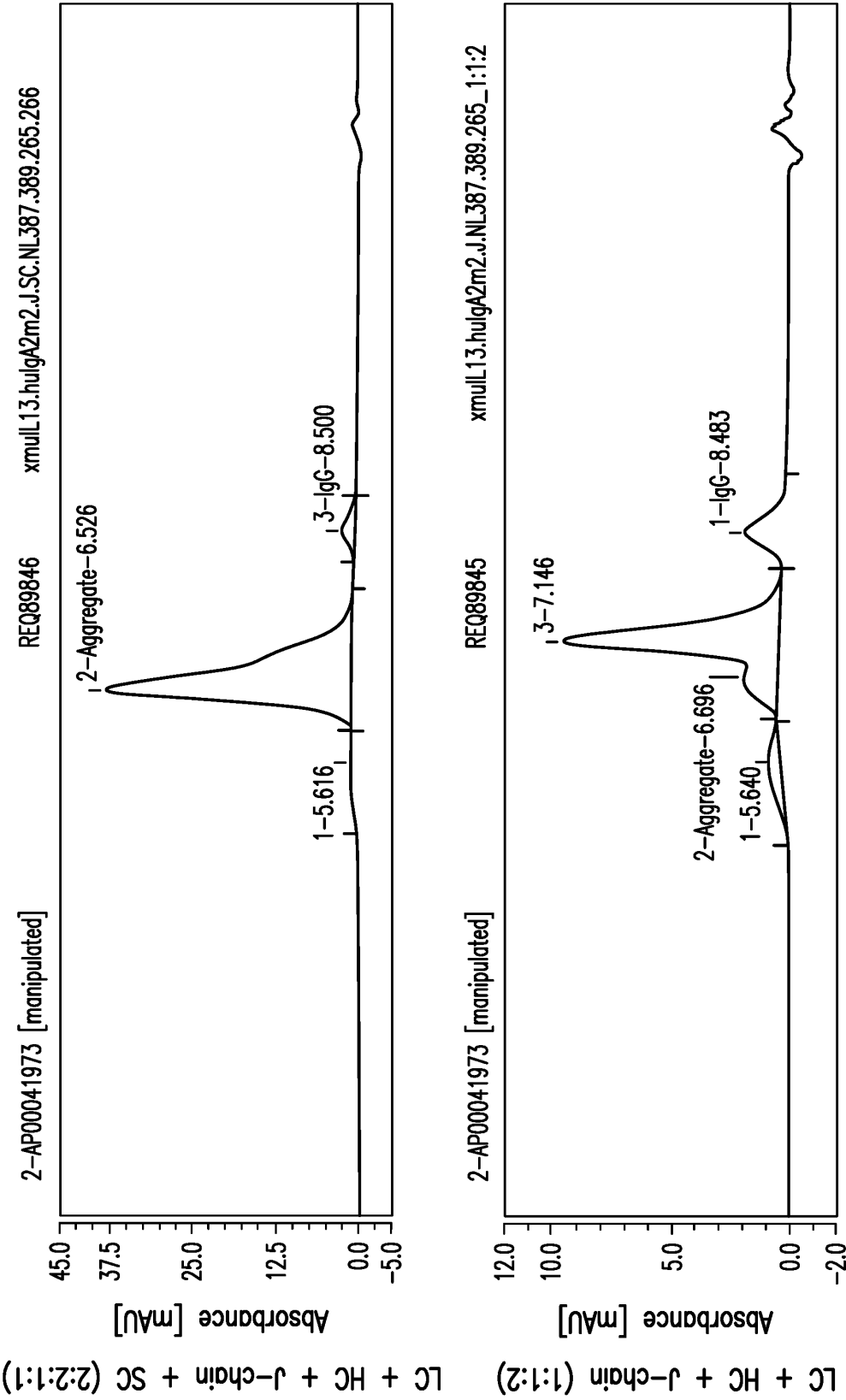


AP00055699

Figure 17A continued

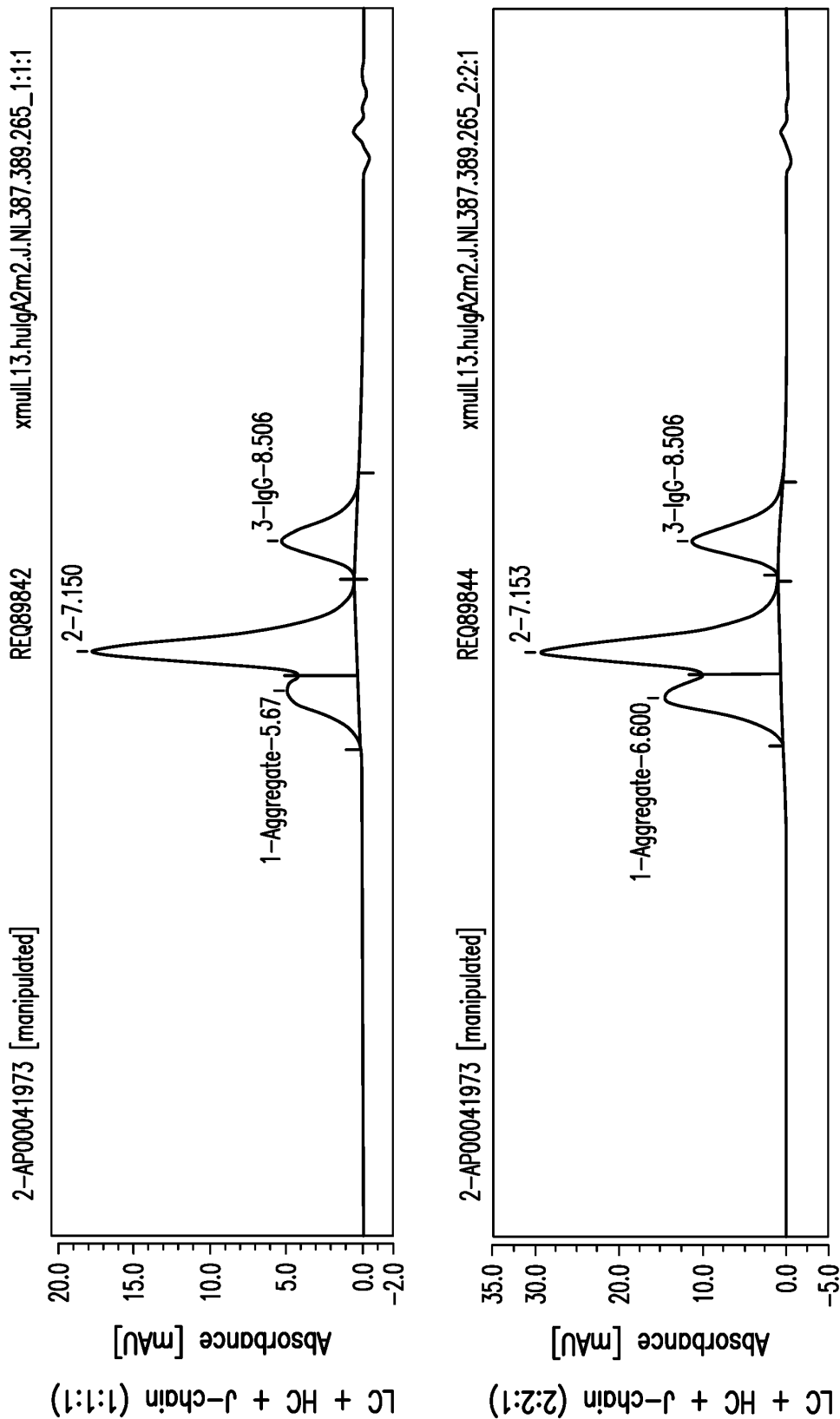
xmull13.IgA2m1 .P221RLC:HC:J- chain Ratio	Dimer Rel Area %	Dimer Area mAU/min	Tetramer Rel Area %	Tetramer Area mAU/min	Total Yield (mg)
1:1:0	8.42	2.414	5.75	1.648	0.58
1:1:1	73.22	12.833	13.09	2.294	0.29
1:1:2	75.9	6.829	11.11	1	0.20
1:1:3	77.75	4.241	10.4	0.567	0.13
1:1:4	85.52	7.982	6.53	0.609	0.11
1:1:5	77.56	2.258	8.97	0.261	0.06

Figure 17B



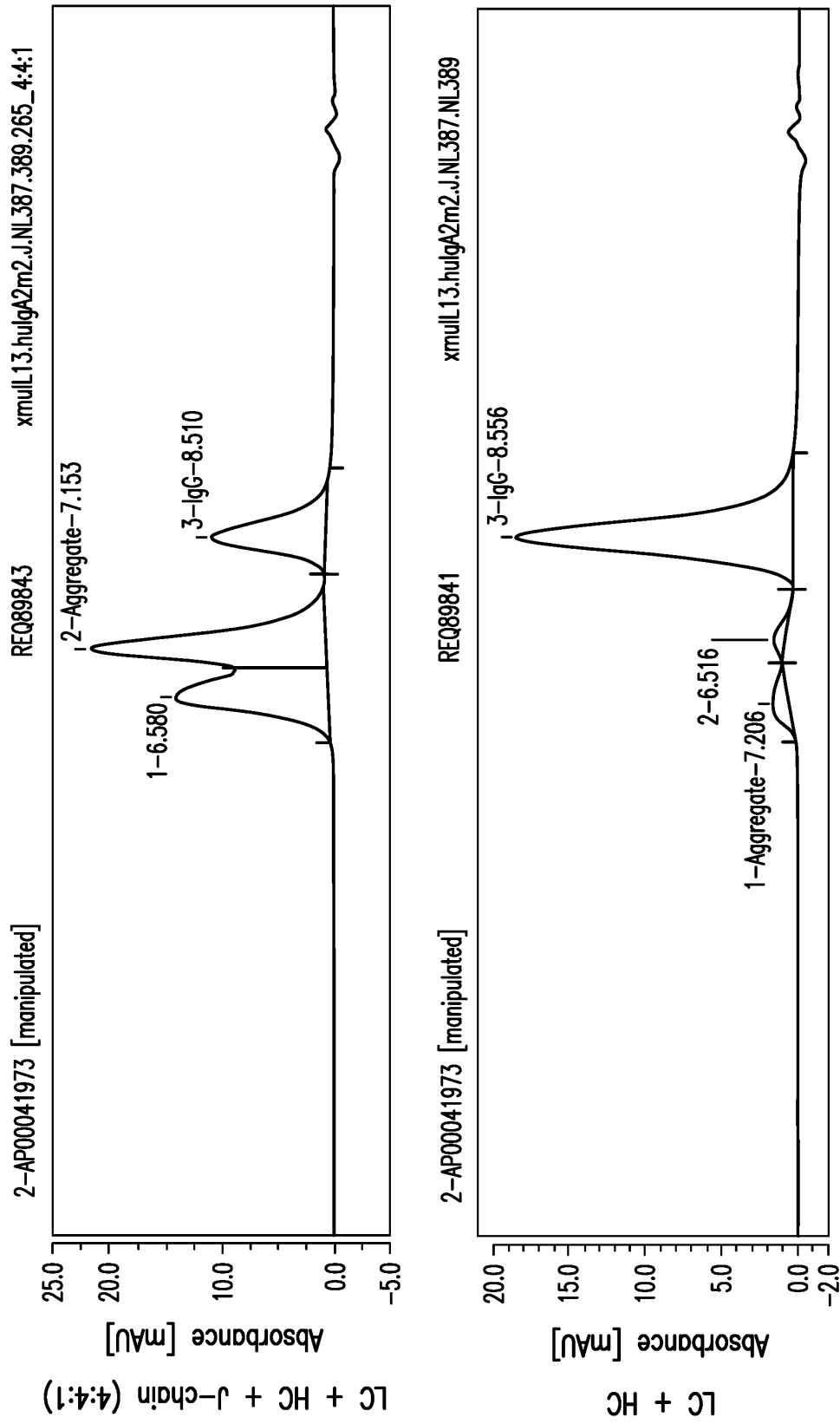
AP00055587

Figure 18A



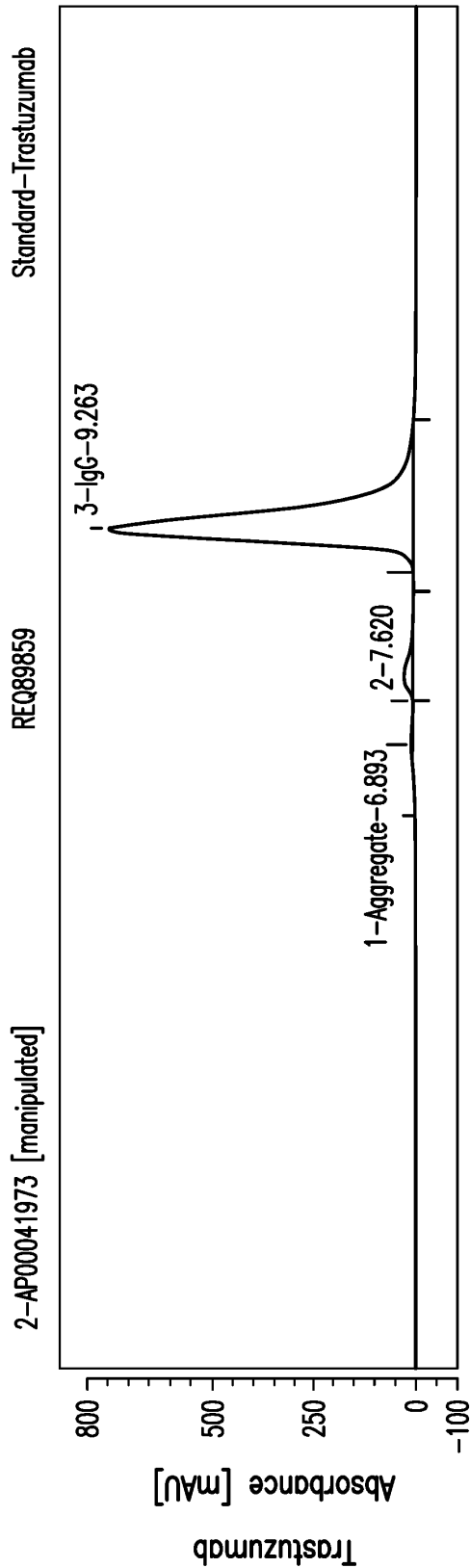
AP00055587

Figure 18A continued



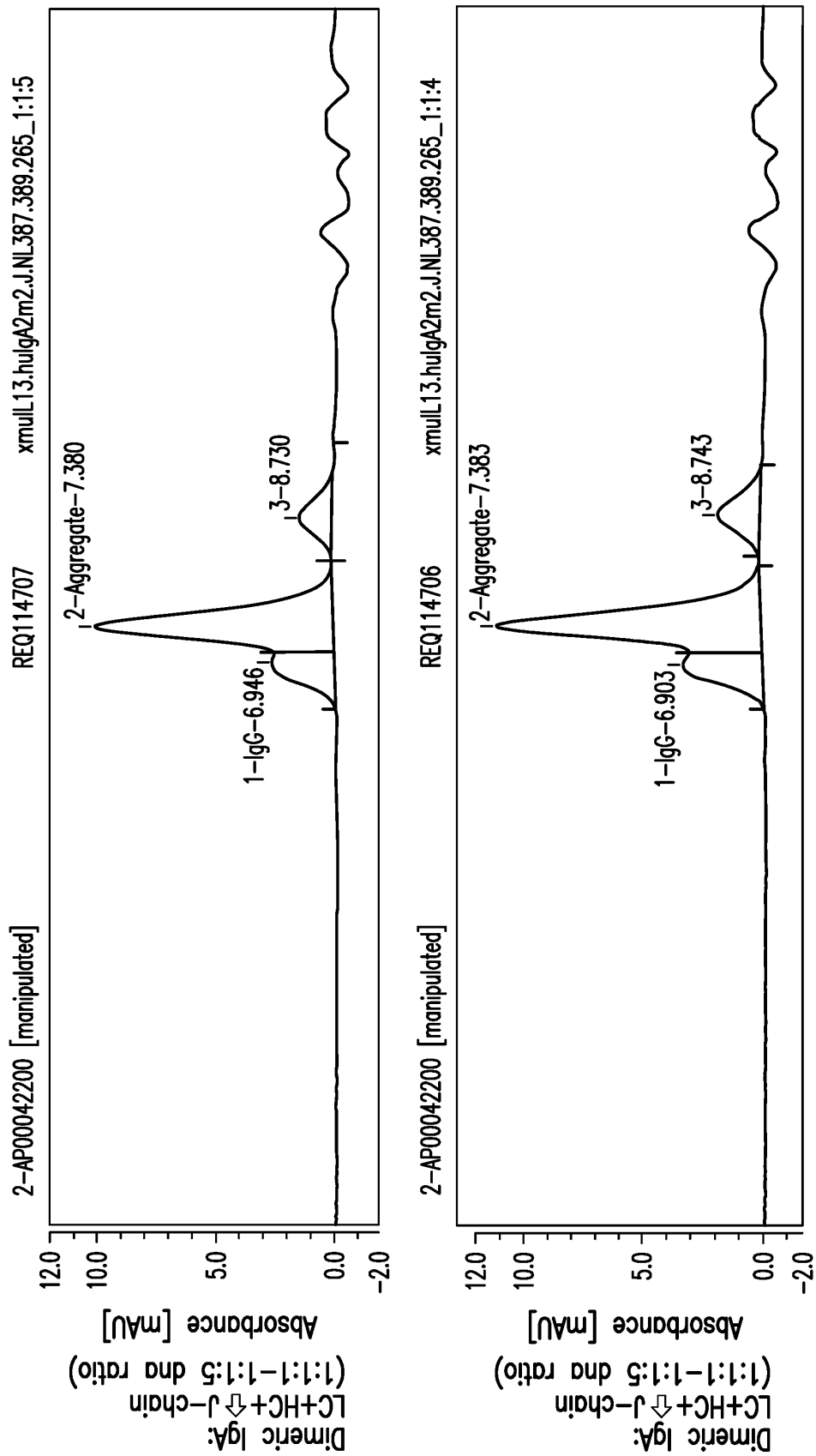
AP00055587

Figure 18A continued



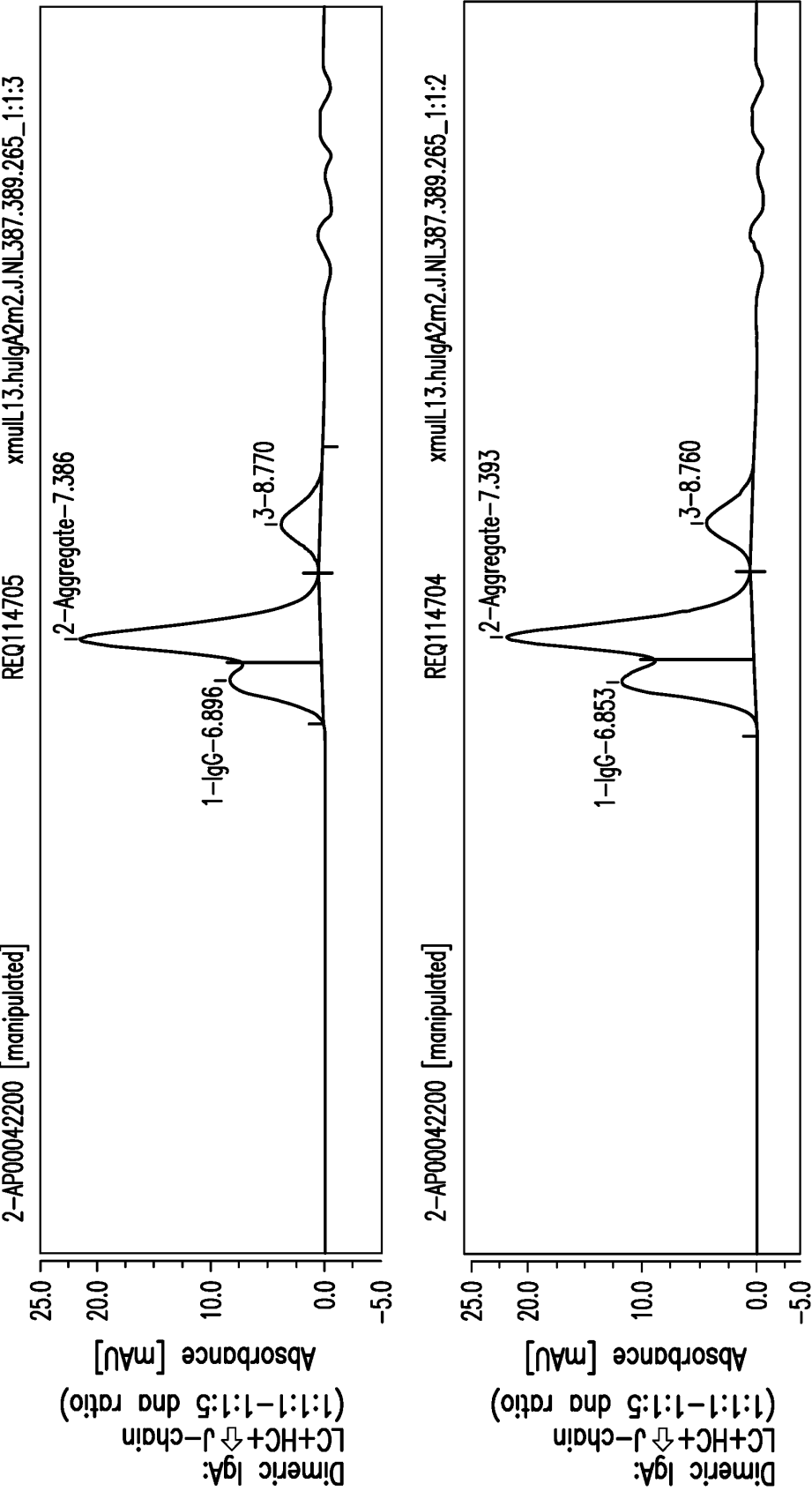
AP00055587

Figure 18A continued



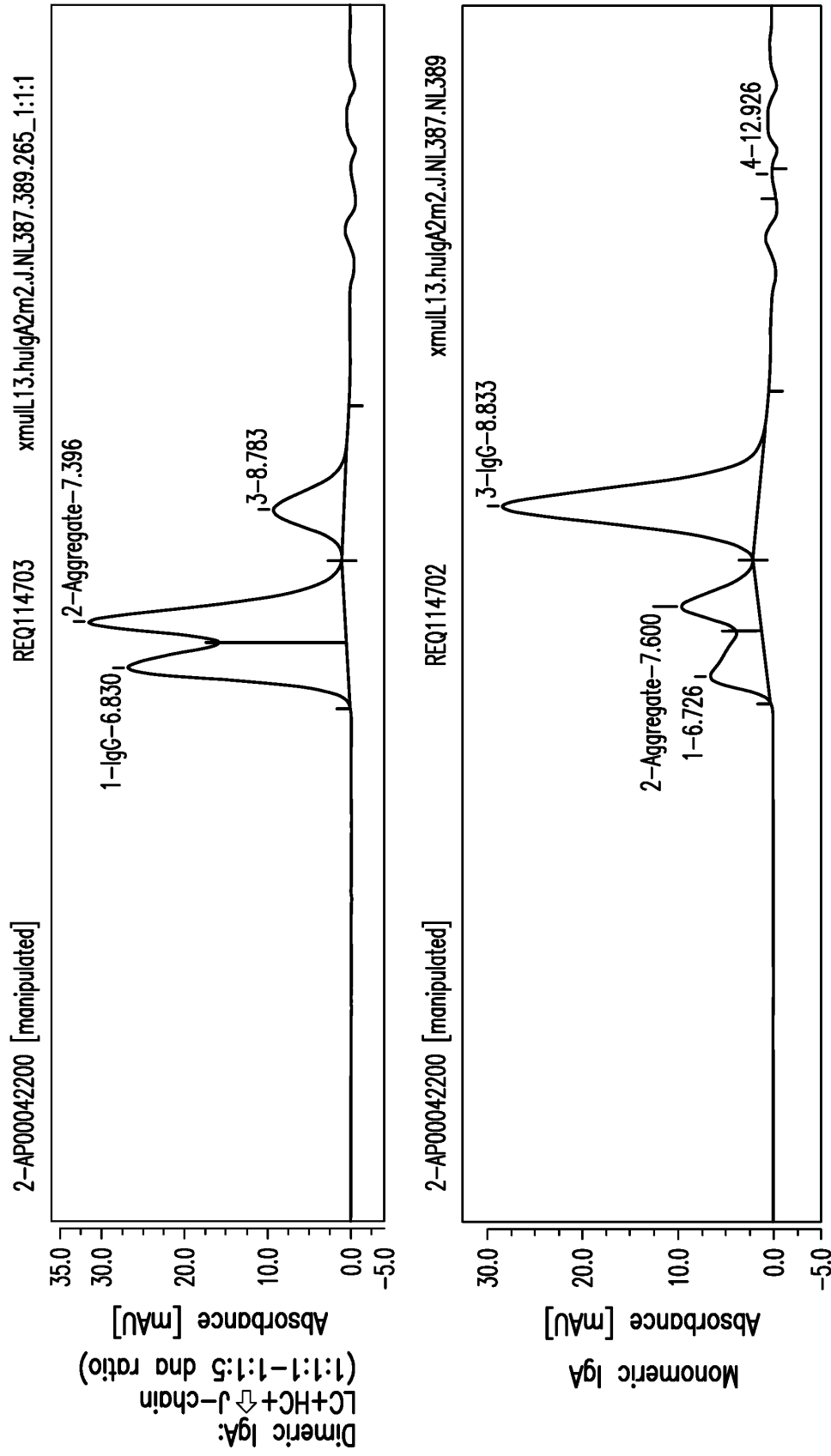
AP00055699

Figure 18B



AP00055699

Figure 18B continued



AP00055699

Figure 18B continued

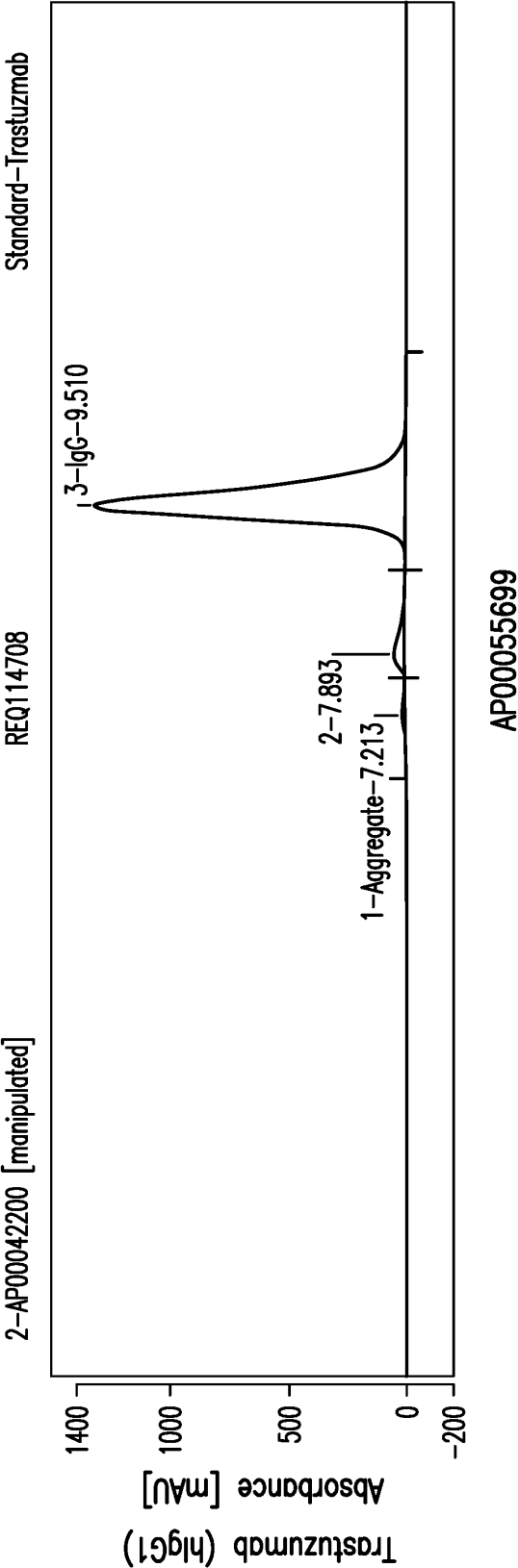
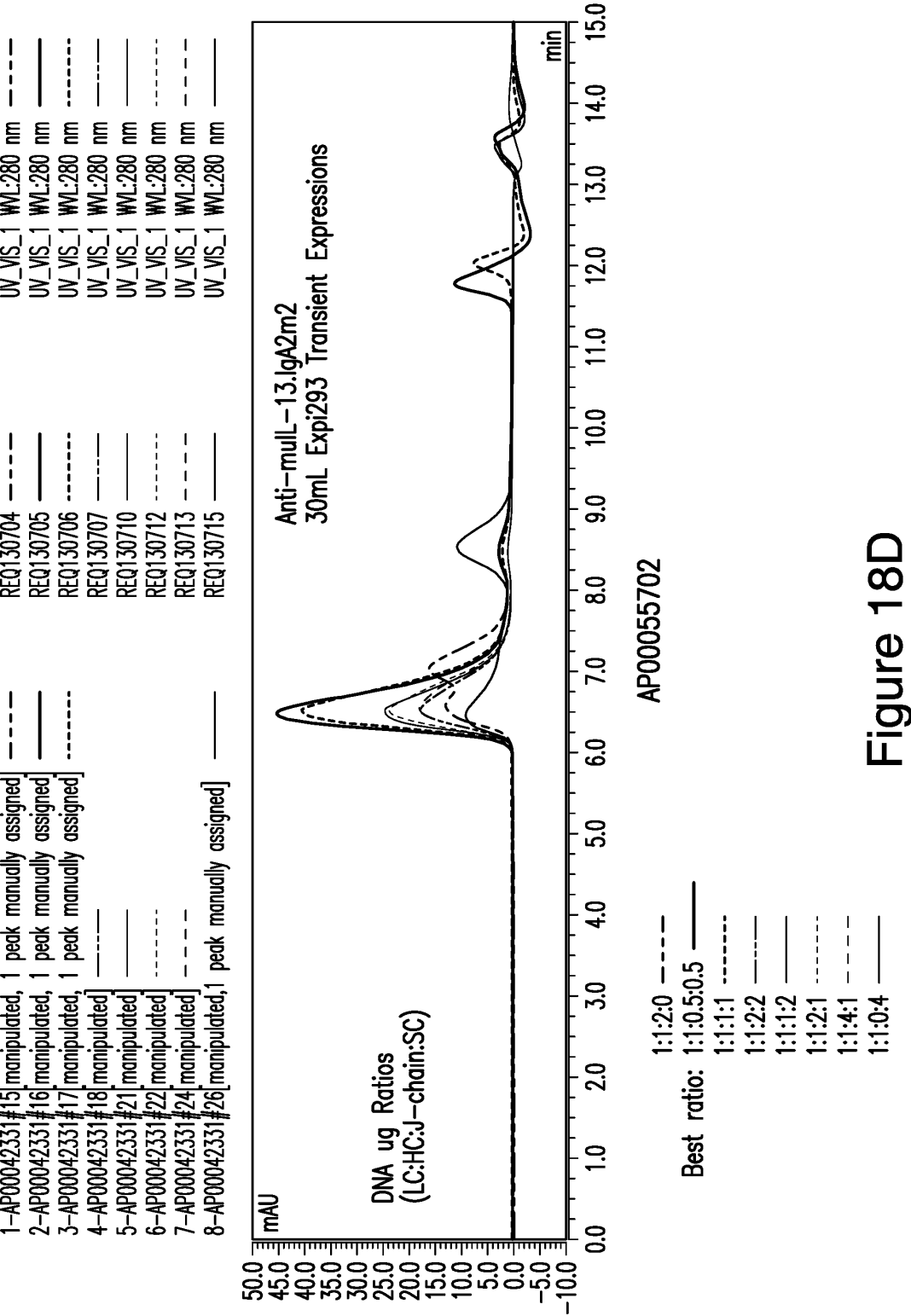


Figure 18B continued

xmull13.IgA2m2 LC:HC:J-chain Ratio	Dimer Rel Area %	Dimer Area mAU/min	Tetramer Rel Area %	Tetramer Area mAU/min	Total Yield (mg)
1:1:0	16.33	3.531	15.37	3.322	0.41
1:1:1	45.87	13.326	38.7	11.243	0.52
1:1:2	56.08	9.184	31.33	5.131	0.32
1:1:3	61.93	8.873	25.53	3.658	0.27
1:1:4	68.17	4.683	19.01	1.305	0.13
1:1:5	70.39	4.212	18	1.077	0.11

Figure 18C



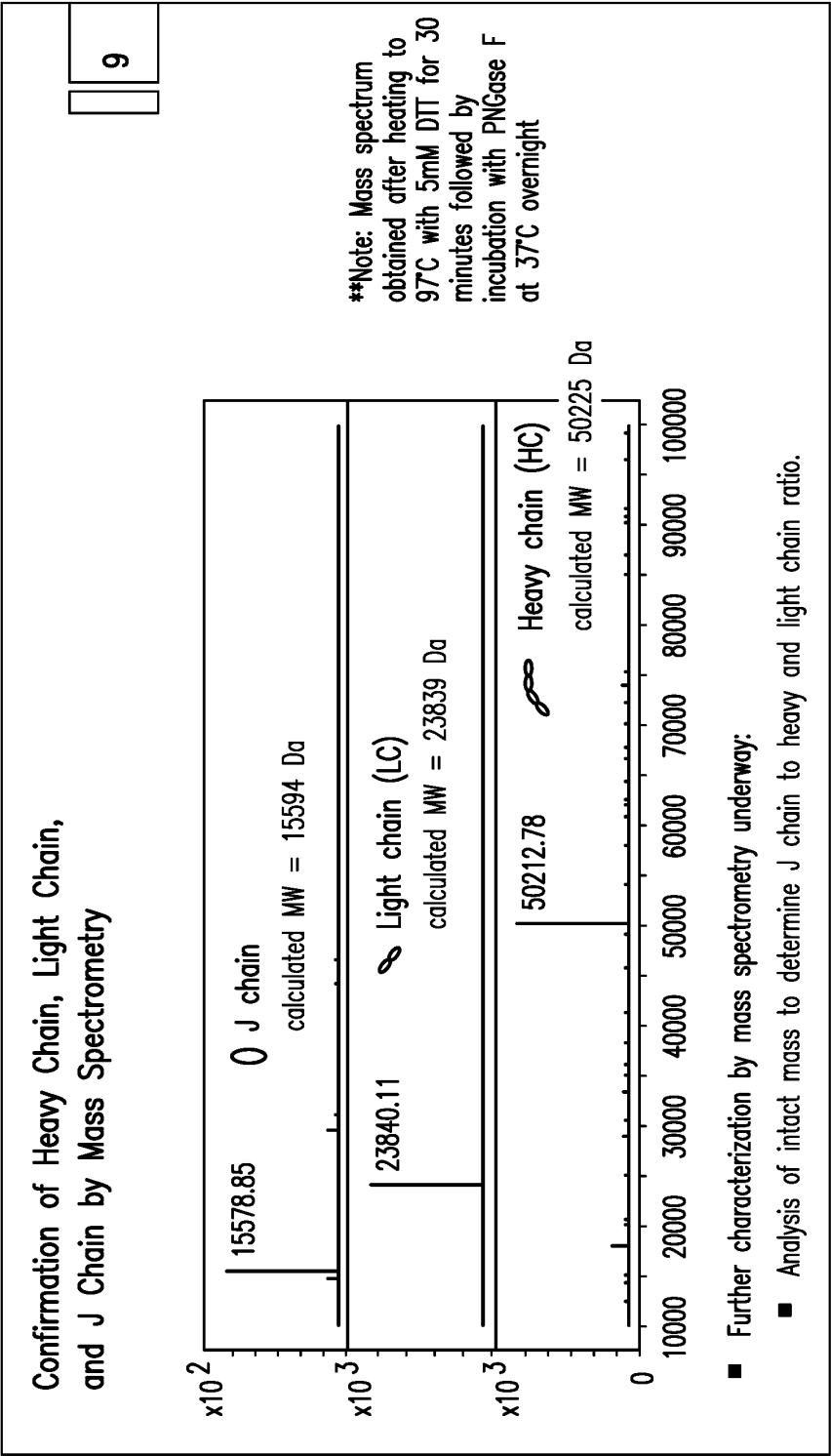


Figure 18E

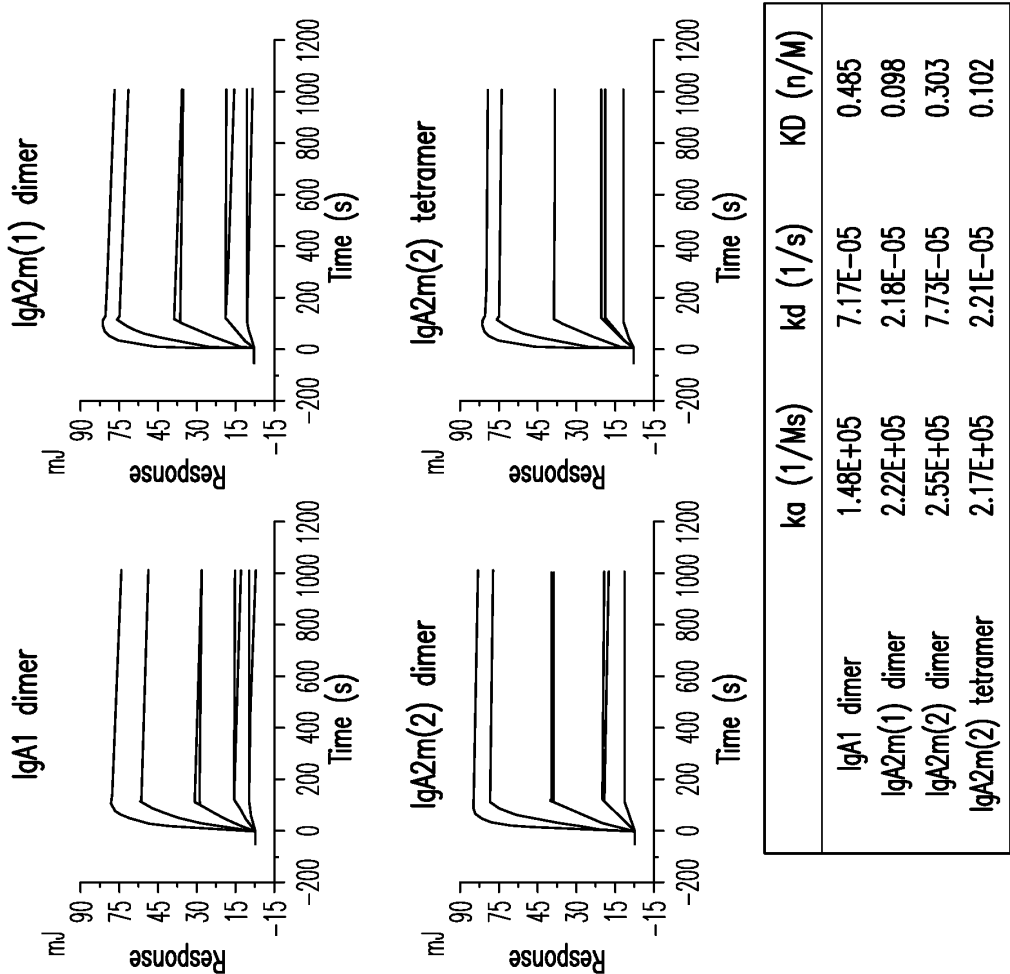
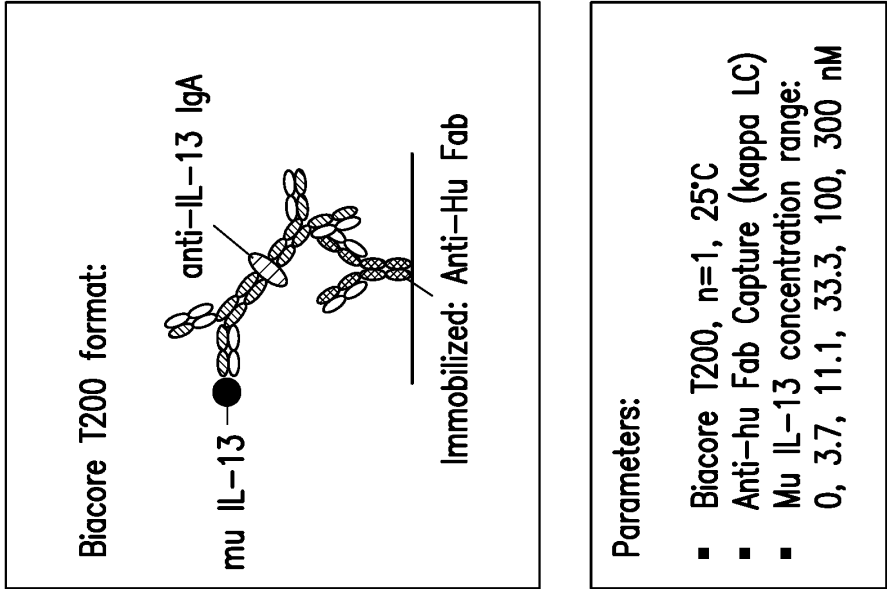
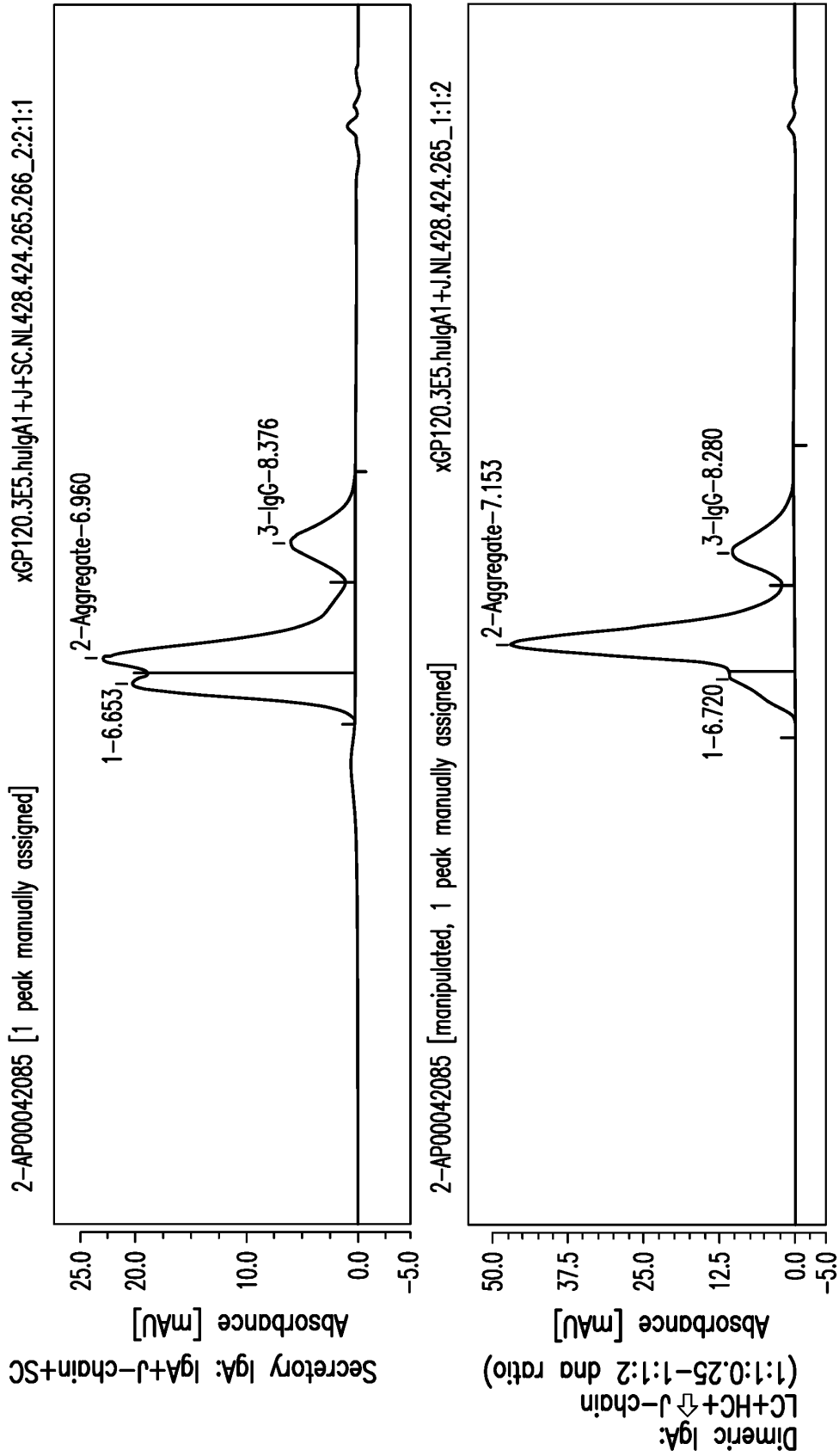
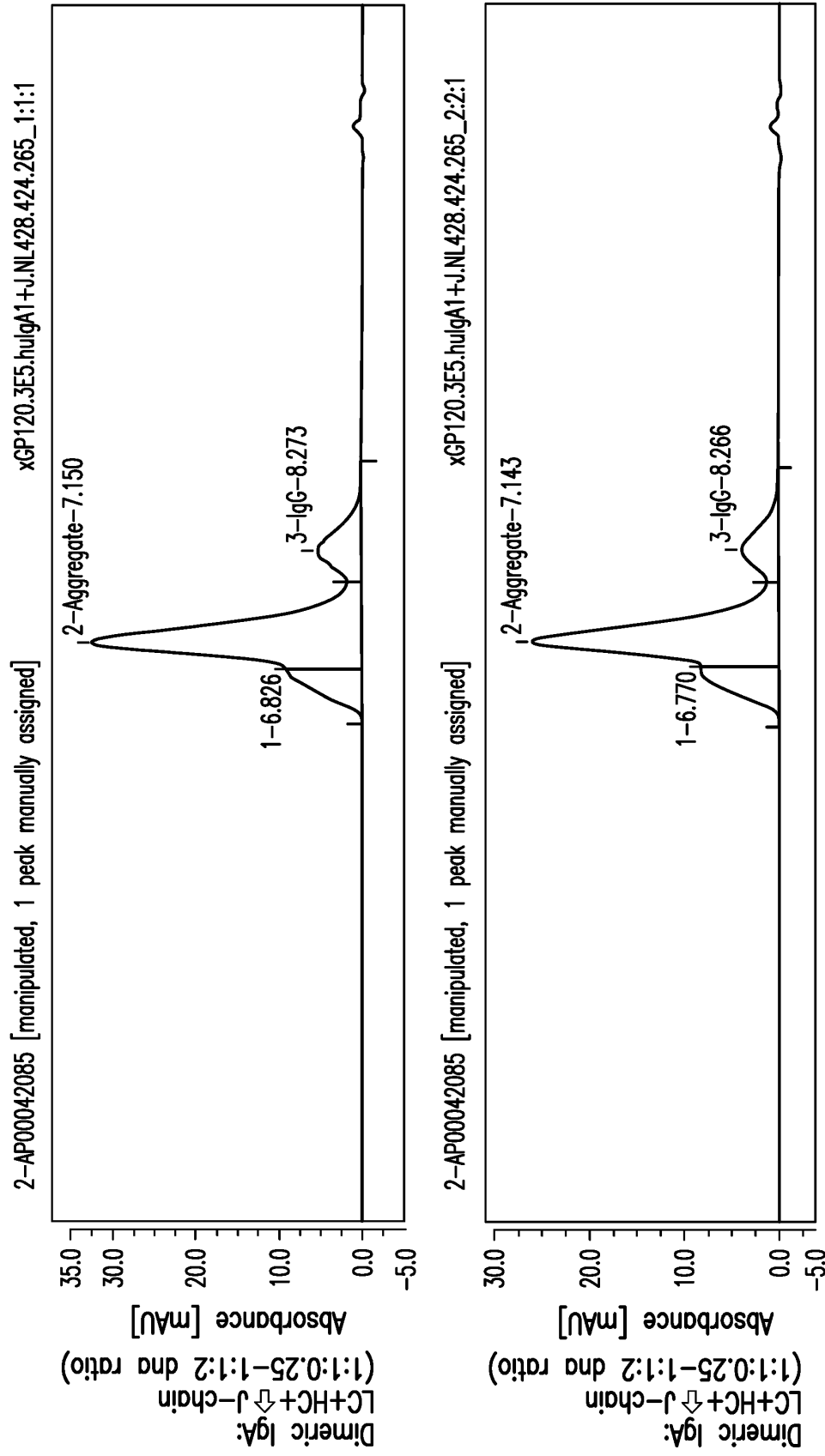


Figure 19



AP00055705

Figure 20A



AP00055705

Figure 20A continued

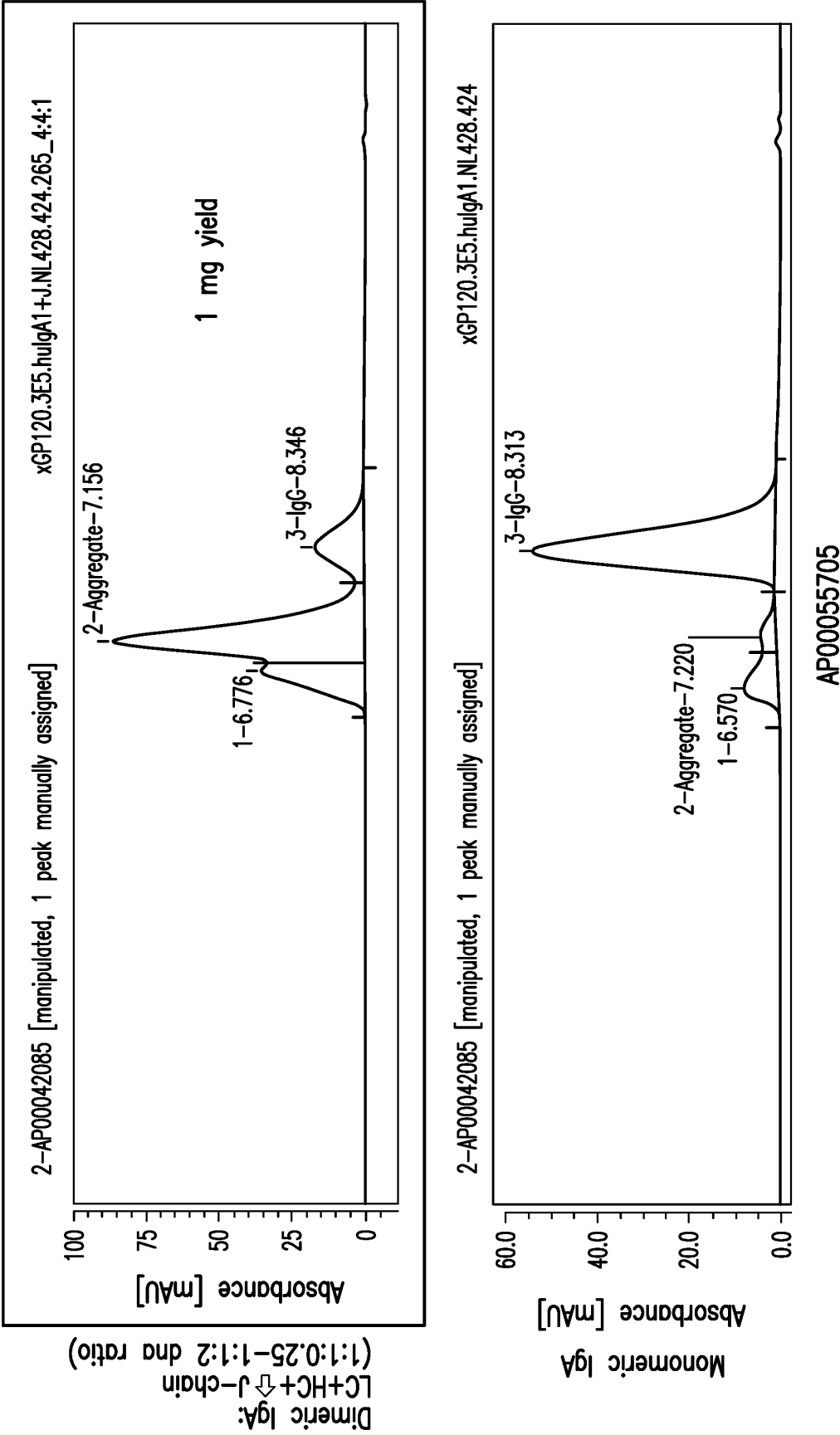
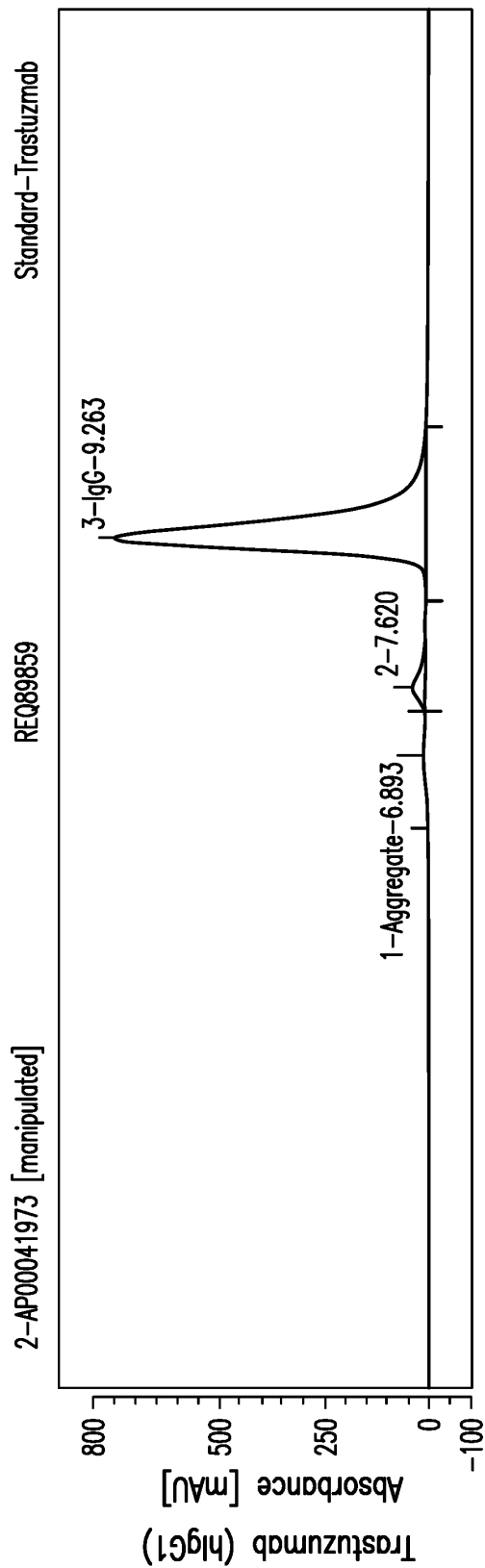
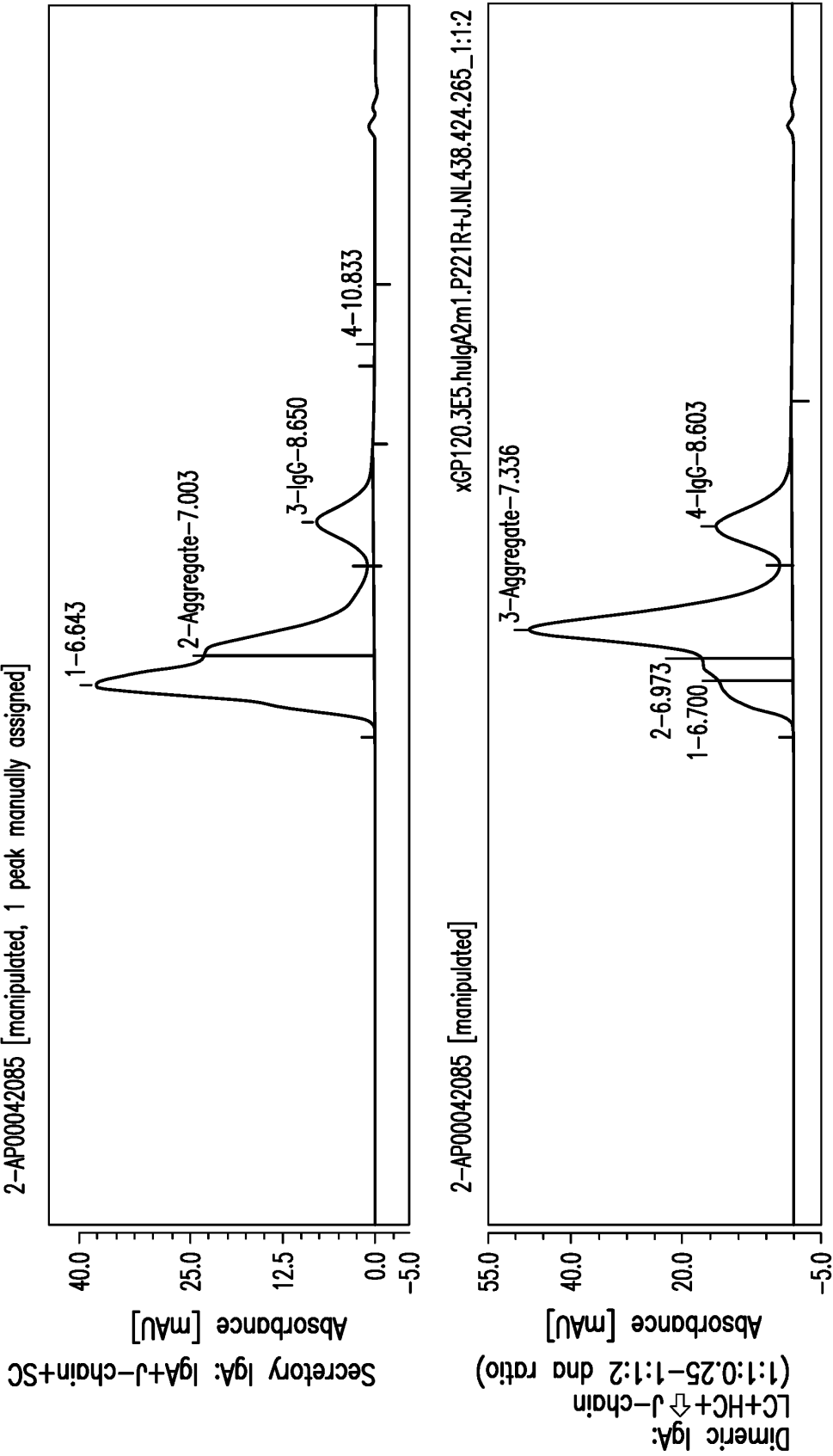


Figure 20A continued



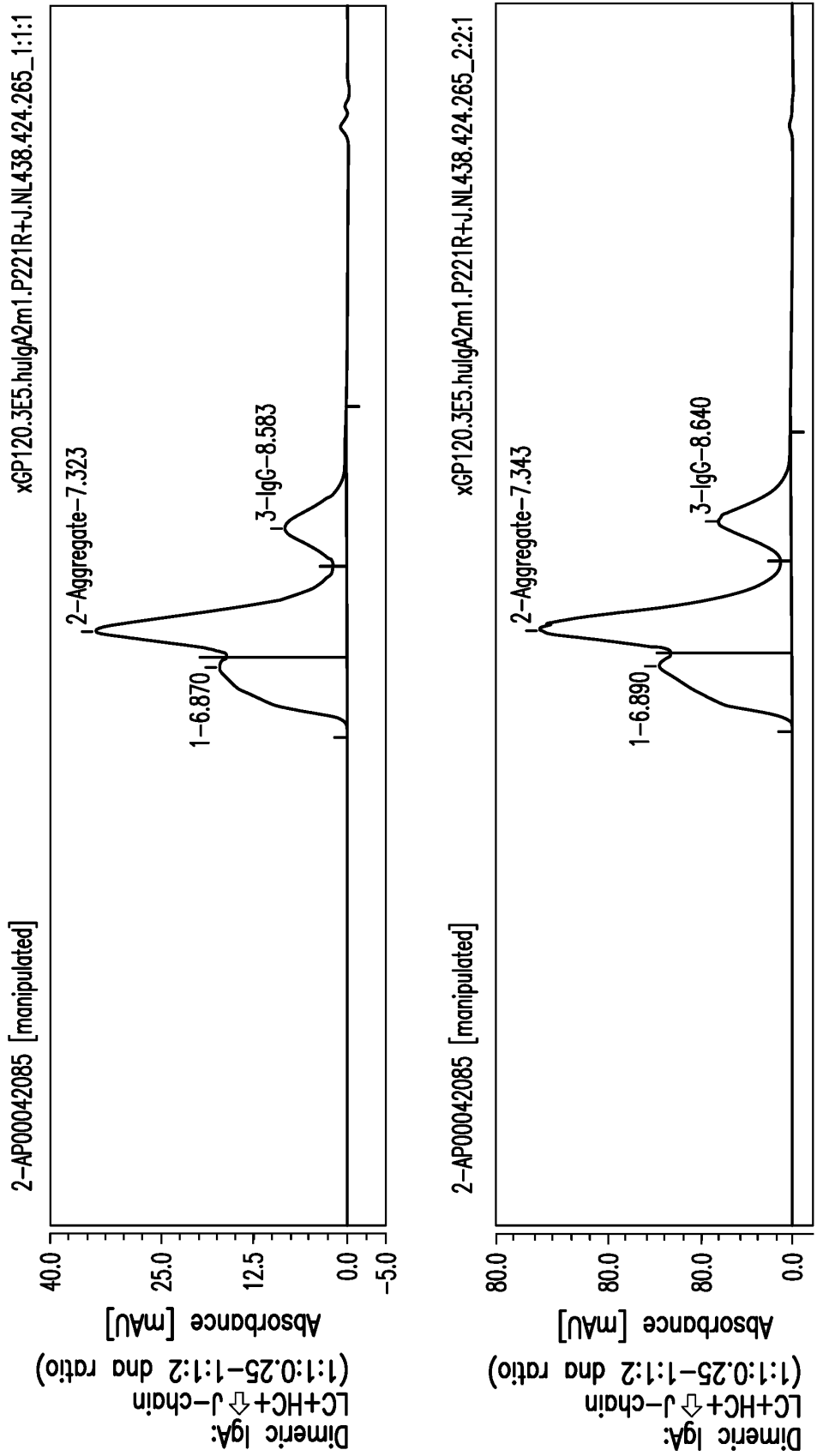
AP00055705

Figure 20A continued



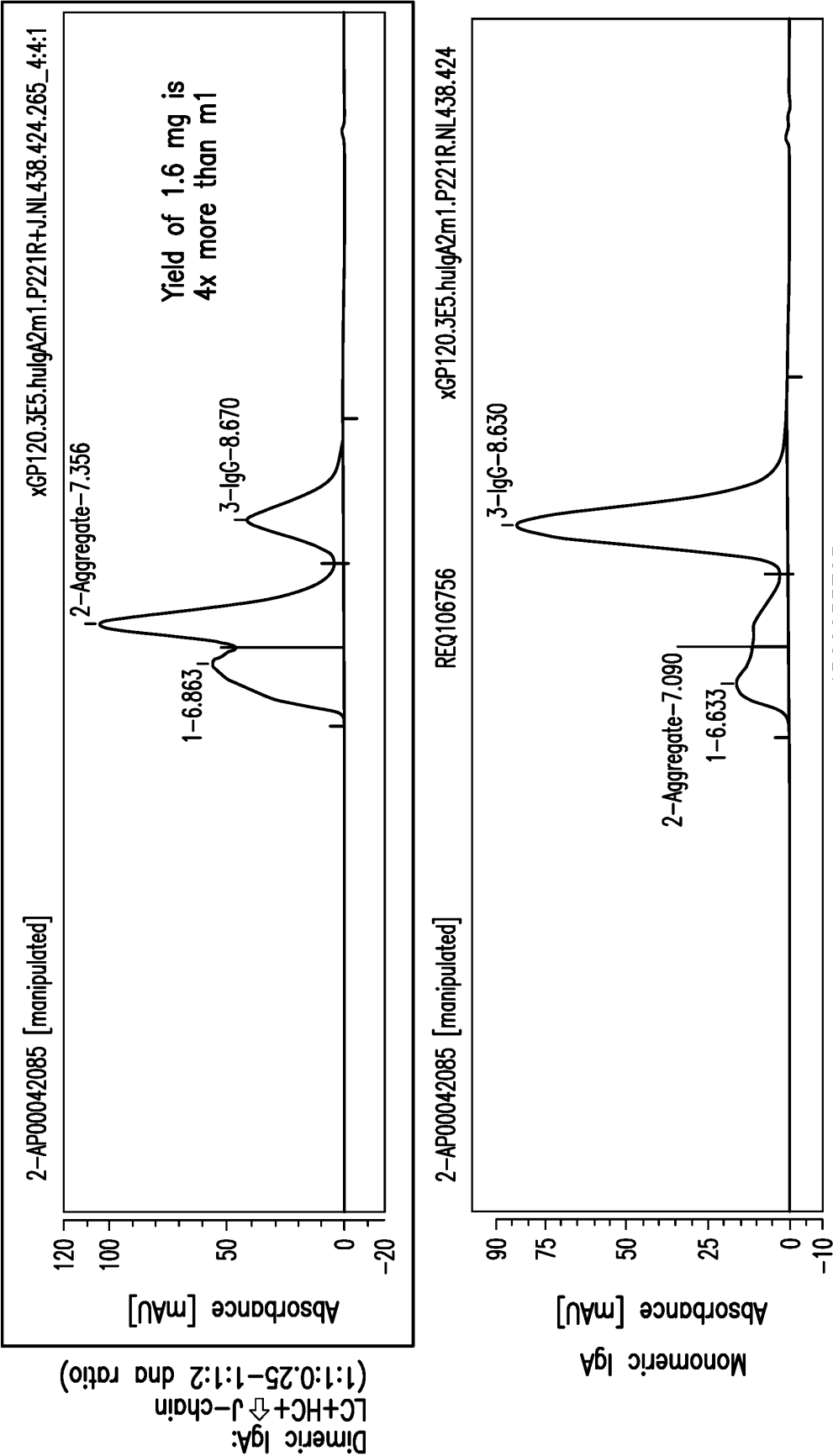
AP00055705

Figure 20B



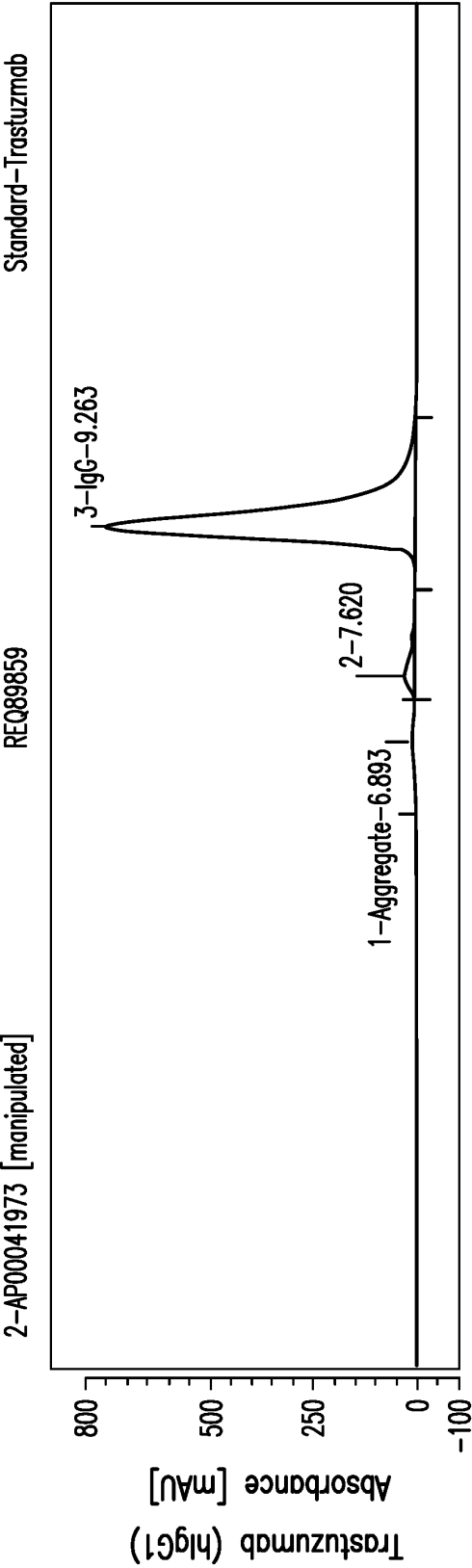
AP00055705

Figure 20B continued



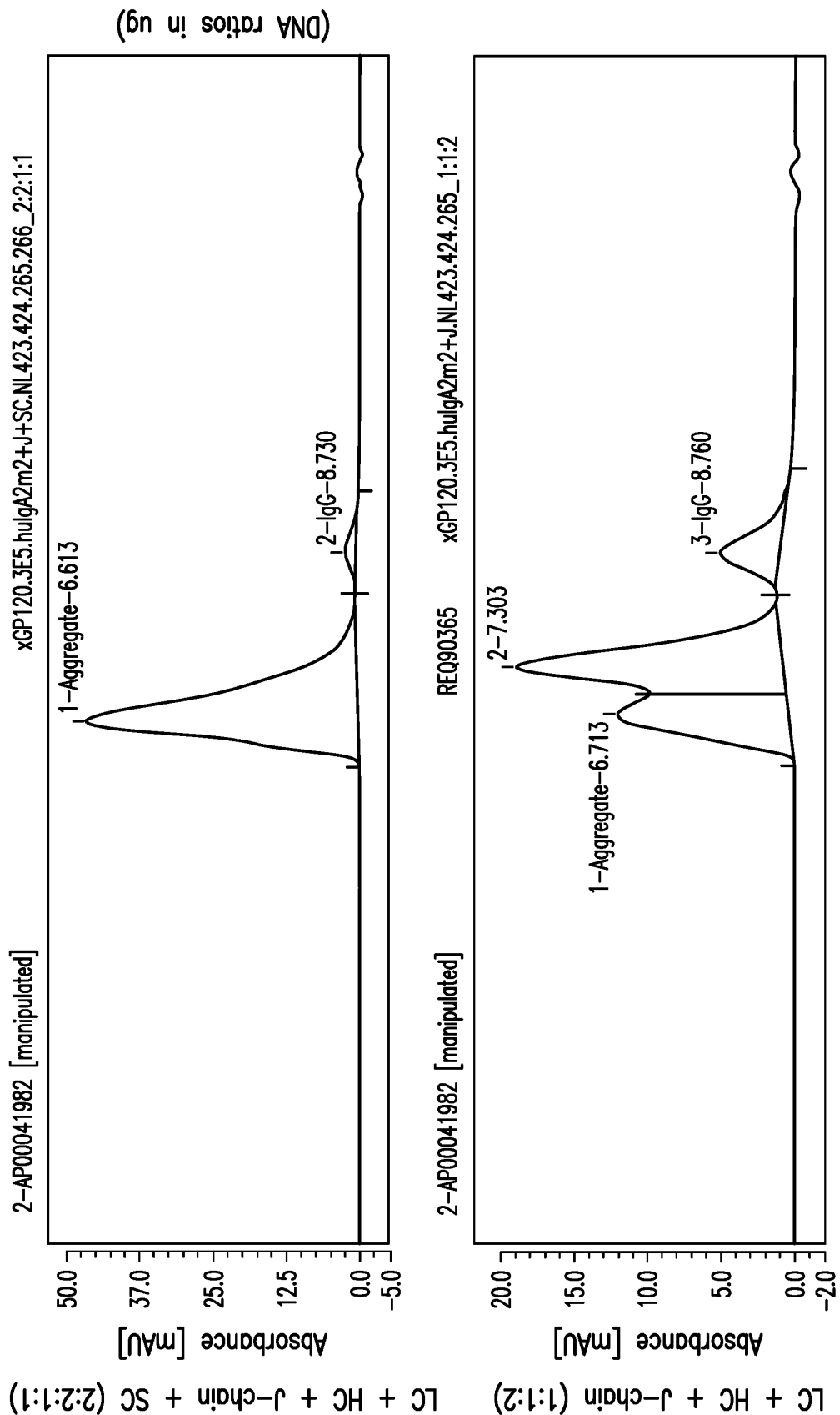
AP00055705

Figure 20B continued



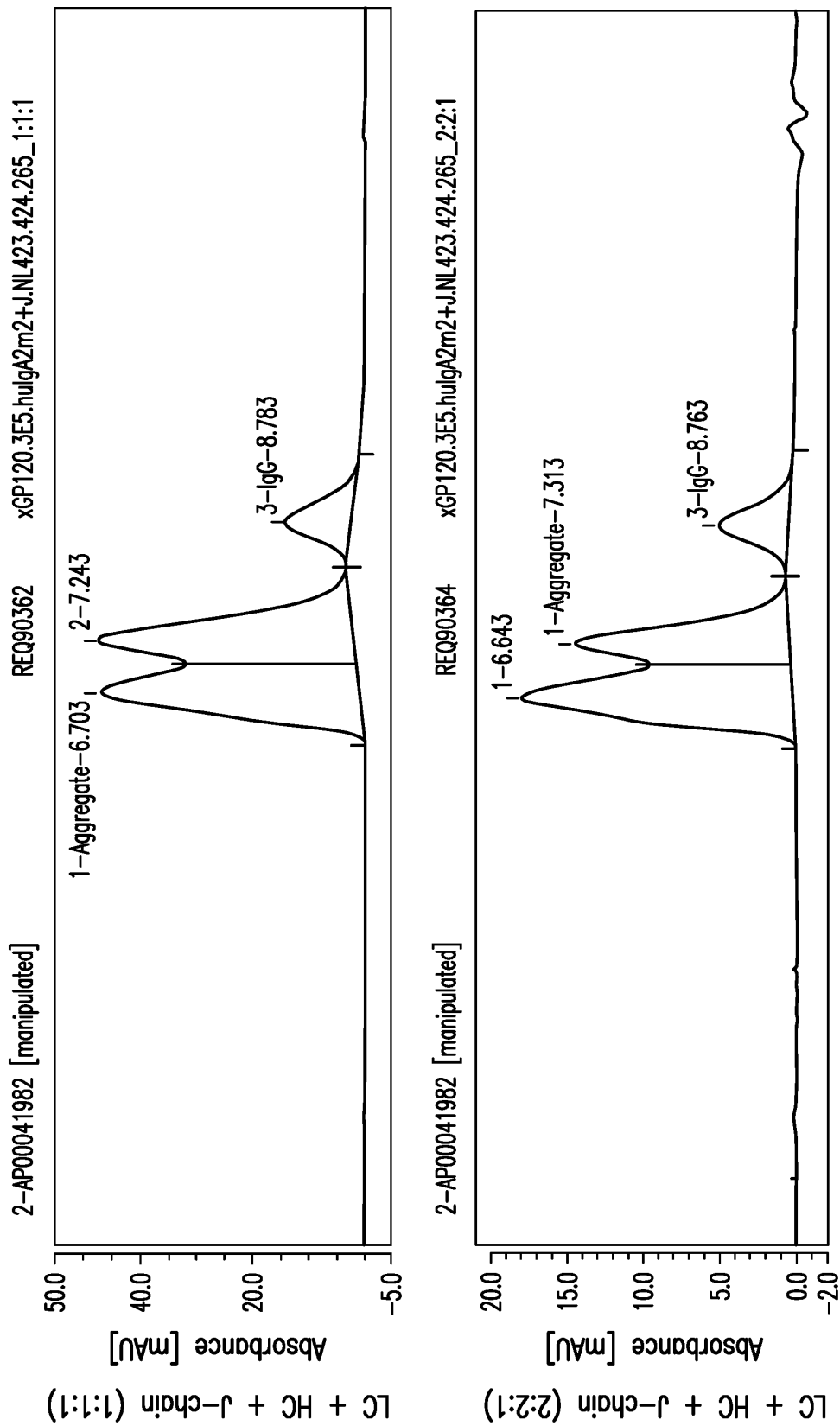
AP00055705

Figure 20B continued



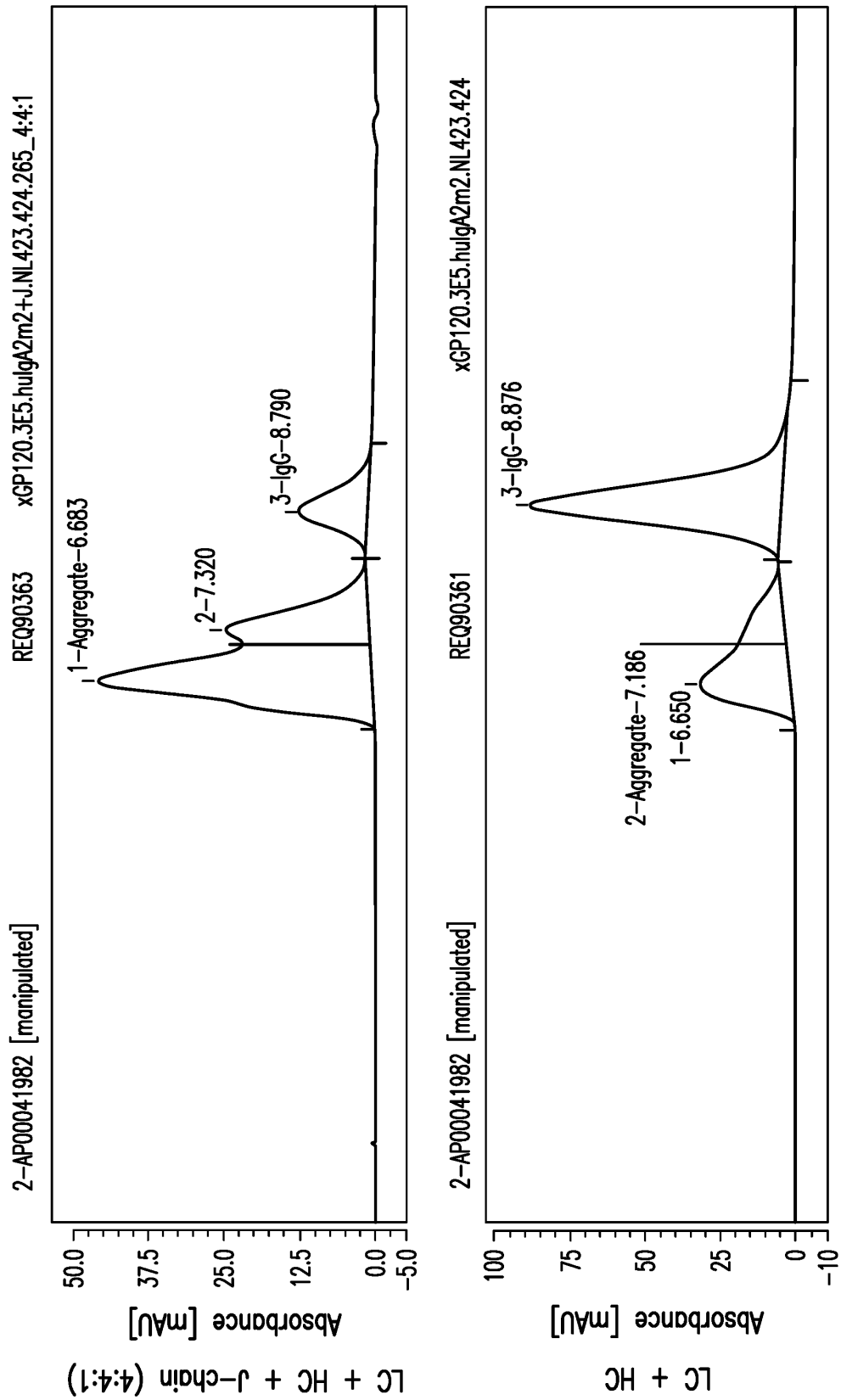
AP00055586

Figure 21



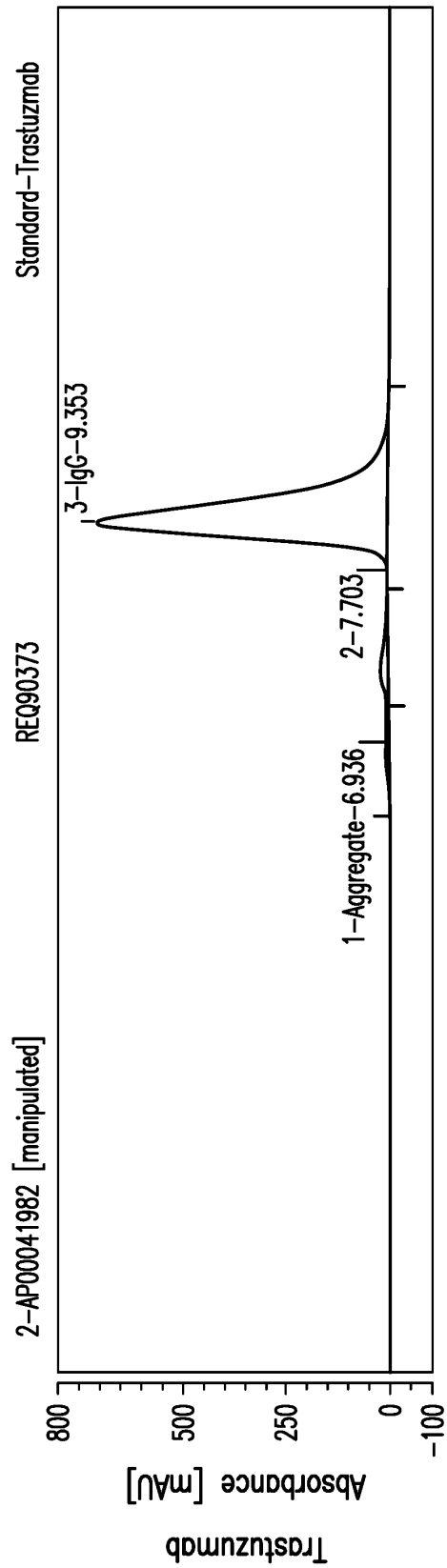
AP00055586

Figure 21 continued



AP00055586

Figure 21 continued



AP00055586

Figure 21 continued

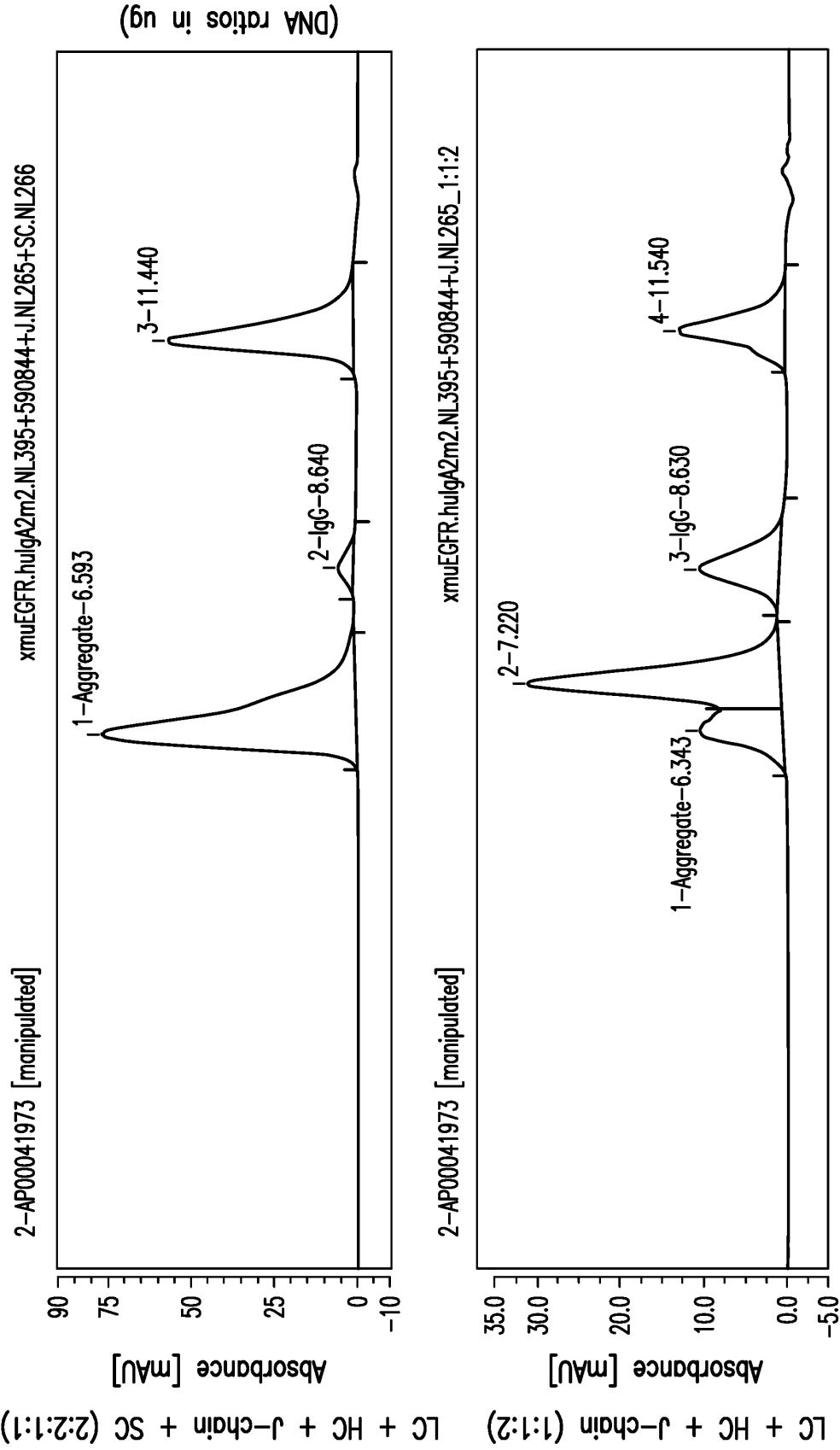


Figure 22

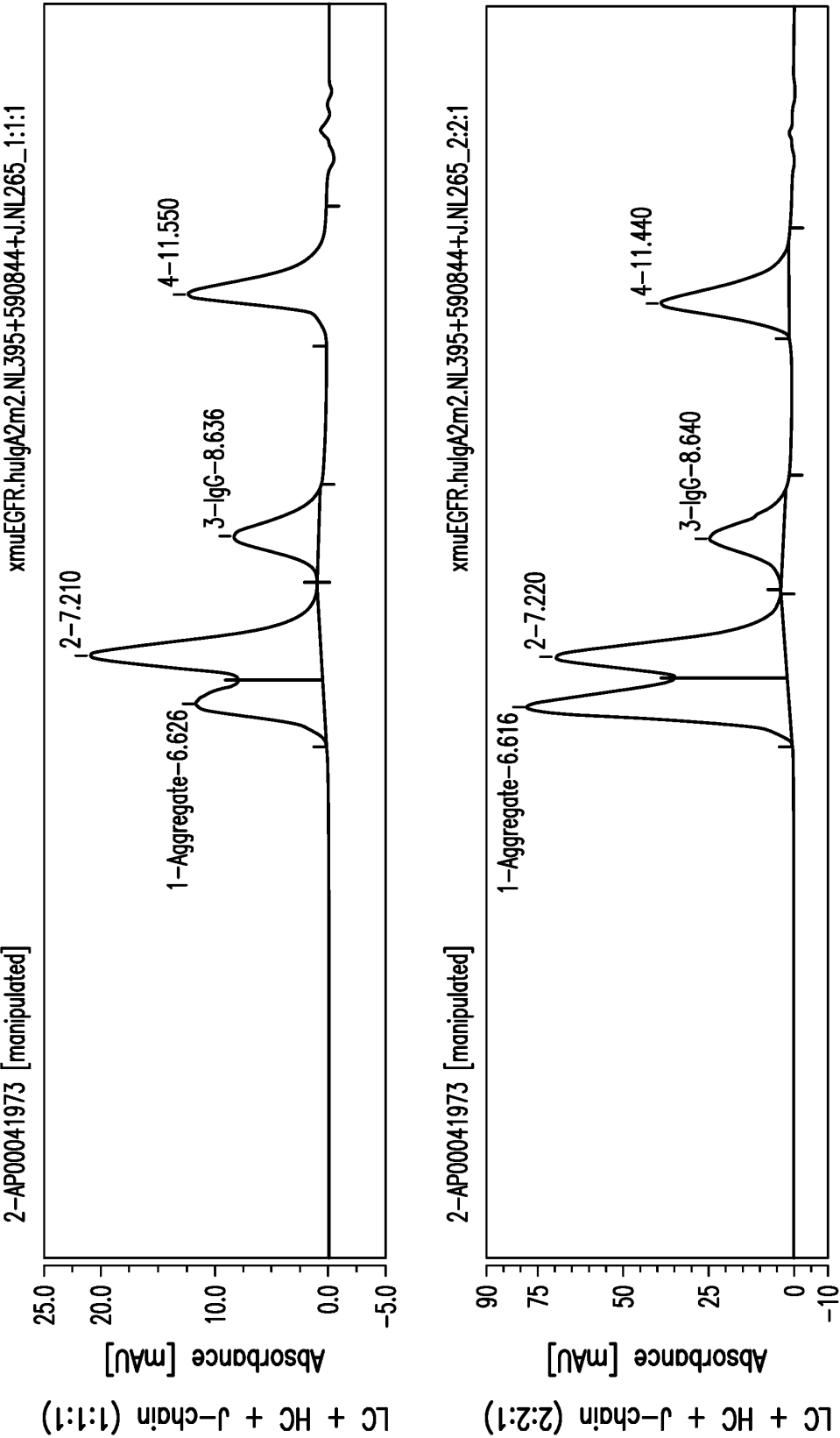
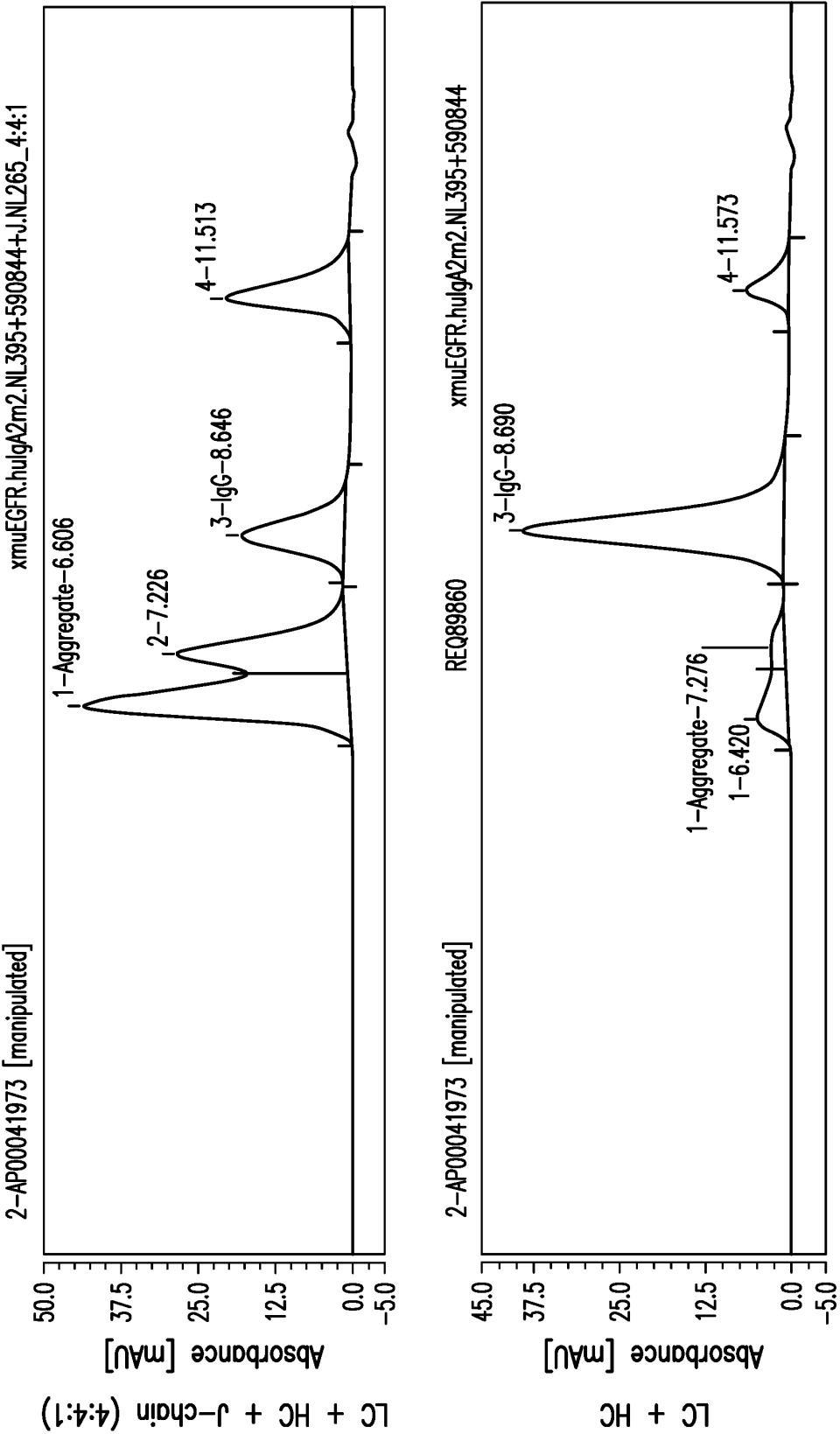
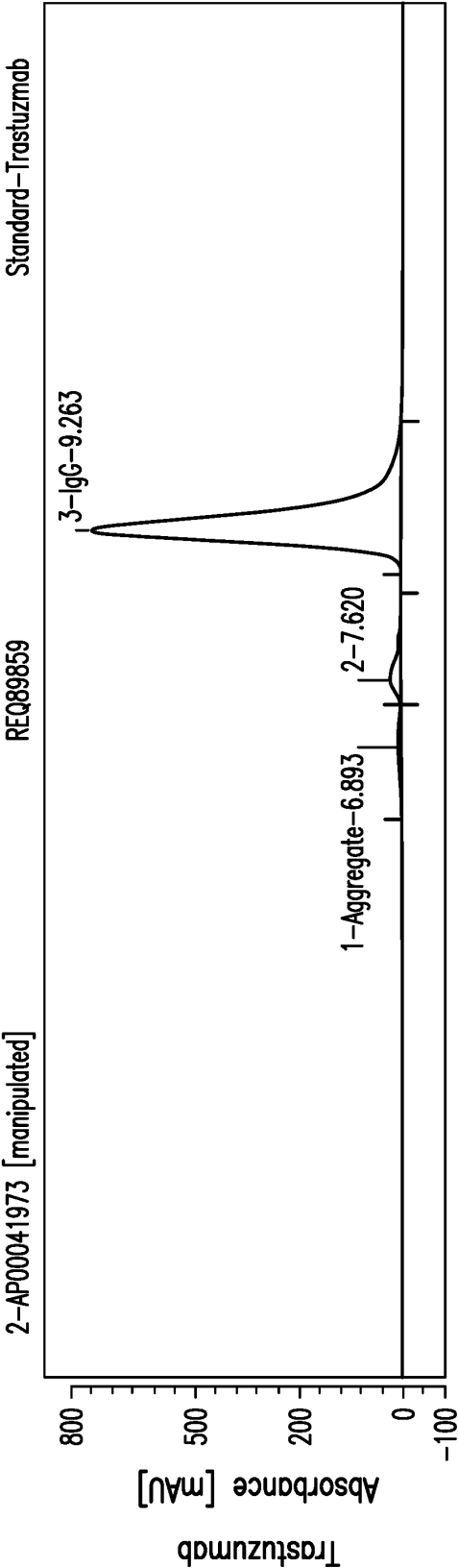


Figure 22 continued



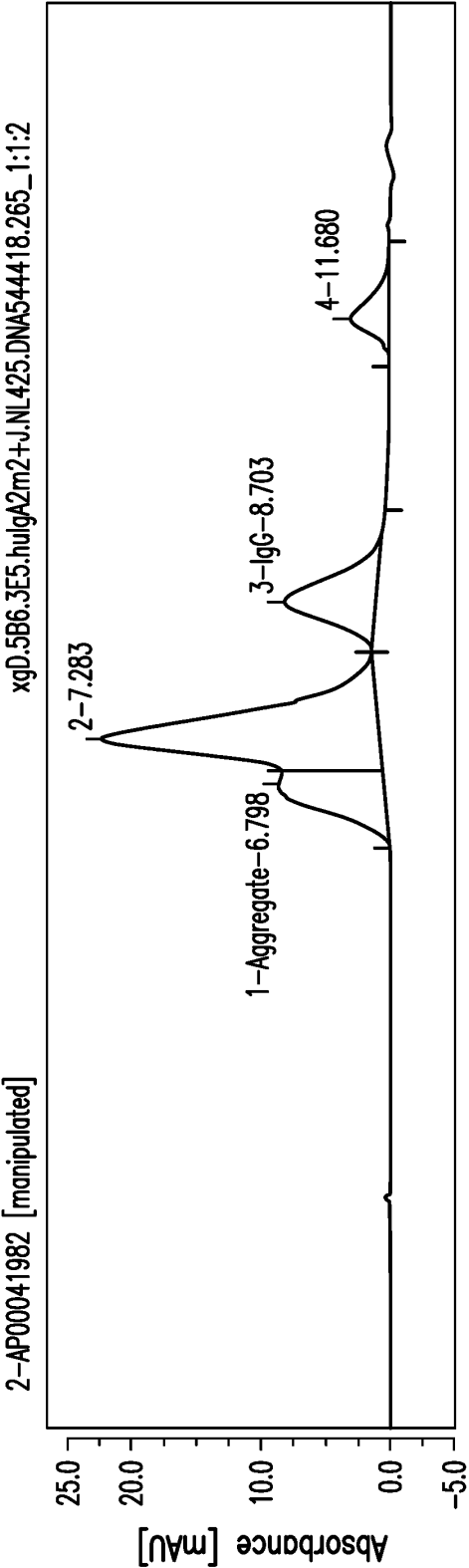
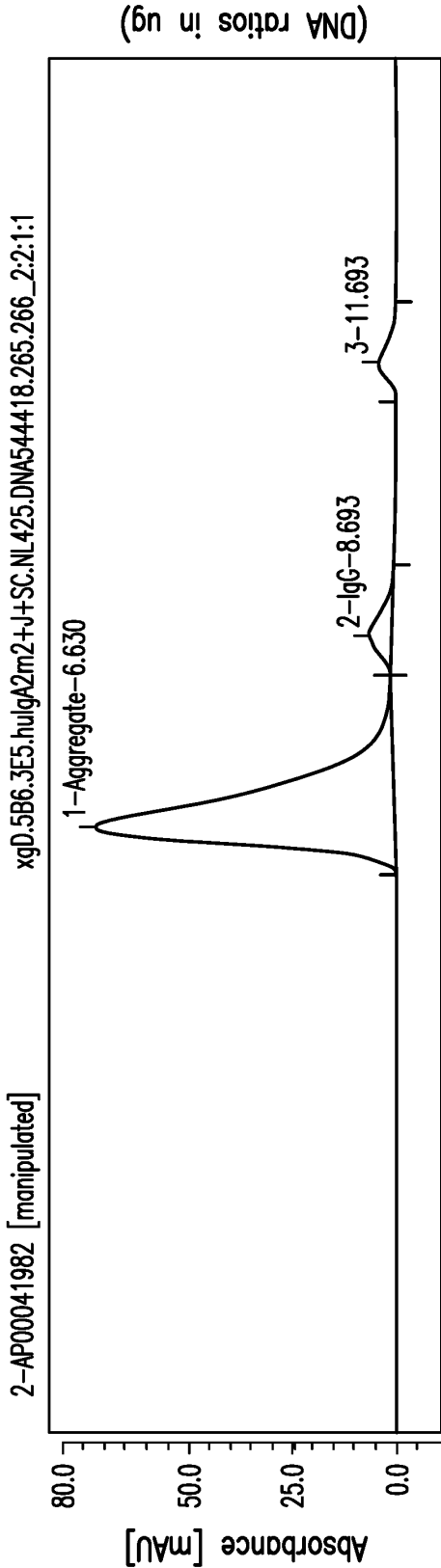
AP00055587

Figure 22 continued



AP00055587

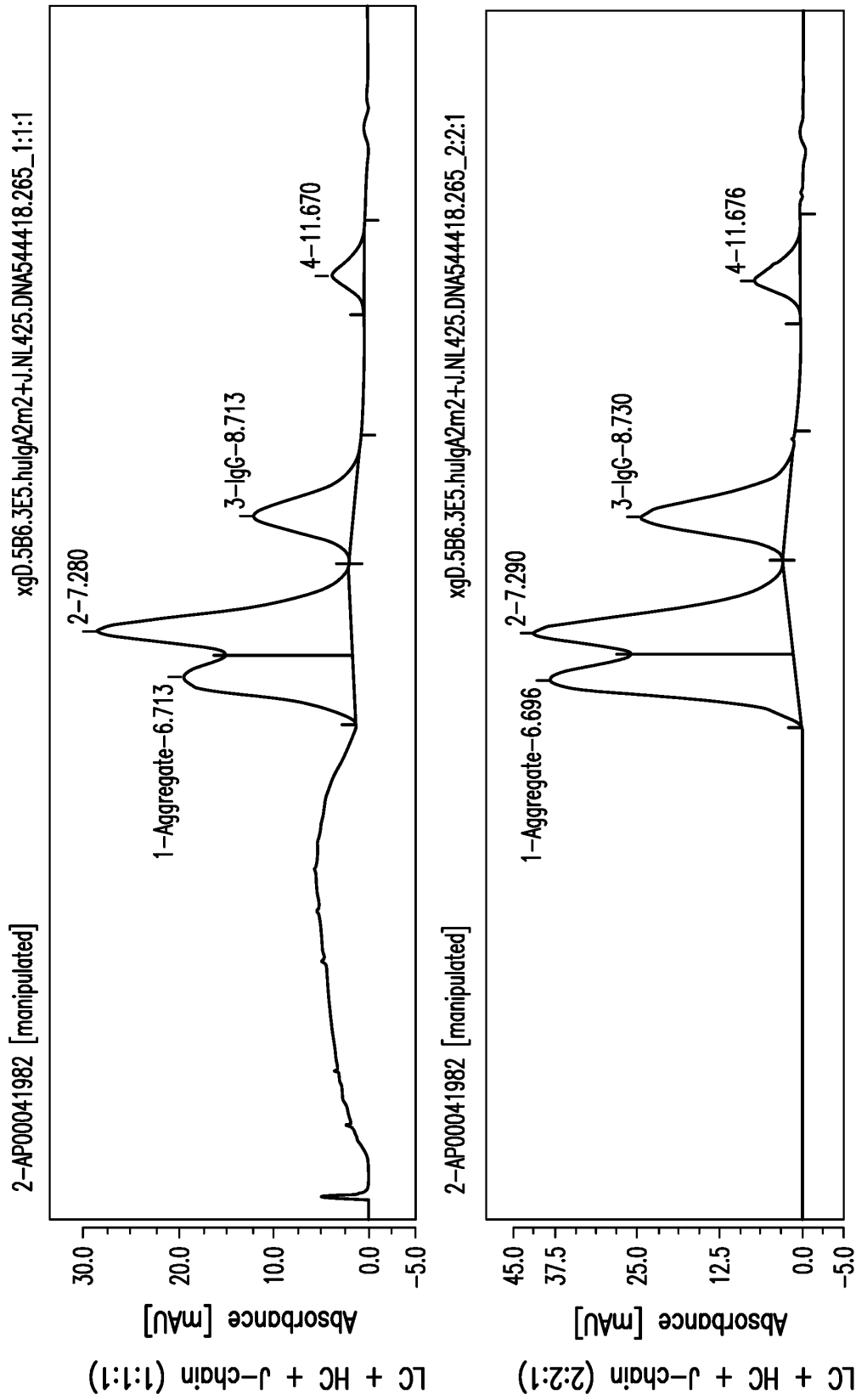
Figure 22 continued



LC + HC + J-chain + SC (2:2:1:1)

LC + HC + J-chain (1:1:2)

AP00055586
Figure 23



AP00055586

Figure 23 continued

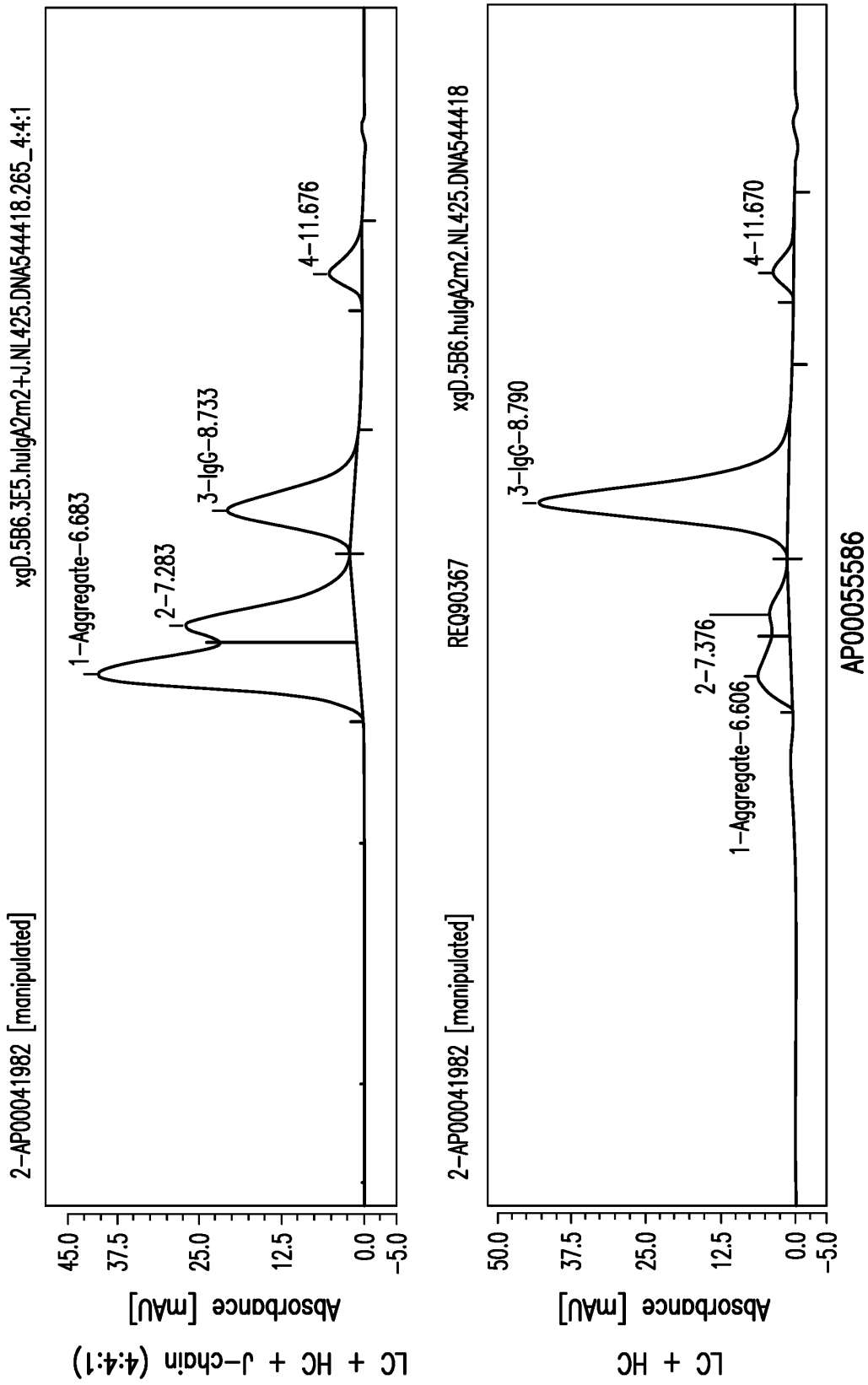
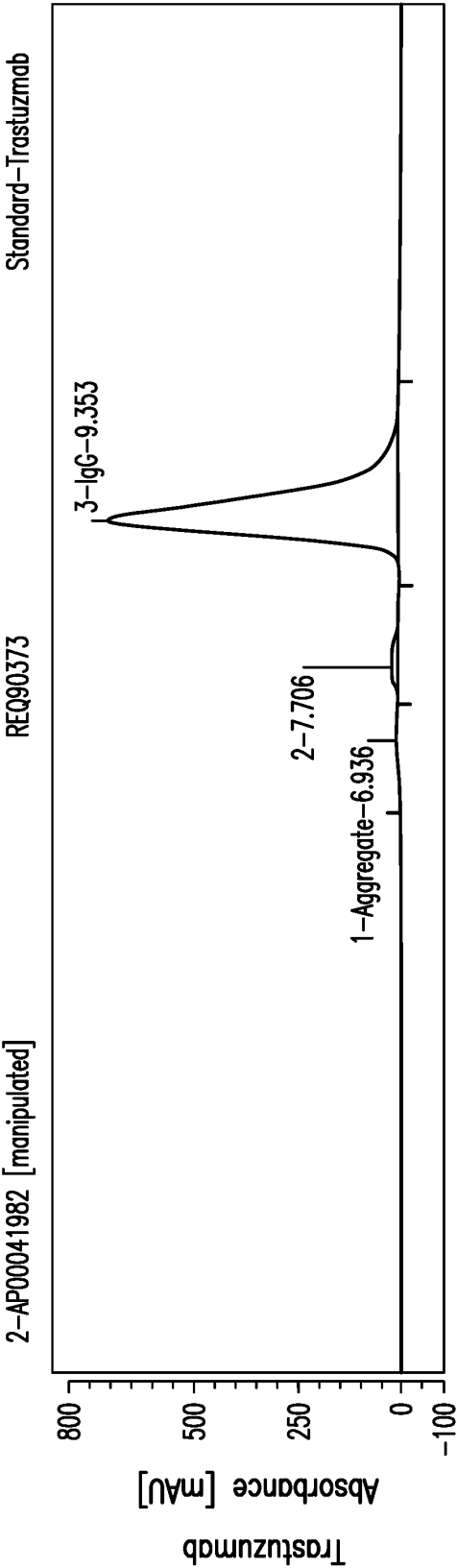
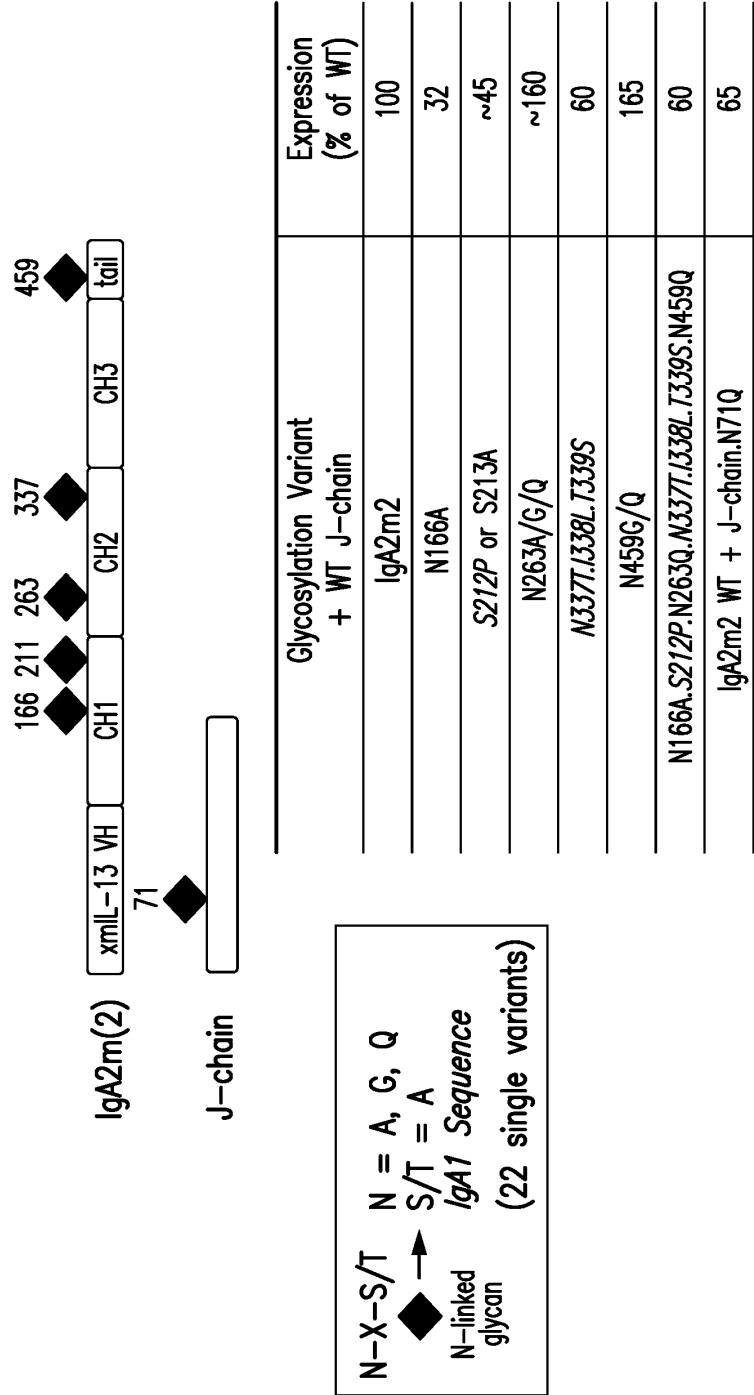


Figure 23 continued



AP00055586

Figure 23 continued



Avg OD280		Normalized to WT
xmull13.hulgA2m2.NL387.389.265	0.30	100
xmull13.lgA2m2.N166A.NL801.389.265	0.10	32
xmull13.lgA2m2.N166G.802.389.265 (IgA1 Sequence)	0.03	9
xmull13.lgA2m2.N166Q.NL803.389.265	0.03	10
xmull13.lgA2m2.T168A.NL804.389.265	0.04	12
xmull13.lgA2m2.N211A.NL805.389.265	0.05	15
xmull13.lgA2m2.N211G.NL806.389.265	0.02	5
xmull13.lgA2m2.N211Q.NL807.389.265	0.04	13
xmull13.lgA2m2.N213A.NL808.389.265	0.15	48
xmull13.hulgA2m2.S212P.NL654.389.265 (IgA1 Sequence)	0.14	45
xmull13.hulgA1.N263A.NL.647.389.265	0.49	163
xmull13.hulgA1.N263G.NL648.389.265	0.47	156
xmull13.hulgA1.N263Q.NL649.389.265	0.48	157
xmull13.lgA2m2.T265A.NL888.389.265	0.28	91
xmull13.lgA2m2.N337A.NL809.389.265	0.14	47
xmull13.lgA2m2.N337G.NL810.389.265	0.02	7
xmull13.lgA2m2.N337Q.NL811.389.265	0.08	26
xmull13.lgA2m2.T339A.NL812.389.265	0.07	23
xmull13.hulgA2m2.N337T.I338L.T339S.NL658.389.265 (IgA1 sequence)	0.18	59
xmull13.hulgA2m2.N459A.NL659.389.265	0.13	144
xmull13.hulgA2m2.N459G.NL660.389.265	0.38	125
xmull13.hulgA2m2.N459Q.NL661.389.265	0.50	165
xmull13.lgA2m2.S461A.NL889.389.265	0.25	81

AP00043335, AP00043337 (4 x 30 mL Exp'293 replicates)

Figure 24B

IgA2m2 with WT J-chain		AVG OD280	Normalized to WT	Ext. Coeff	mg/mL	Yield mg	
AP000043458	xuLL13.IgA2m2 WT						
	xmuLL13.IgA2m2.N168G.S214P.N252G.N461G (4)	0.24	100	1.15	0.21	0.30	xmuLL13.hulIgA2m2.NL387.389.265
	xmuLL13.IgA2m2.N168G.S214P.N252G.N326G (4)	0.03	12	1.15	0.03	0.04	A2m2.4mer.NL872.389.265
	xmuLL13.IgA2m2.N168G.S214P.N252G.N326G (4)	0.06	25	1.15	0.05	0.07	A2m2.4mer.NL905.389.265
	xmuLL13.IgA2m2.N168G.S214P.N326G.N461G (4)	0.06	25	1.15	0.05	0.07	A2m2.4mer.NL906.389.265
	xmuLL13.IgA2m2.N168G.N252G.N326G.N461G (4)	0.02	8	1.15	0.02	0.02	A2m2.4mer.NL907.389.265
	xmuLL13.IgA2m2.S214P.N252G.N326G.N461G (4)	0.08	31	1.15	0.07	0.09	A2m2.4mer.NL908.389.265
	xmuLL13.IgA2m2.N168G.S214P.N252G.N326A.N461G (5)	0.02	7	1.15	0.02	0.02	A2m2.5mer.NL873.389.265
	xmuLL13.IgA2m2.N168G.S214P.N252G.N326G.N461G (5)	0.03	11	1.15	0.02	0.03	A2m2.5mer.NL874.389.265
	xmuLL13.IgA2m2.N168G.S214P.N252G.N326Q.N461G (5)	0.03	10	1.15	0.02	0.03	A2m2.5mer.NL875.389.265
	xmuLL13.IgA2m2.N168G.S214P.N252G.T328A.N461G (5)	0.03	10	1.15	0.02	0.03	A2m2.5mer.NL876.389.265
	xmuLL13.IgA2m2.N168G.S214P.N252G.N326T.N461G (5)	0.10	42	1.15	0.09	0.12	A2m2.5mer.NL877.389.265
AP000043466	xmuLL13.IgA2m2.N168A.S215A.N252Q.N326T.I327L.T328S.N461G (5)	0.01	3	1.15	0.01	0.01	xmuLL13.IgA2m2.5mer.NL987.389.265
	xmuLL13.IgA2m2.N168A.S215A.N252A.N326T.I327L.T328S.N461Q (5)	0.08	34	1.15	0.07	0.10	xmuLL13.IgA2m2.5mer.NL1005.389.265
	xmuLL13.IgA2m2.N168A.S215A.N252A.N326T.N461Q 5	0.04	18	1.15	0.04	0.05	xmuLL13.IgA2m2.5mer.NL1006.389.265
	xmuLL13.IgA2m2.N168A.S215A.N252Q.N326T.N461Q (5)	0.05	20	1.15	0.04	0.06	xmuLL13.IgA2m2.5mer.NL1007.389.265
	xmuLL13.IgA2m2.N168A.S214P.N252A.N326T.I327L.T328S.N461Q (5)	0.10	41	1.15	0.08	0.12	xmuLL13.IgA2m2.5mer.NL1008.389.265
	xmuLL13.IgA2m2.N168A.S214P.N252A.N326T.N461Q (5)	0.03	11	1.15	0.02	0.03	xmuLL13.IgA2m2.5mer.NL1009.389.265
	xmuLL13.IgA2m2.N168A.S214P.N252Q.N326T.N461Q (5)	0.05	21	1.15	0.04	0.06	xmuLL13.IgA2m2.5mer.NL1010.389.265
	xmuLL13.IgA2m2.N168A.S214P.N252Q.N326T.I327L.T328S.N461Q (5)	0.17	72	1.15	0.15	0.21	xmuLL13.IgA2m2.5mer.NL1011.389.265
	xmuLL13.IgA2m2.N168G.S214P.N252G.N326T.I327L.T328S.N461G (5)	0.08	33	1.15	0.07	0.10	xmuLL13.IgA2m2.5mer.NL877.389.265
	xmuLL13.IgA2m1.P221R.N168A.N265Q.N326T.I327L.T328S.N461Q (4)	0.08	31	1.15	0.07	0.09	xmuLL13.IgA2m1.P221R.4mer.NL997.389.265
	xmuLL13.IgA2m1.P221R.N265Q.N326T.I327L.T328S.N461Q (3)	0.15	63	1.15	0.13	0.18	xmuLL13.IgA2m1.P221R.IgA2m1.NL1001.389.265

Figure 24C

	pIgR	ASGR/ManR	FcRn
WT IgA2m2 Tetramer (Ran in last mouse PK study)	☒	☒	X
Aglycosylated IgA2m2 Tetramer	☒	X	X
High Sialylation IgA Monomer from serum	X	X	X

	pIgR	Glycosylation	FcRn
WT IgA2m2 Tetramer	☒	41 sites (56 % sialylation)	X
Aglycosylated IgA2m2 Tetramer + glycosylated J chain	☒	1 site on J chain (14 % sialylation)	X
IgA monomer from human serum	X	95%	X

Figure 25

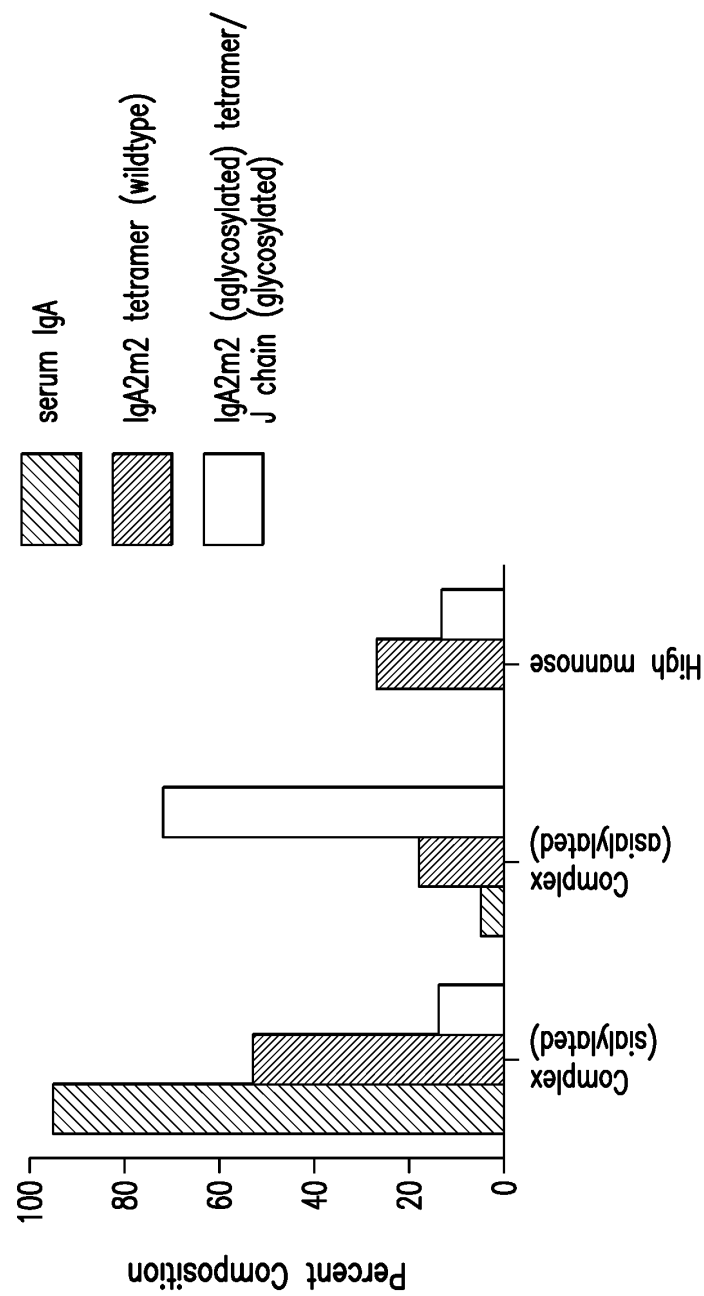
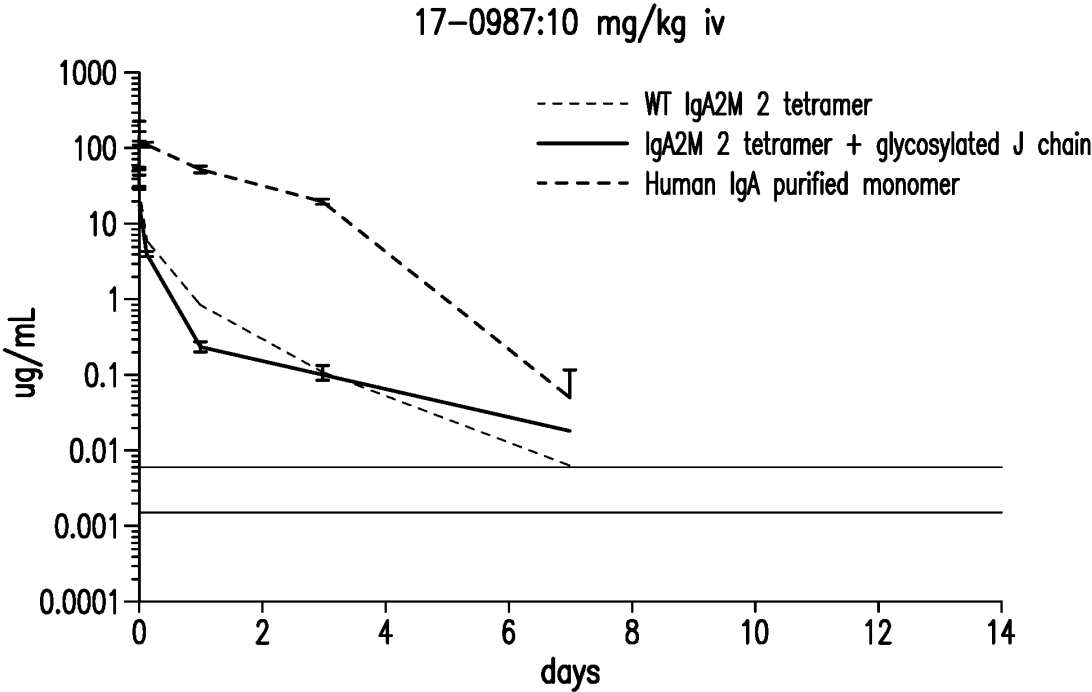
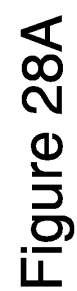


Figure 26



Group	C _{max} (ug/mL)	AUC _{inf} (day*ug/mL)	CL (mL/day/kg)	V _{ss} (mL/kg)
WT IgA2M 2 Tetramer,	121	7.77	1240	446
Glycosylated IgA2M2 tetramer + glycosylated J chain	48.3	4.39	2050	776
Human IgA purified monomer	203	203	45.8	59.8

Figure 27



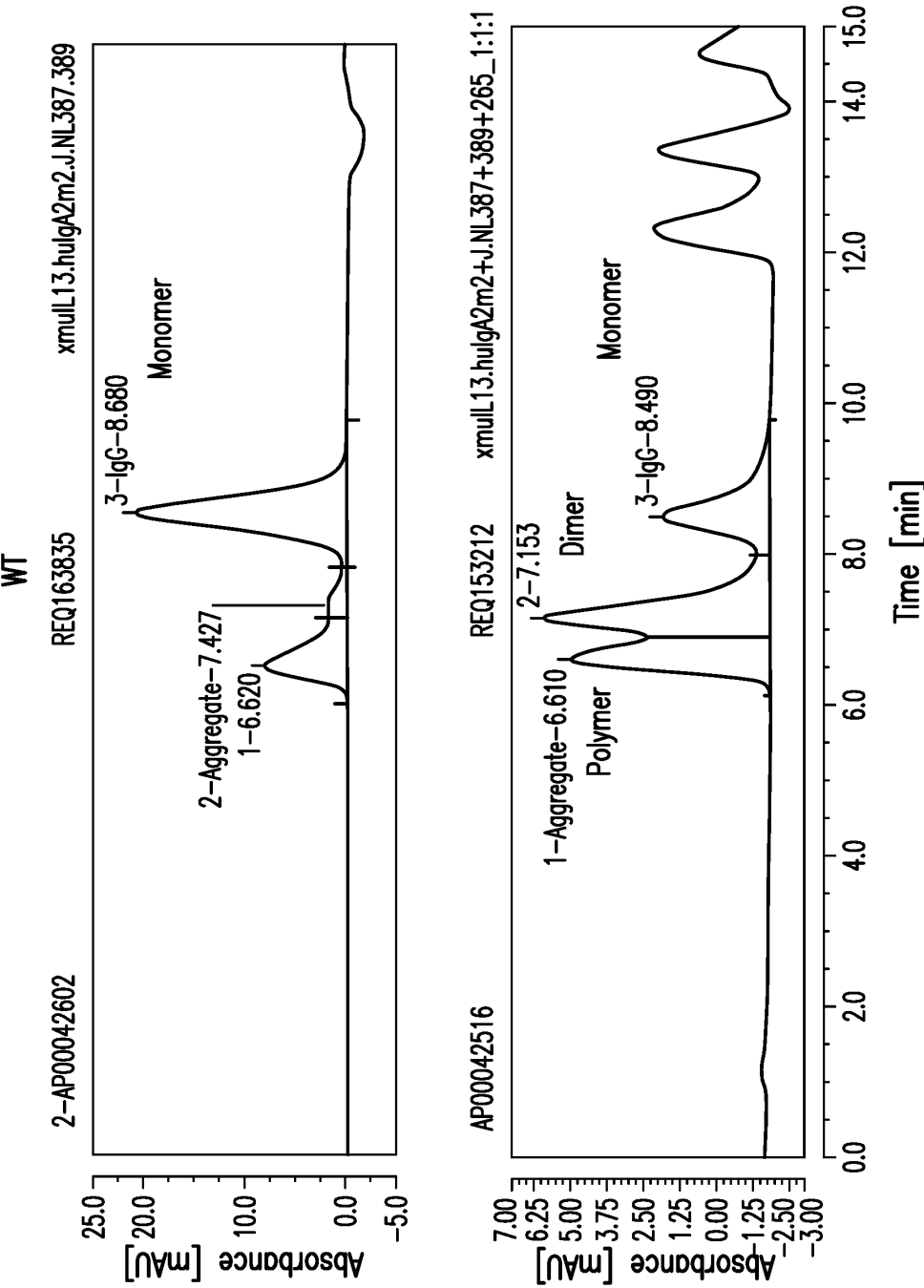
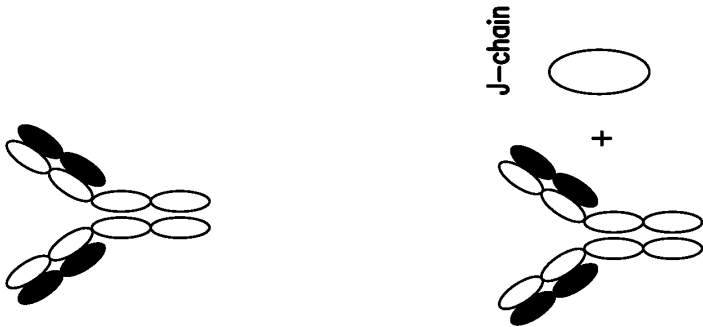
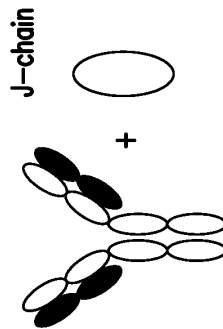
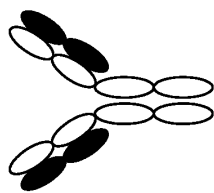
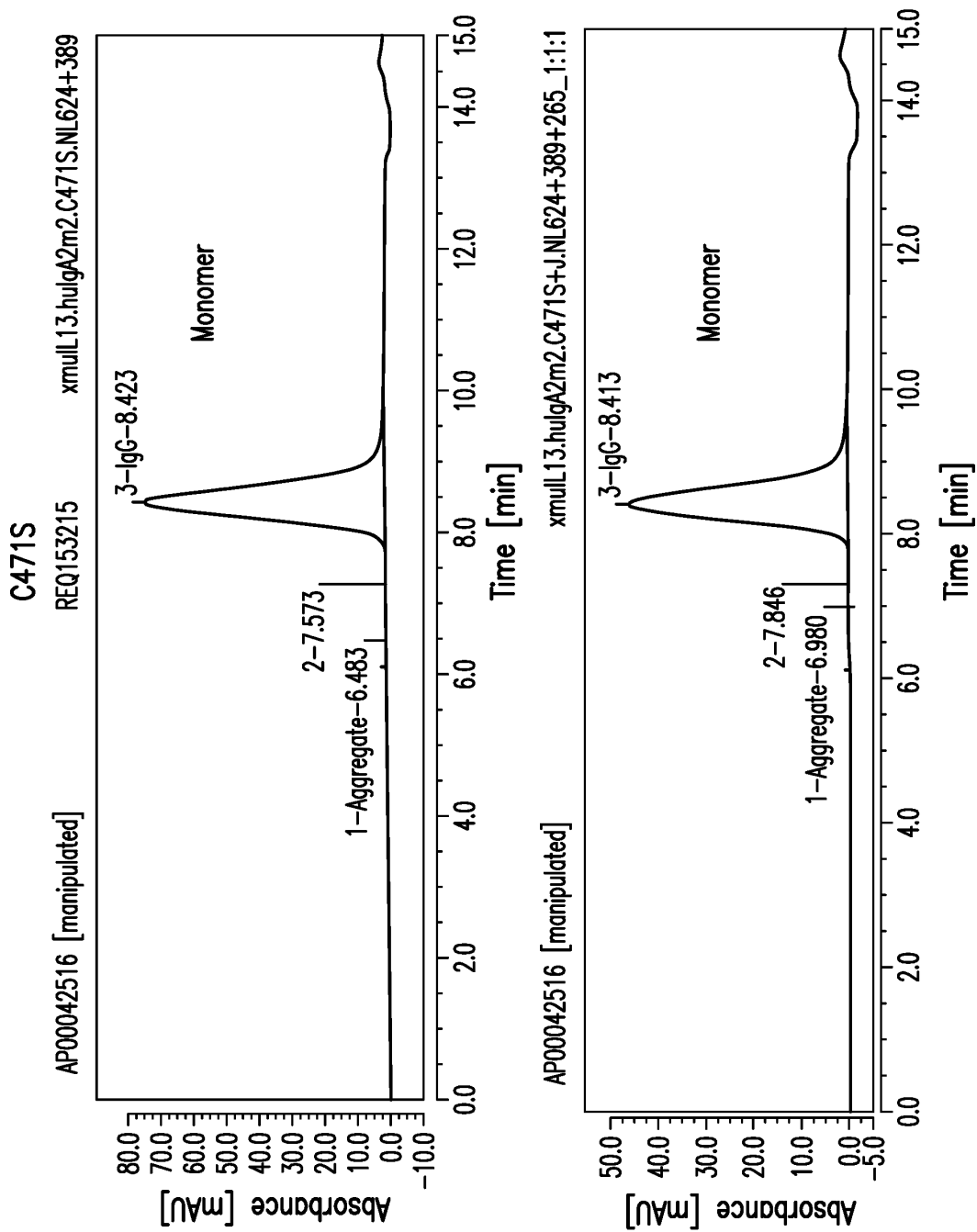


Figure 28B





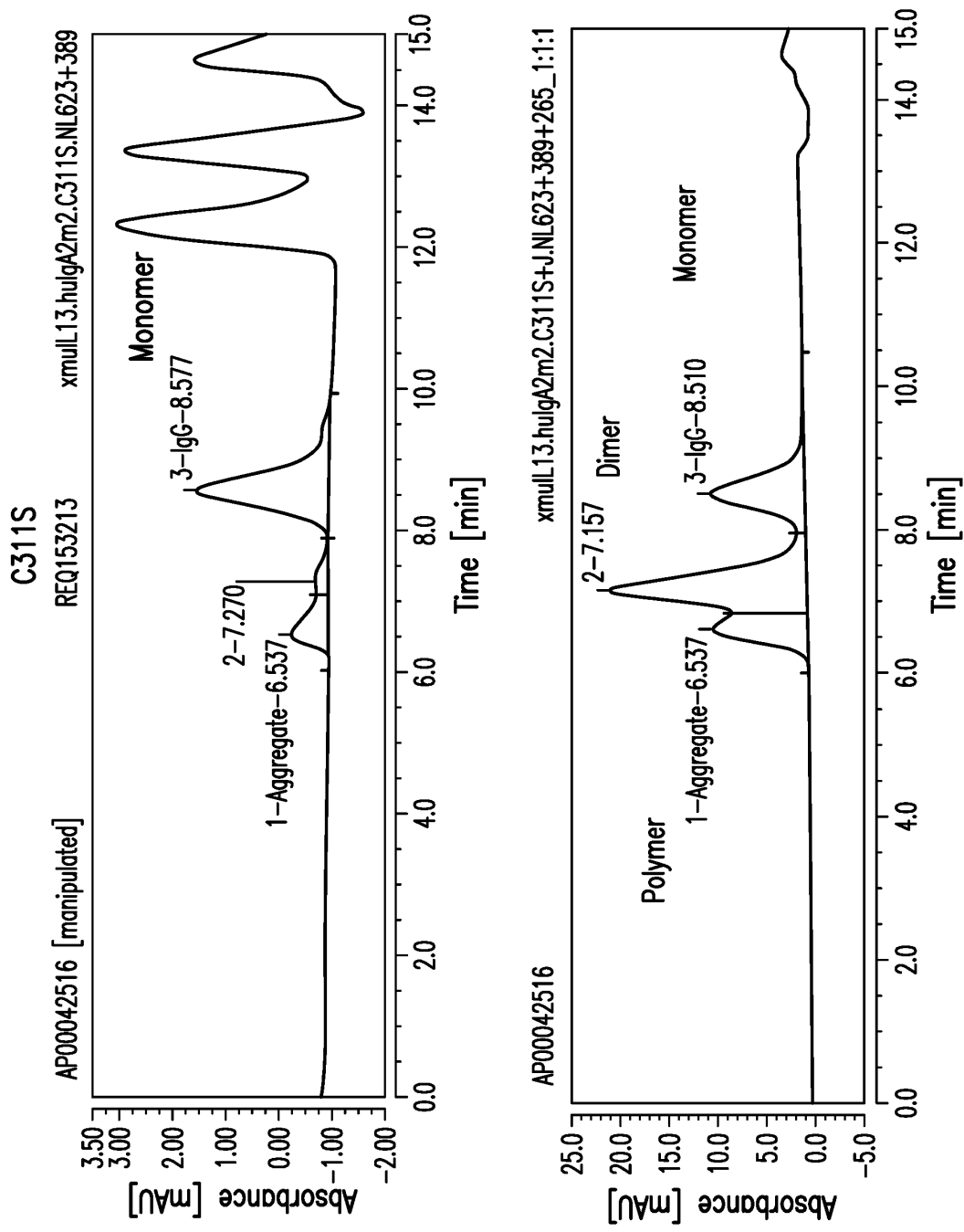
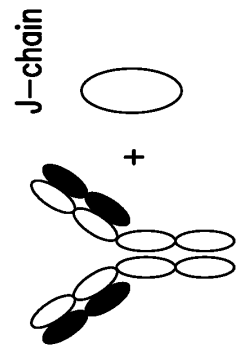
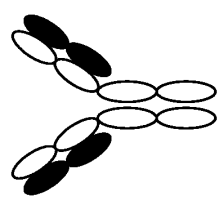


Figure 28B continued



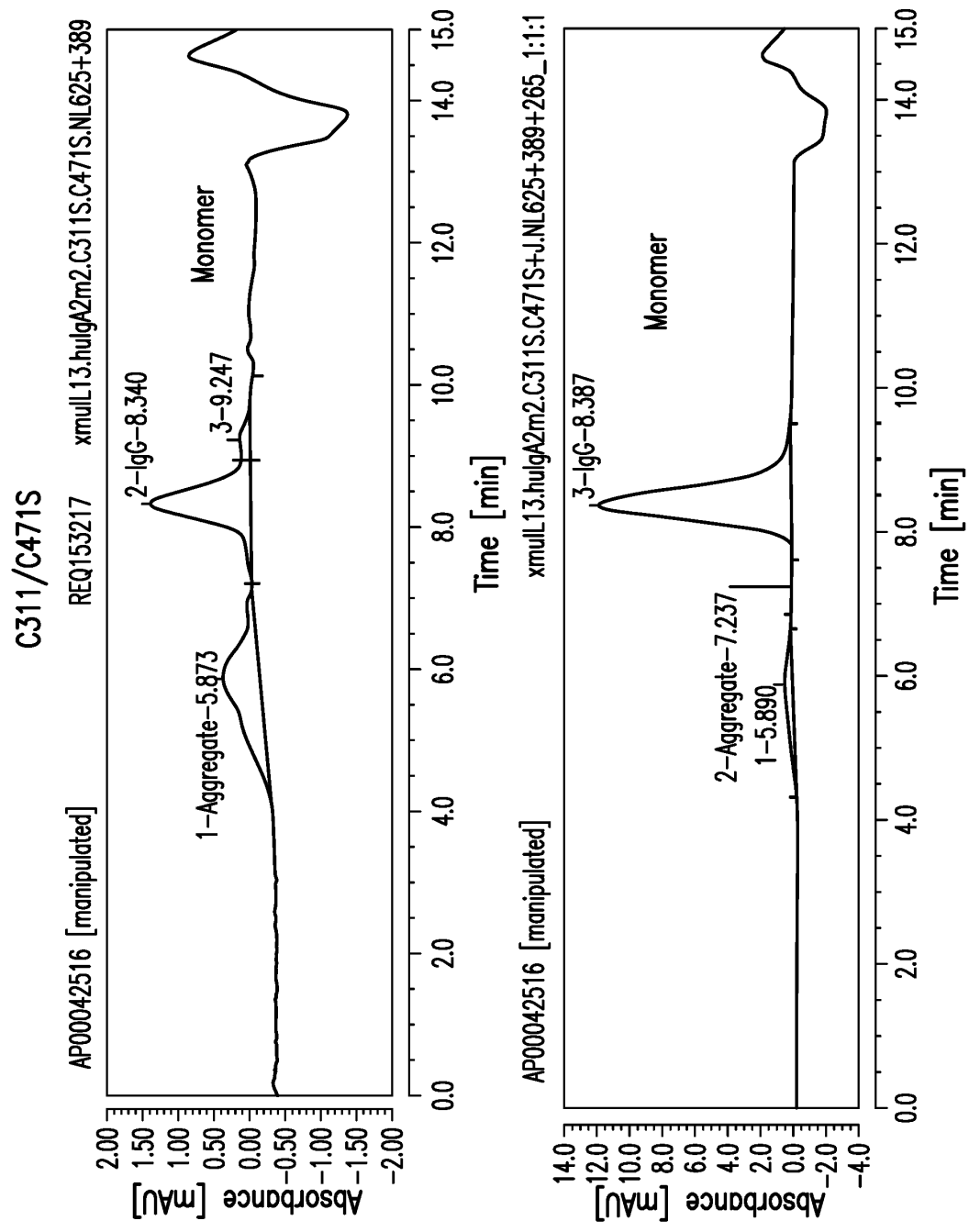
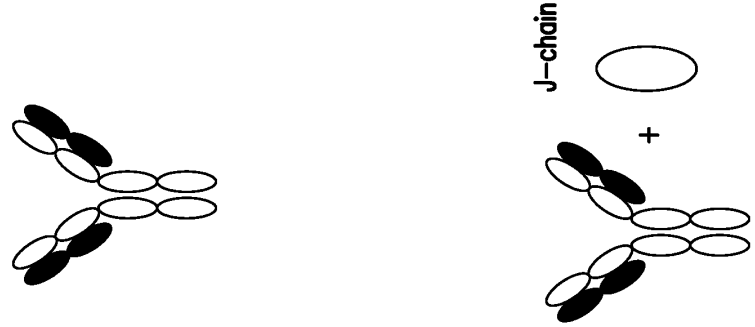


Figure 28B continued



DNA Ratios: LC:HC:J-chain:SC

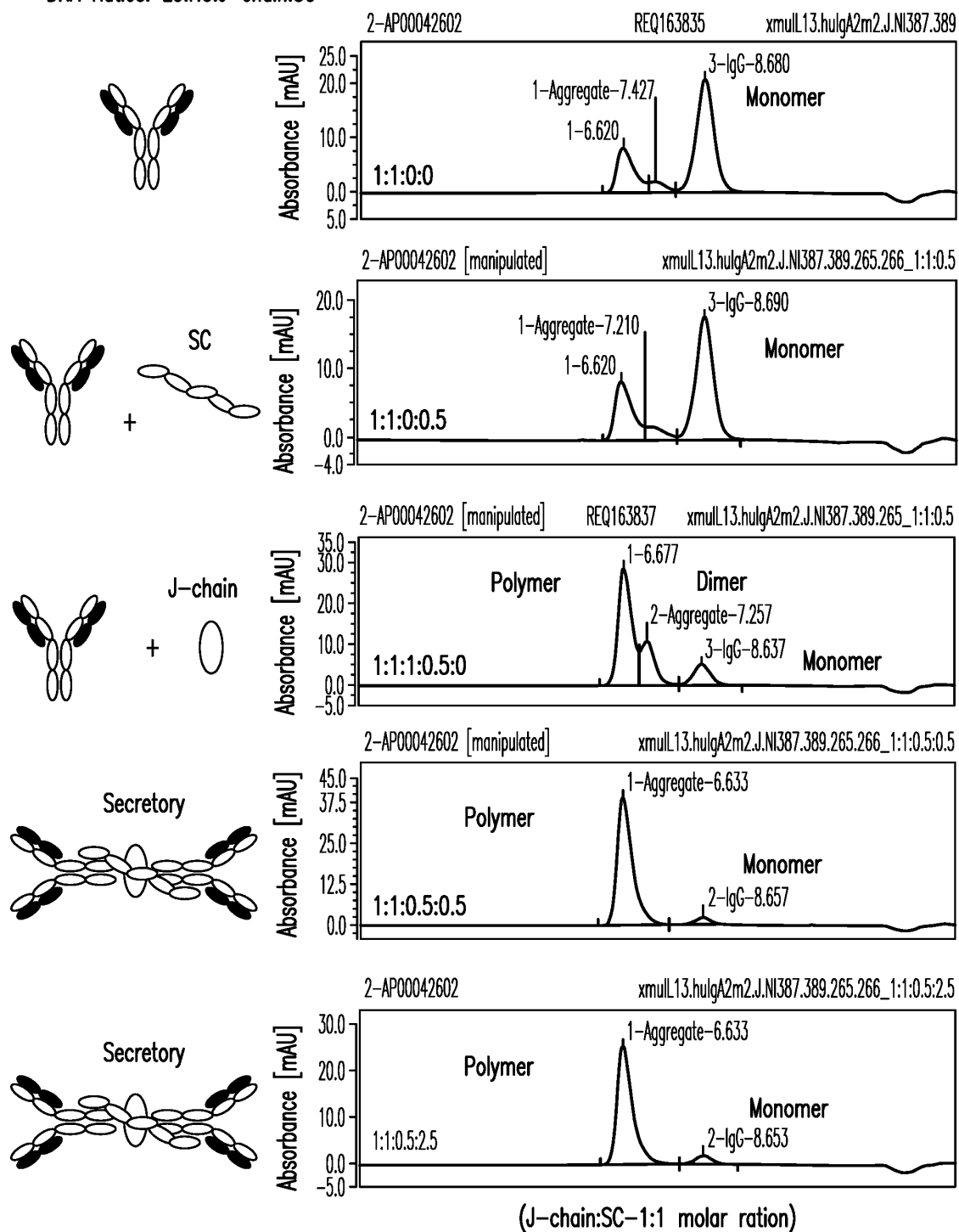


FIG. 29

DNA Ratios: LC:HC:J-chain:SC

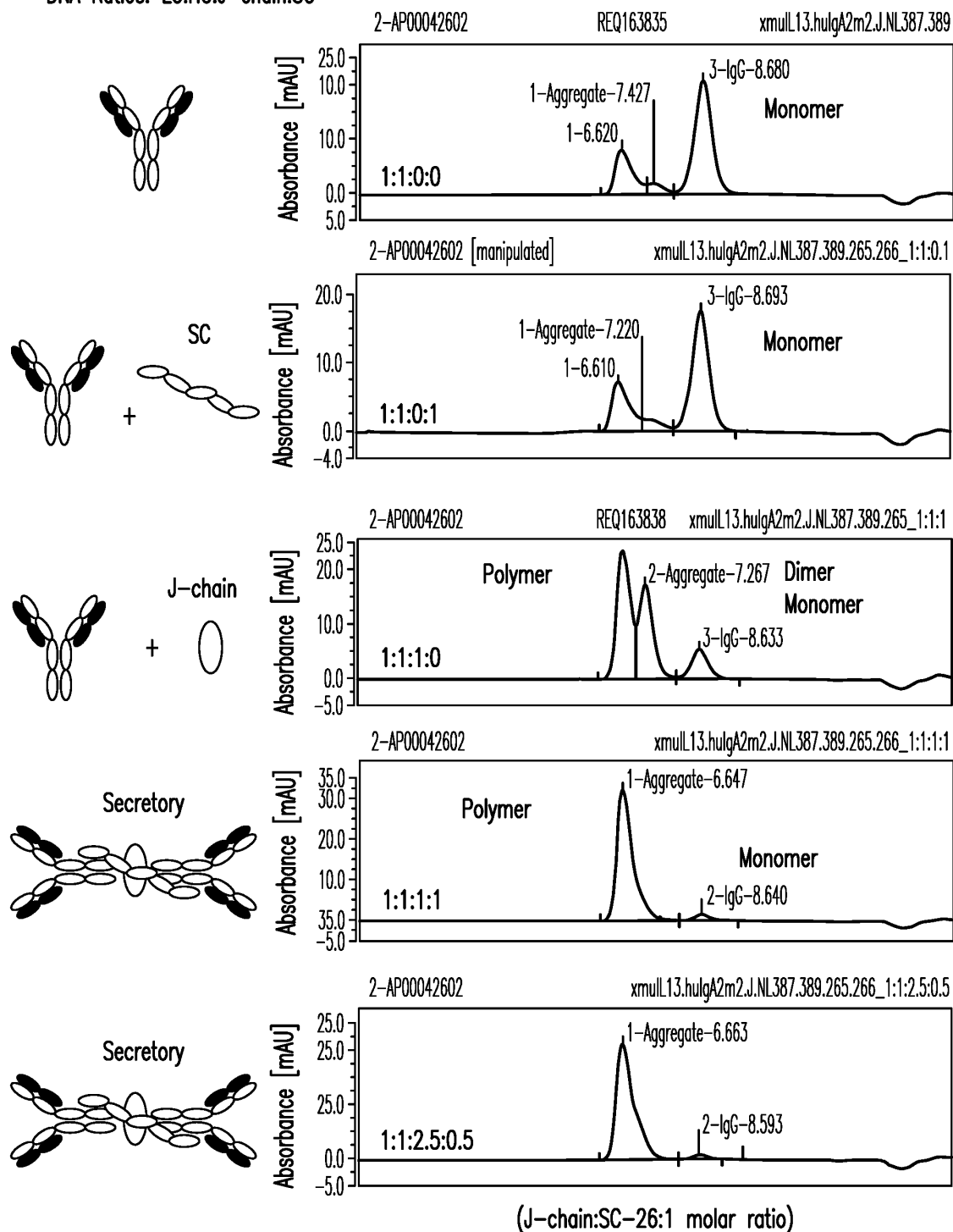


FIG. 29 continued

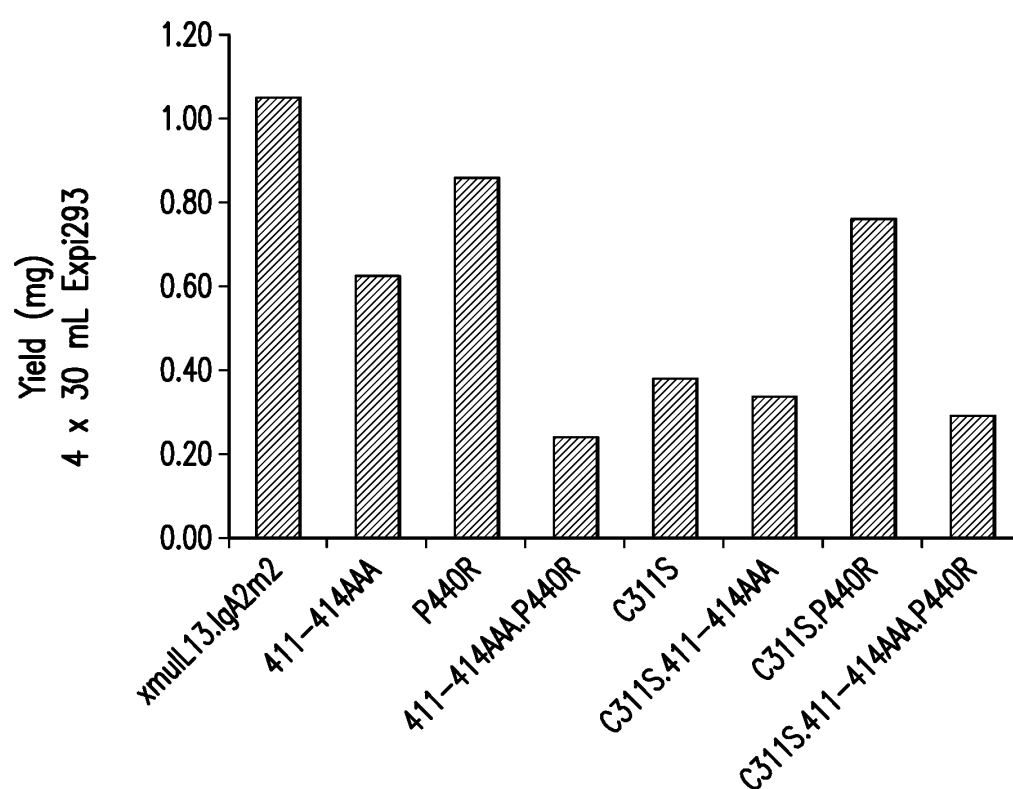
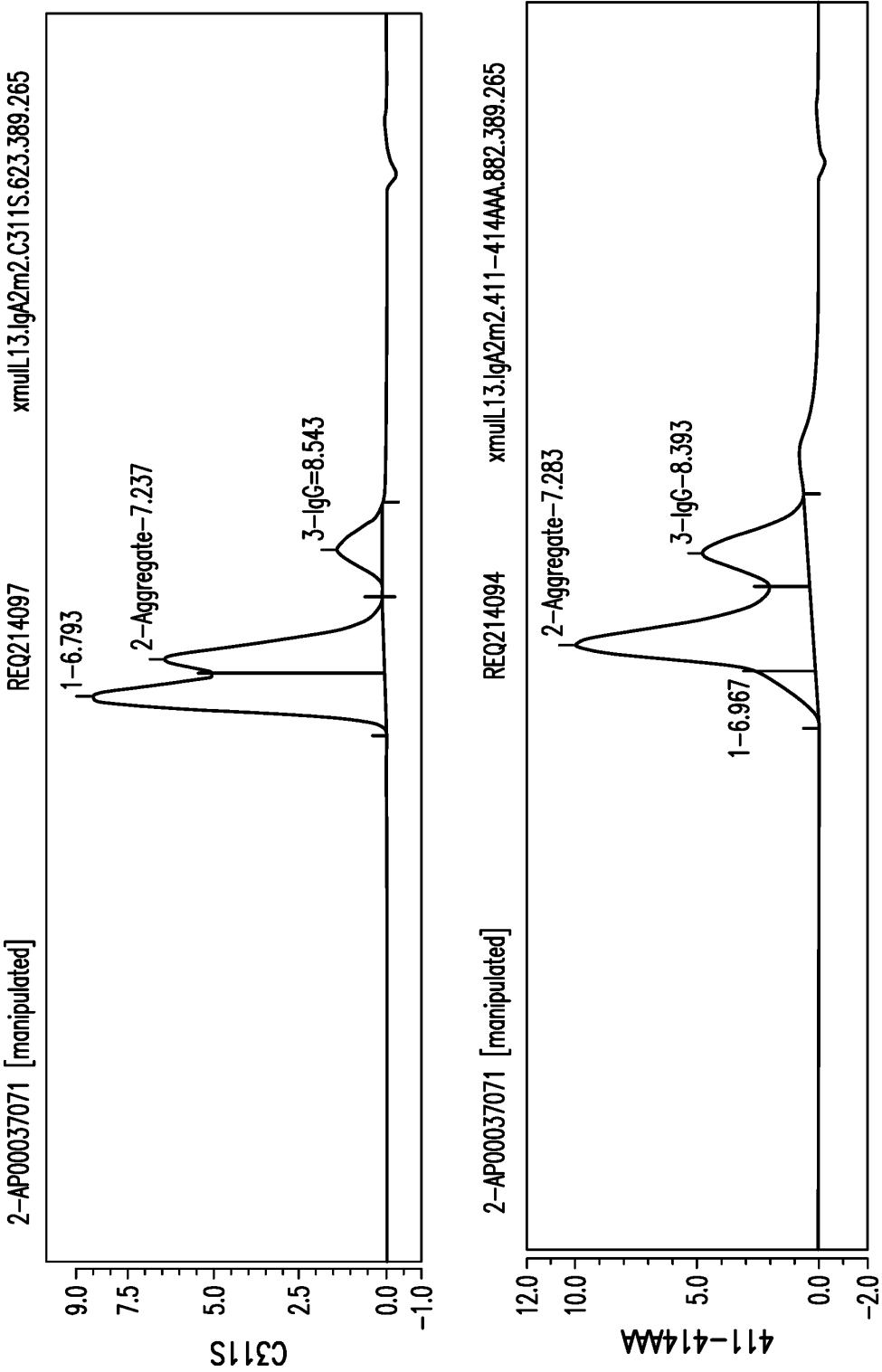
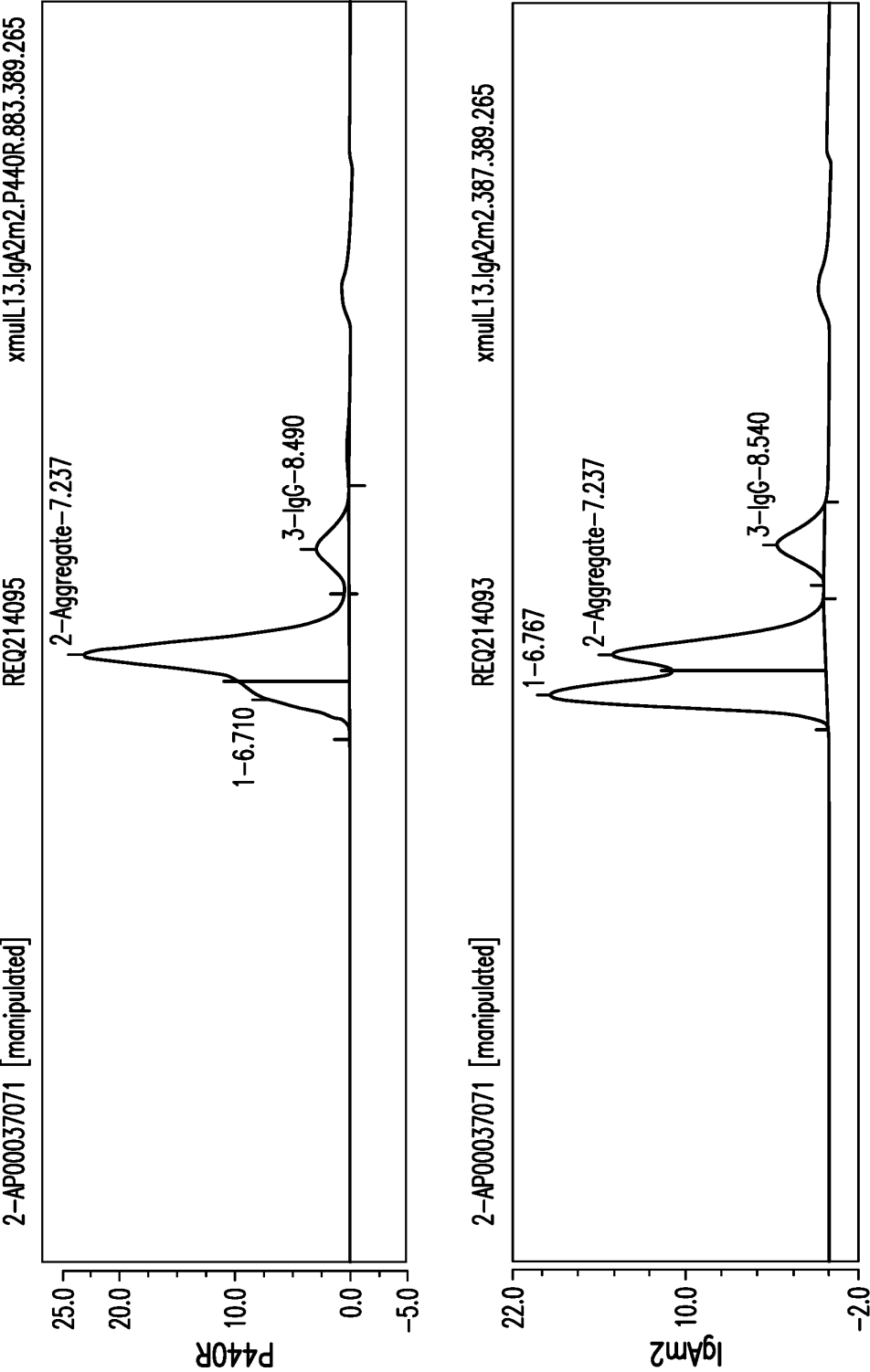


Figure 30A



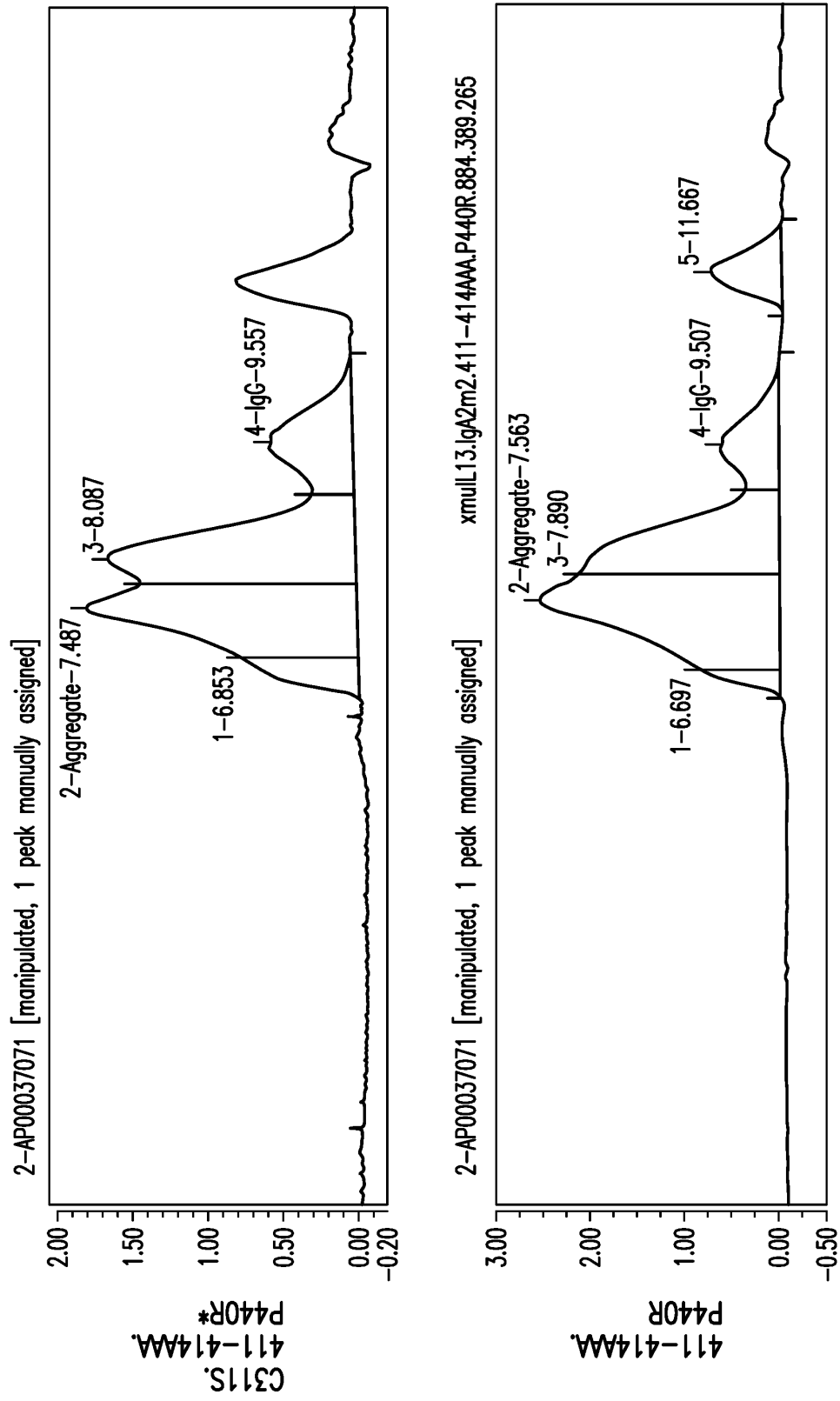
* Poorly behaved by HPLC-SEC

Figure 30B



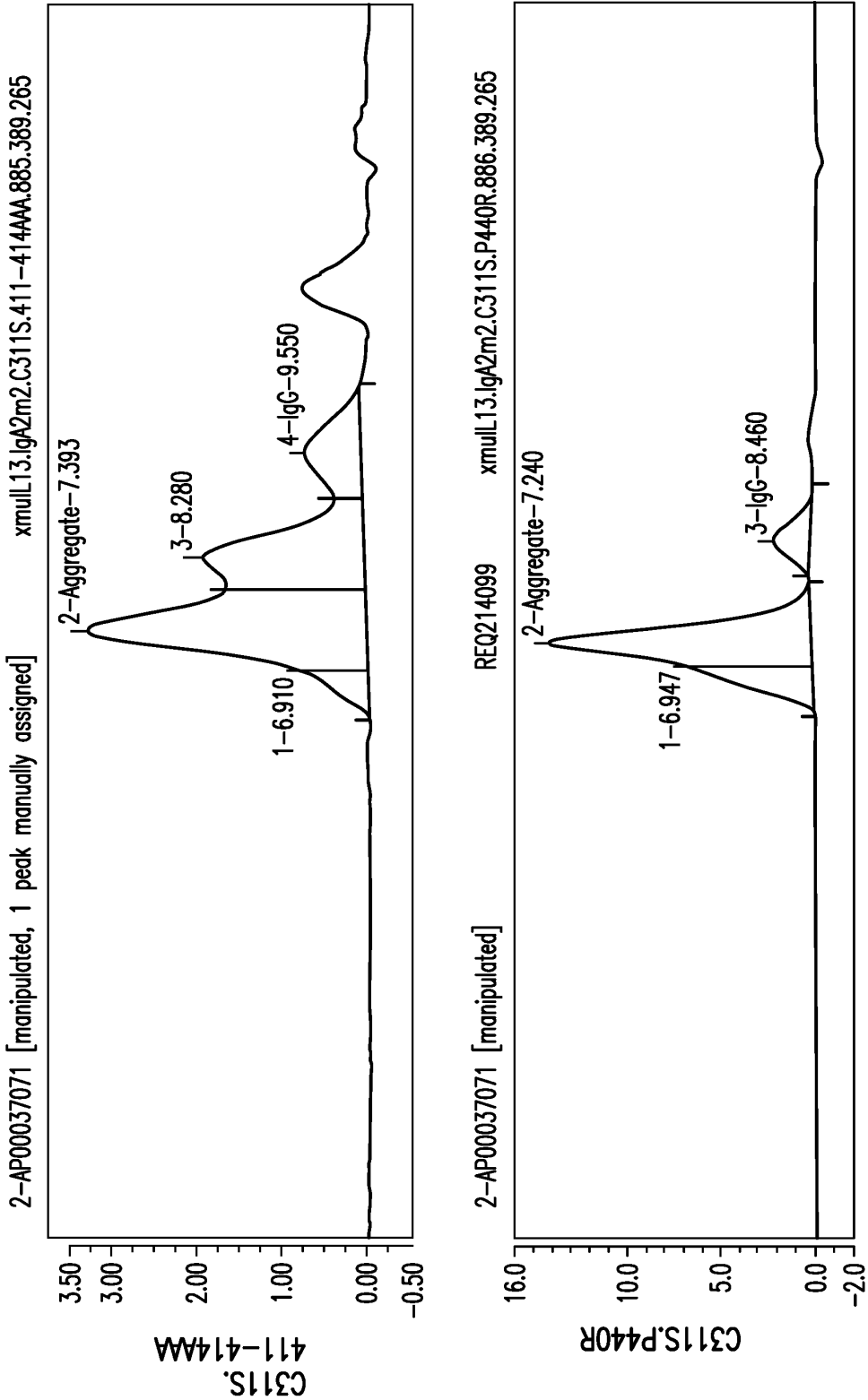
* Poorly behaved by HPLC-SEC

Figure 30B continued



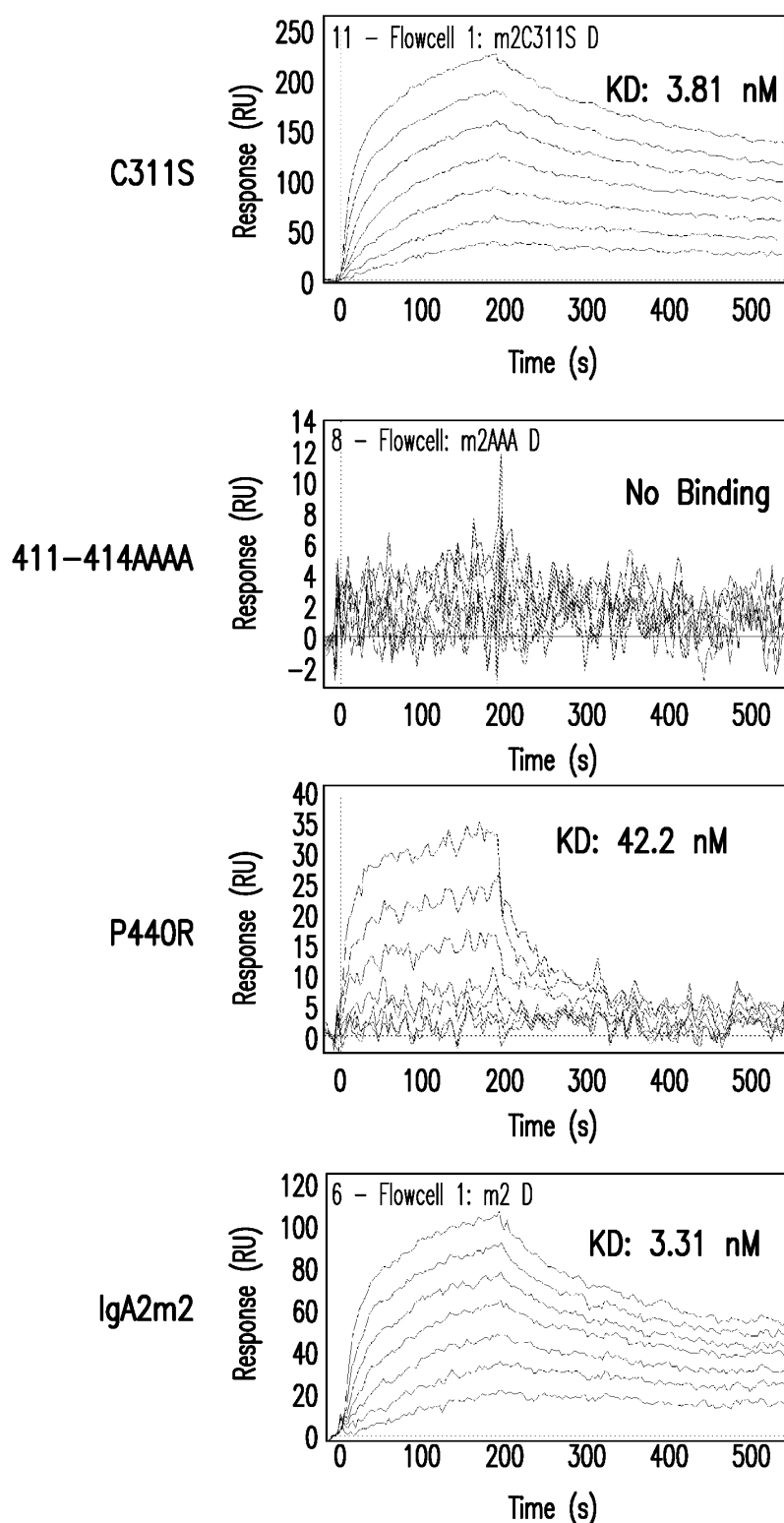
* Poorly behaved by HPLC-SEC

Figure 30B continued



* Poorly behaved by HPLC-SEC

Figure 30B continued



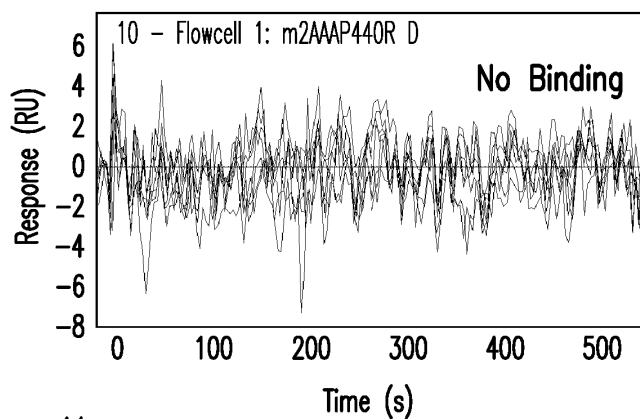
P440R has 10-fold decreased affinity compared to WT

Figure 30C

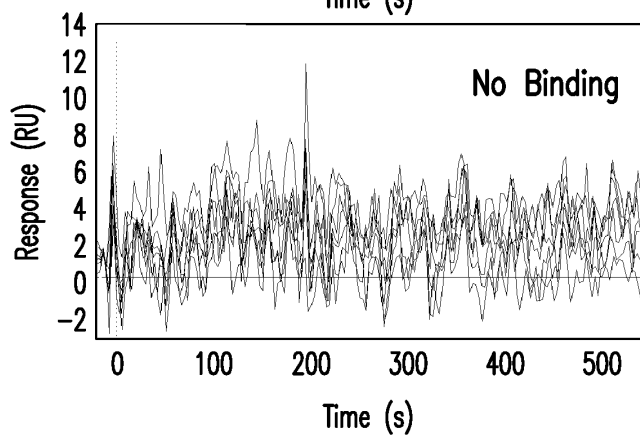
C311S.
411-414AAA.
P440R

Poorly behaved
by HPLC-SEC

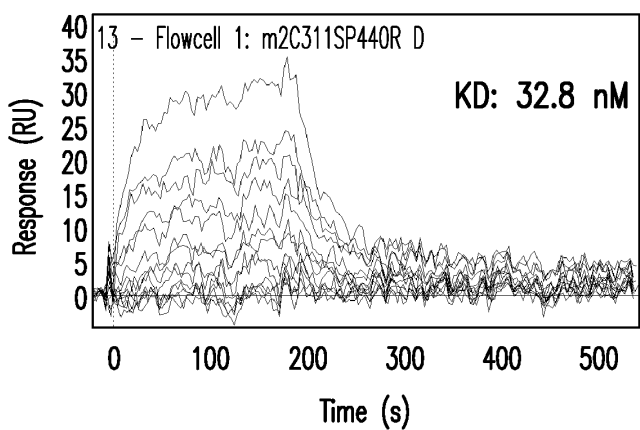
411-414AAA.
P440R



C311S.
411-414AAA

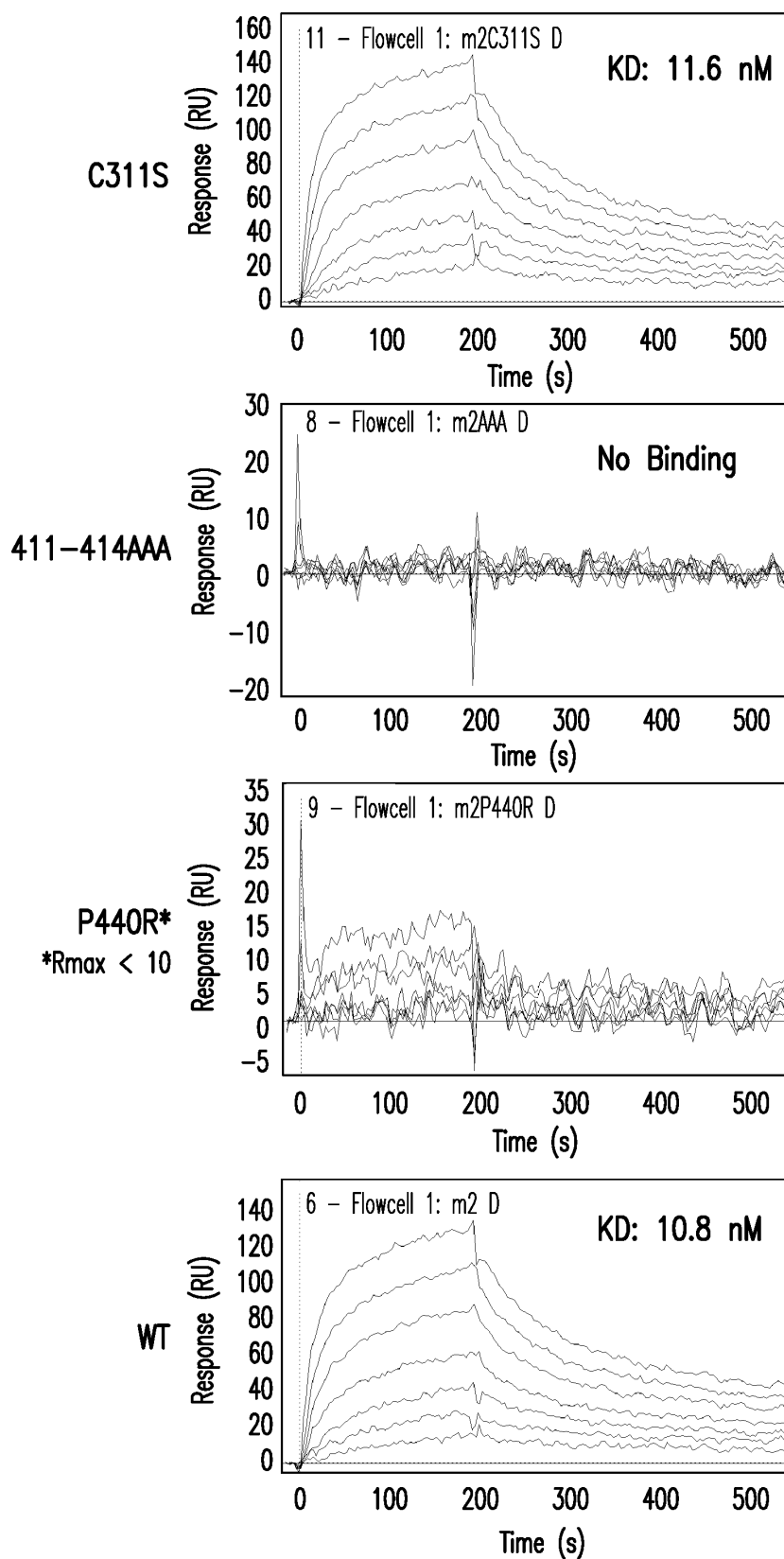


C311S.P440R



Purified IgA2m2 Dimers

Figure 30C continued

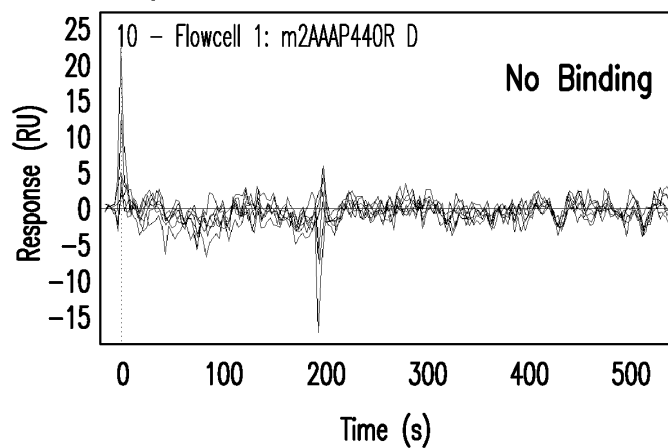
**Figure 30D**

SUBSTITUTE SHEET (RULE 26)

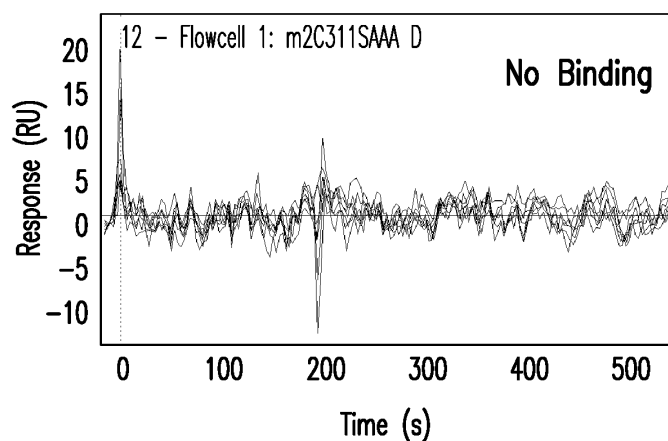
C311S.
411-414AAA.
P440R

Poorly behaved
by HPLC-SEC

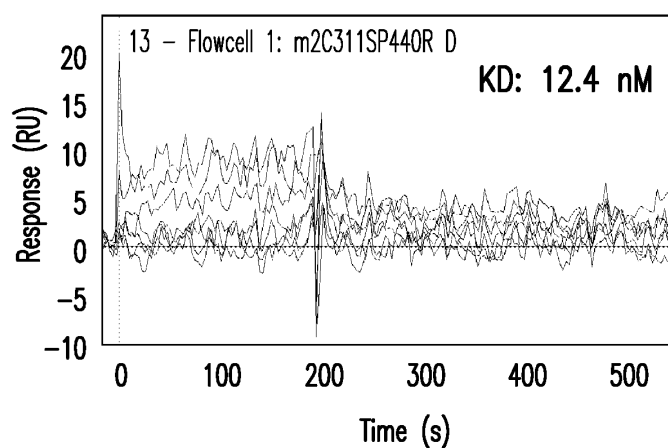
411-414AAA.
P440R



C311S.
411-414AAA

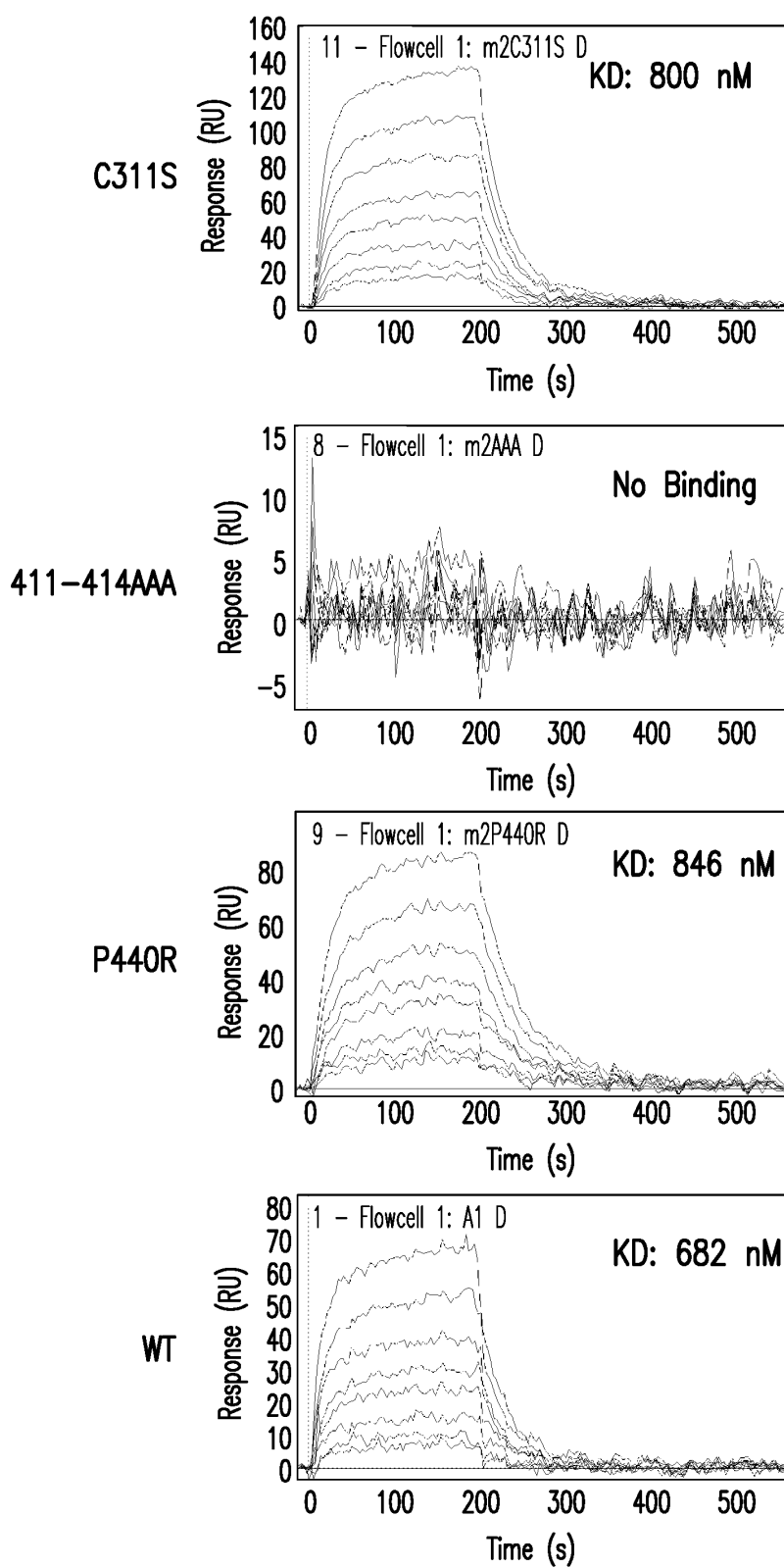


C311S.P440R*



Purified IgA2m2 Dimers

Figure 30D continued

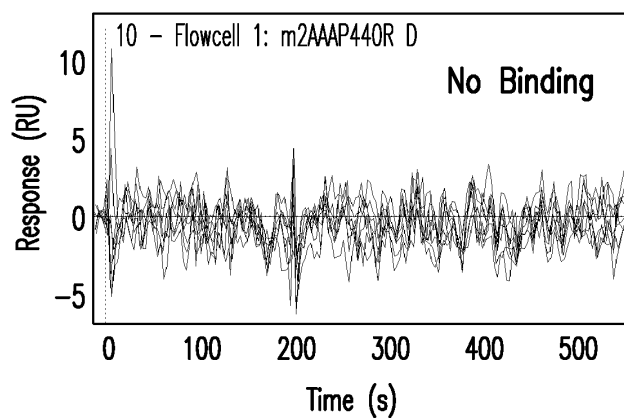
**Figure 30E**

SUBSTITUTE SHEET (RULE 26)

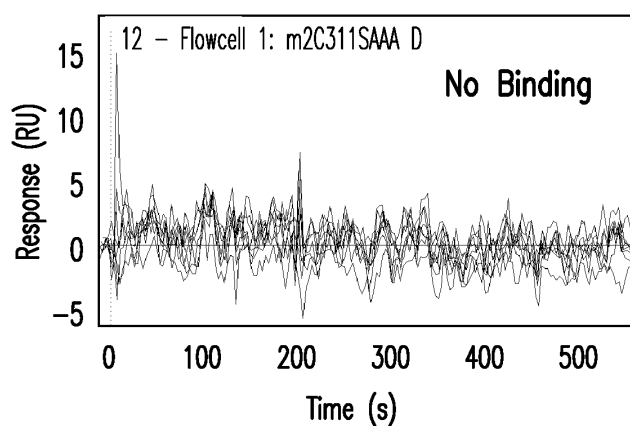
C311S.
411-414AAA.
P440R

Poorly behaved
by HPLC-SEC

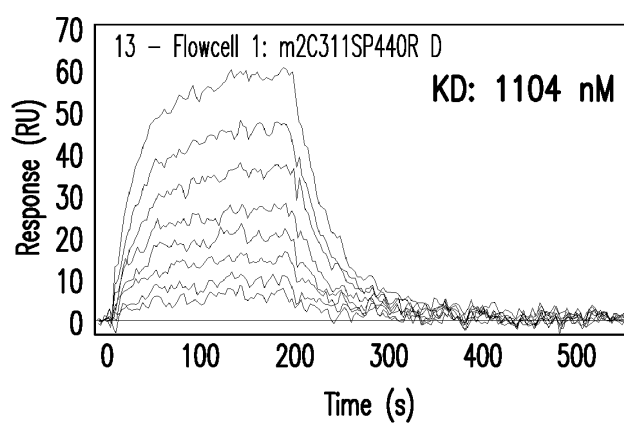
411-414AAA.
P440R



C311S.
411-414AAA



C311S.P440R



Purified IgA2m2 Dimers

Figure 30E continued

SUBSTITUTE SHEET (RULE 26)

020718 IgA stable conditions to increase sialylation

anti-Jag1.2B3.IgA2m2 WT

front cassette: LC+HC+J-chain

back cassette: ST or GT or ST/GT

For 100mL – 400 mg Galactose (4 g/L Gal) (VWR – 220178–524)

For 100mL – 220 mg ManNAc (2.2 g/L ManNAc) (Sigma A8176)

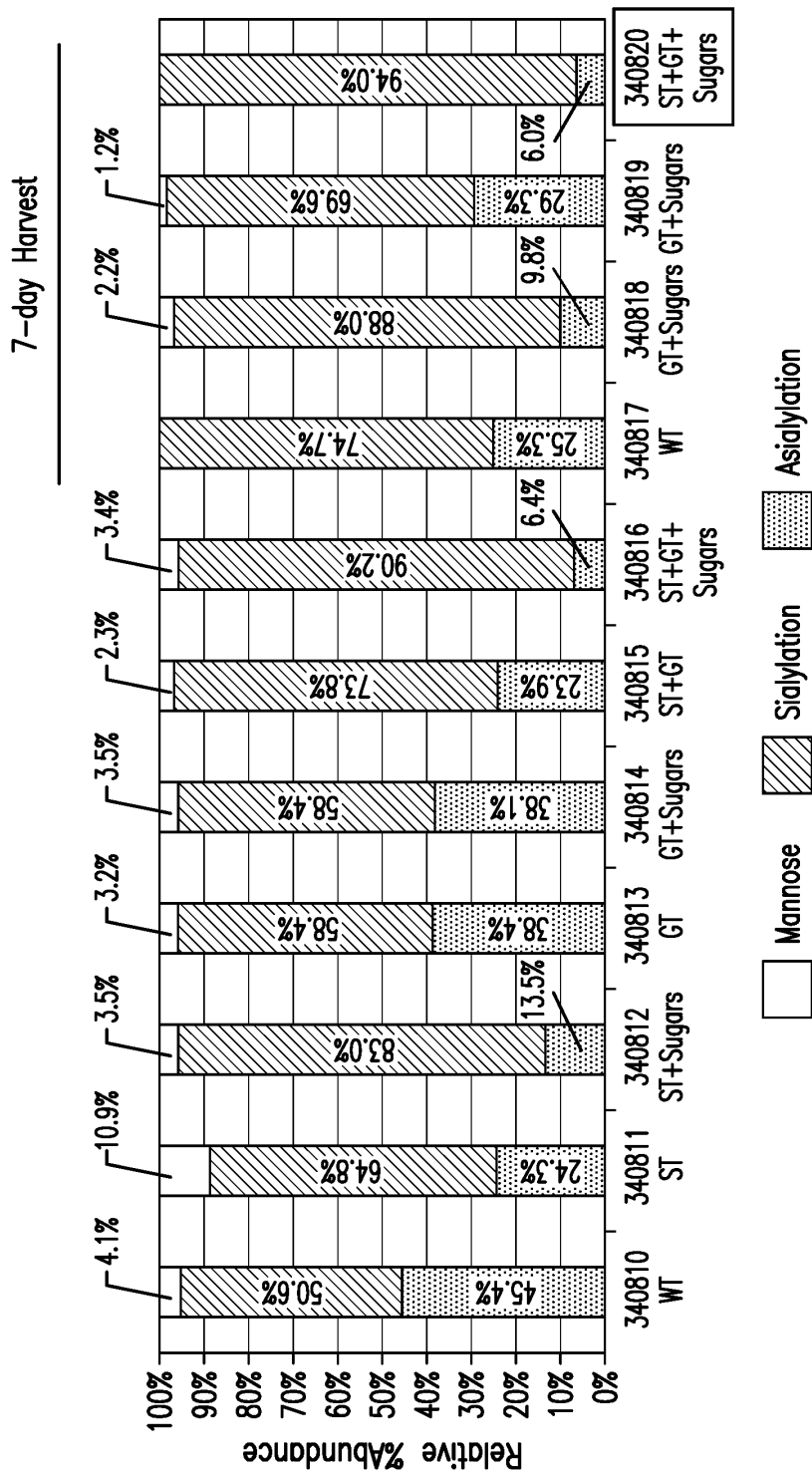
ST: Sialyltransferase

GT: Galactosyltransferase

clone	Req.#	Notes
xJAG1.2B3.hlgA2m2	340810	
xJAG1.2B3.hlgA2m2+ST	340811	
xJAG1.2B3.hlgA2m2+ST+Sugars	340812	Galactose 400mg/100 ml, ManNAc 220 mg/100 ml.
xJAG1.2B3.hlgA2m2+GT	340813	
xJAG1.2B3.hlgA2m2+GT+Sugars	340814	Galactose 400mg/100 ml, ManNAc 220 mg/100 ml.
xJAG1.2B3.hlgA2m2+ST+GT	340815	
xJAG1.2B3.hlgA2m2+ST+GT+Sugars	340816	Galactose 400mg/100 ml, ManNAc 220 mg/100 ml.
xJAG1.2B3.hlgA2m2 (7 day harvest)	340817	
xJAG1.2B3.hlgA2m2 (7 day harvest)+ST+Sugars	340818	Galactose 400mg/100 ml, ManNAc 220 mg/100 ml.
xJAG1.2B3.hlgA2m2 (7 day harvest)+GT+Sugars	340819	Galactose 400mg/100 ml, ManNAc 220 mg/100 ml.
xJAG1.2B3.hlgA2m2 (7 day harvest)+ST+GT+Sugars	340820	Galactose 400mg/100 ml, ManNAc 220 mg/100 ml.

Oligomers were purified away from monomer

Figure 31A



- 7-day vs 14-day harvest gives higher sialylation
- ST+GT+Sugars gives highest sialylation compared to other conditions

Figure 31B

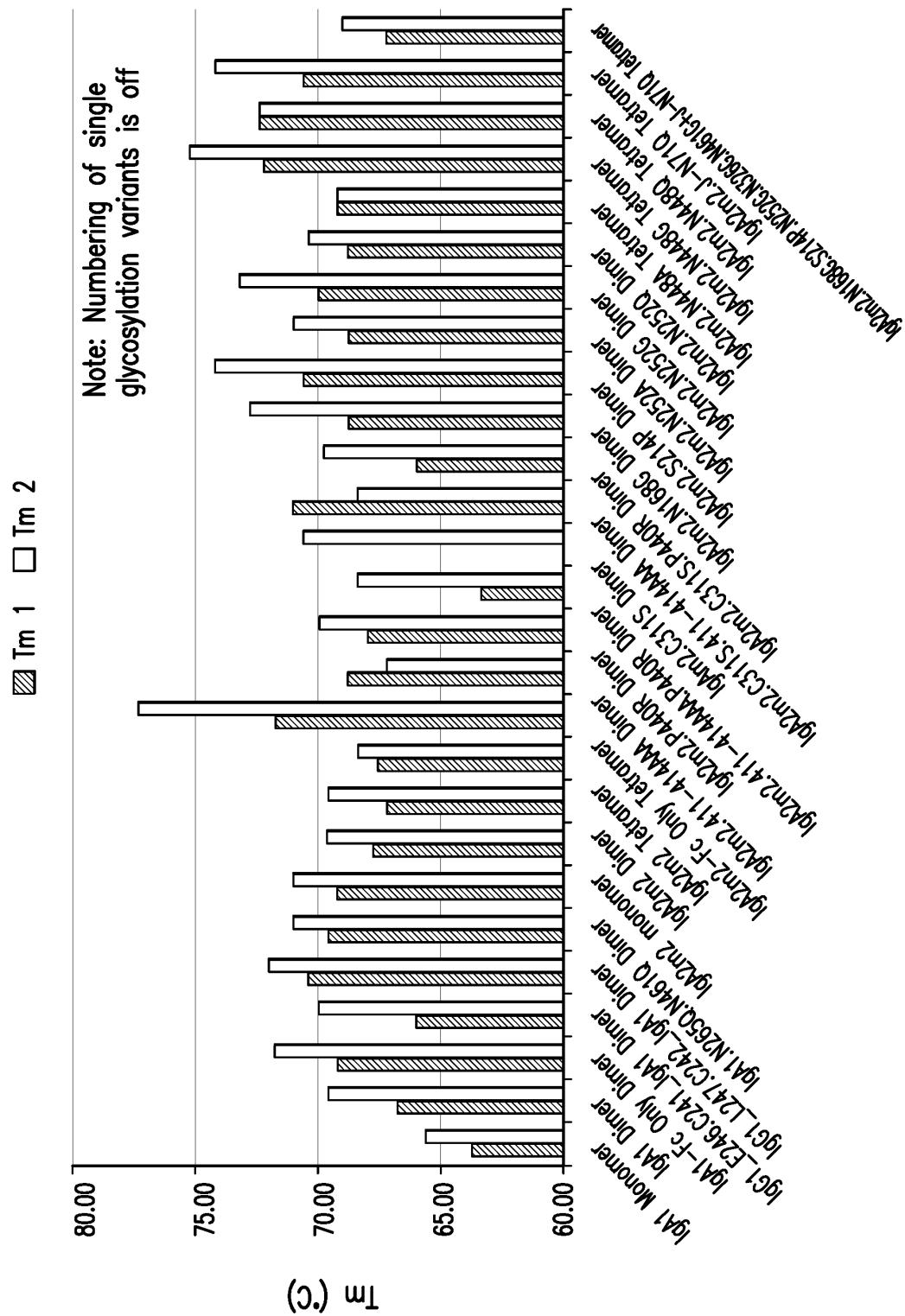


Figure 32

104/150

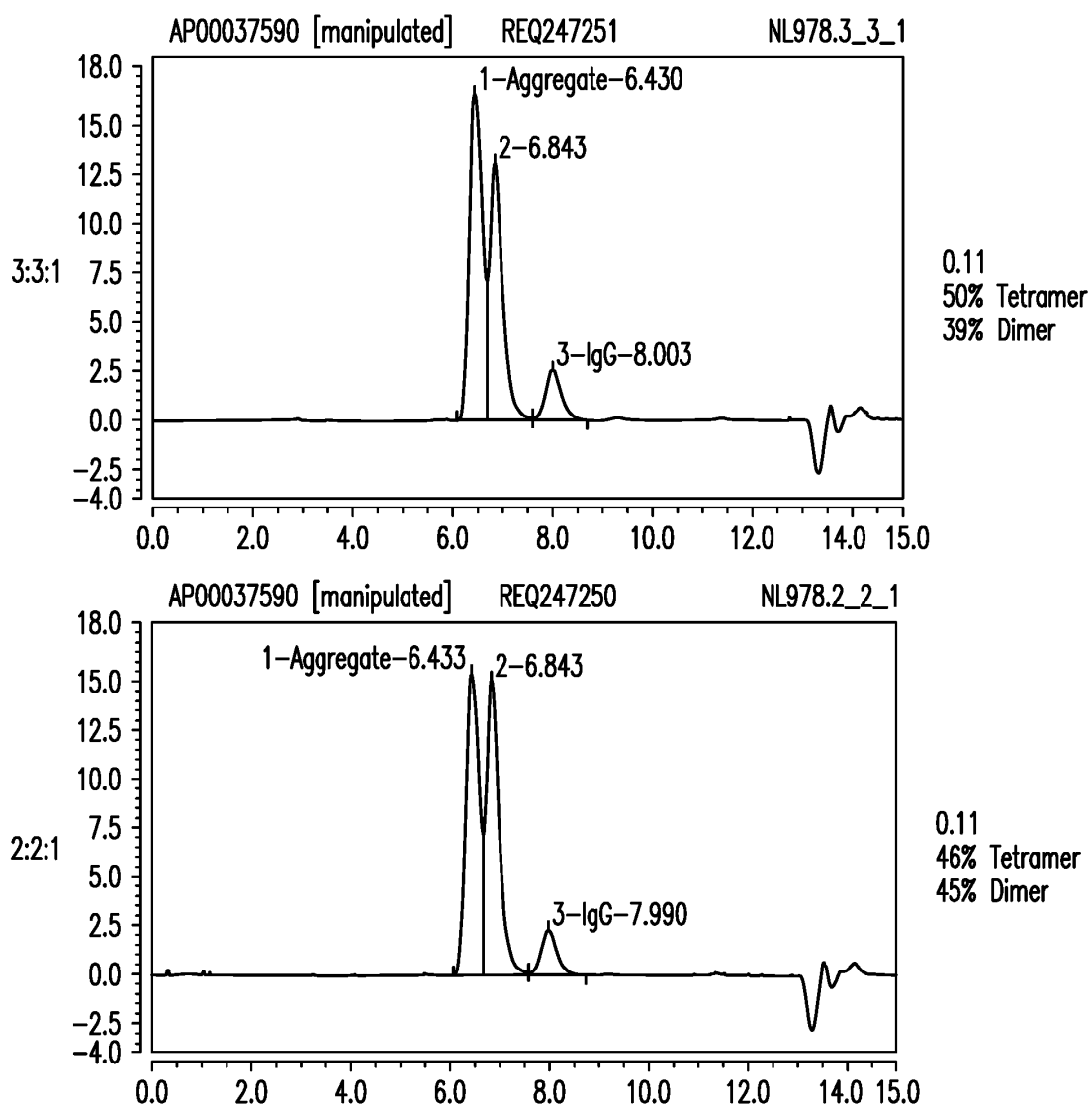
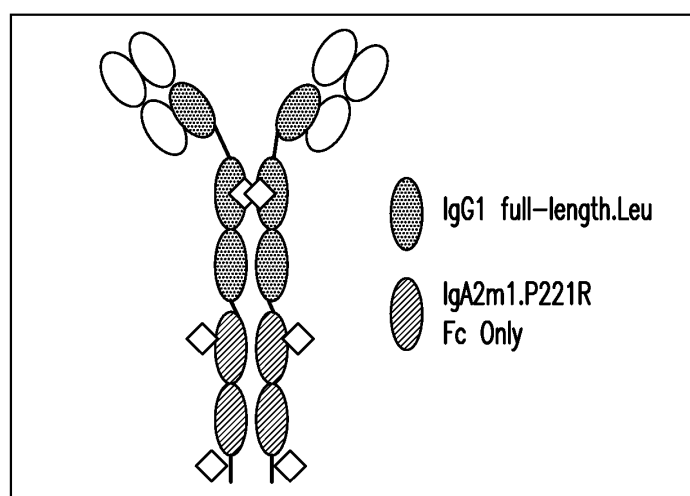


Figure 33A

SUBSTITUTE SHEET (RULE 26)

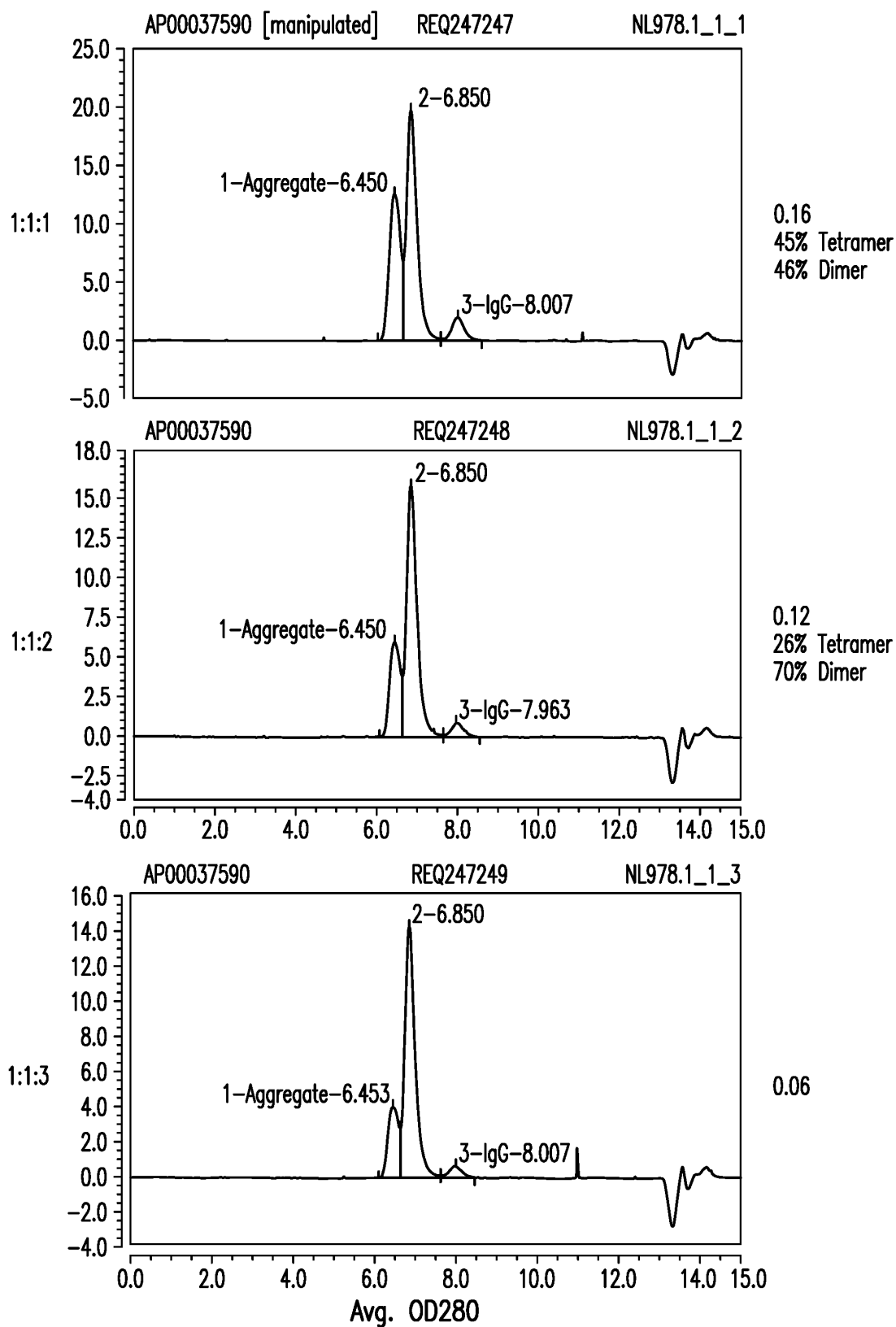


Figure 33A continued

SUBSTITUTE SHEET (RULE 26)

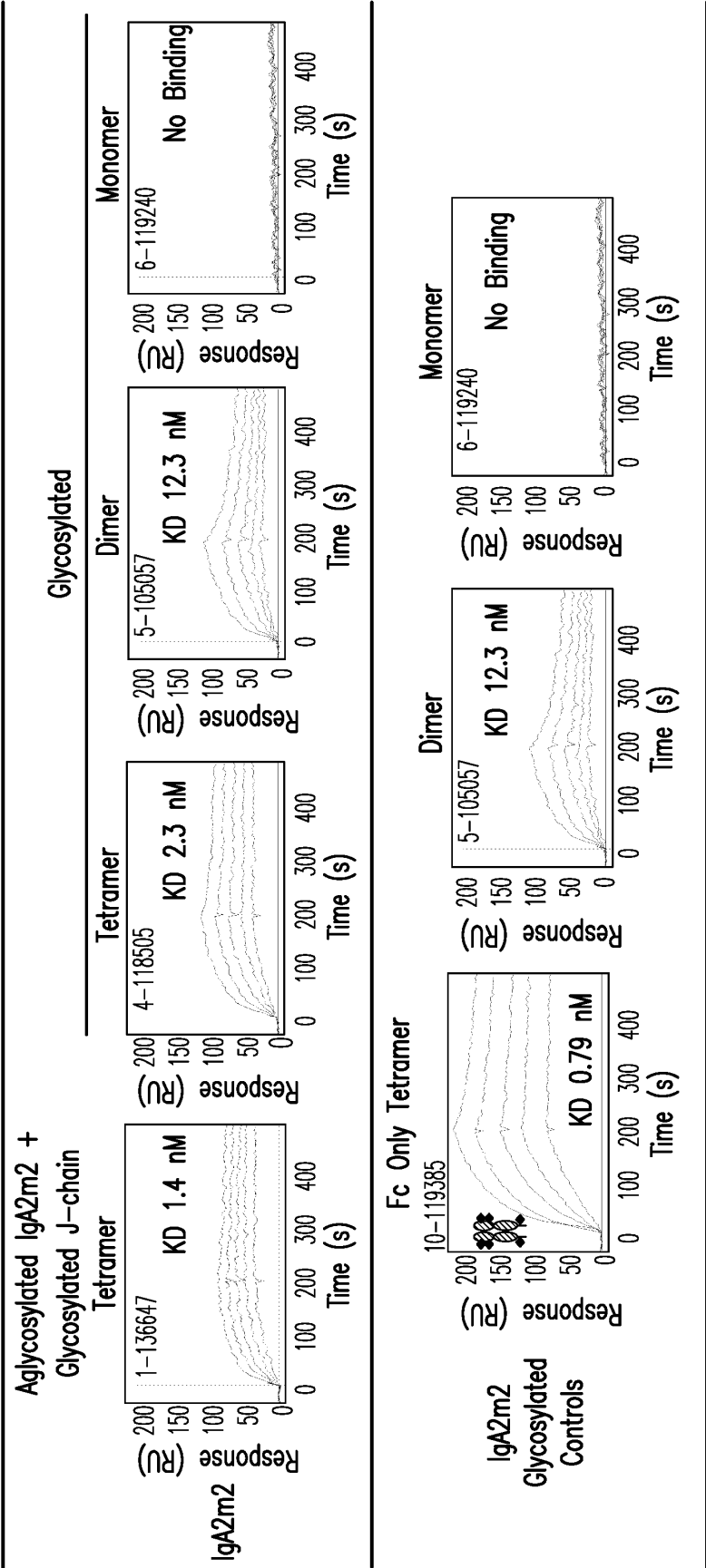


Figure 33B

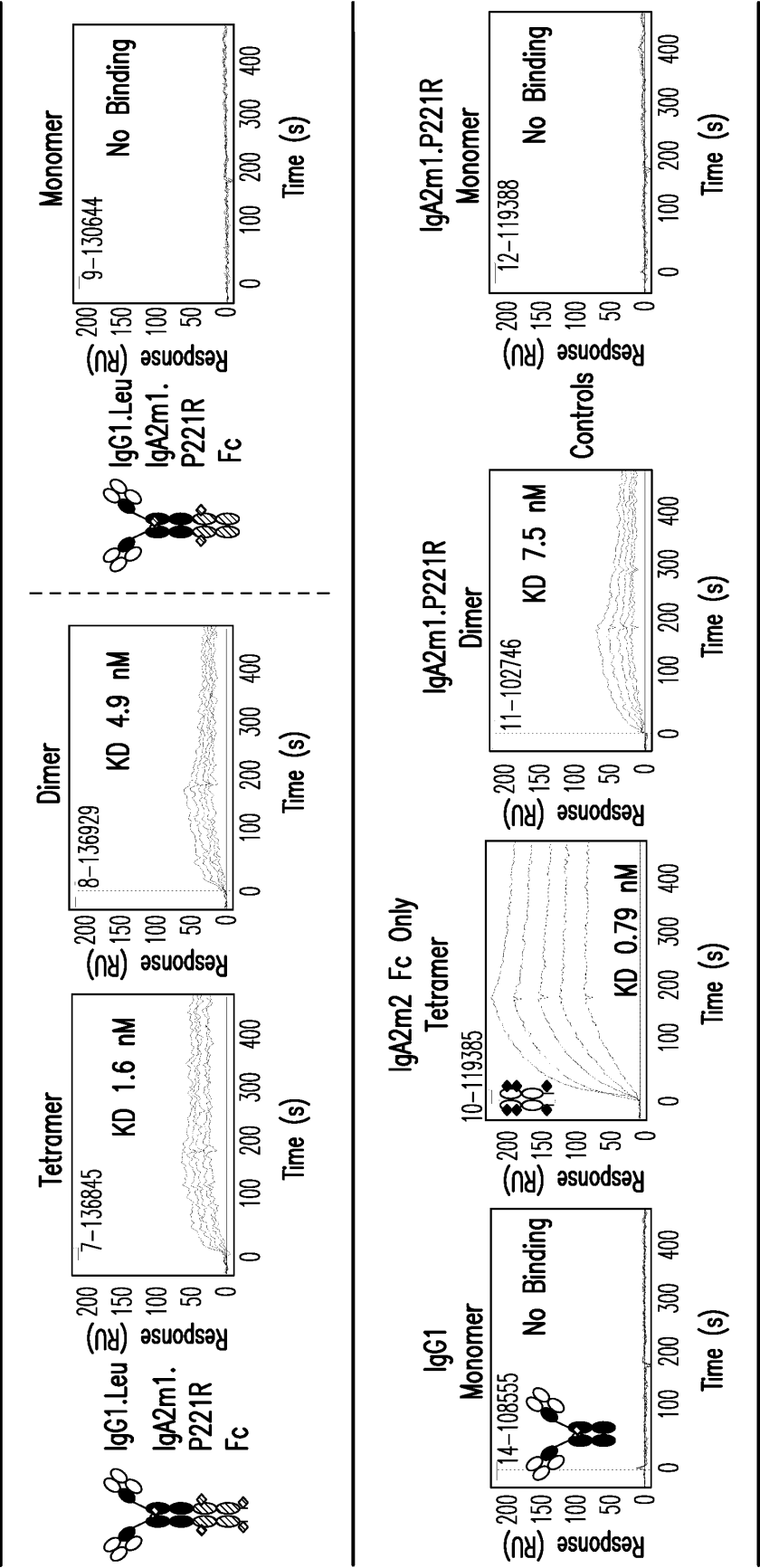


Figure 33B continued

PUR	PUR	Mouse plgR				Human plgR			
		ka	kd	Rmax	KD	ka	kd	Rmax	KD
136645	Aglycosylated IgA2m2 + Glycosylated J-chain-Tetramer (NL1011.389.265)	4.62e5	5.82e-4	102.67	1.26nM	6.85(2)e5	5.43(5)e-4	100.4(1)	792pM
118505	Glycosylated IgA2m2 Tetramer (NL387.389.265)	3.48e5	8.14e-4	122.2	2.34nM	4.69(2)e5	9.06(9)e-4	114.9(2)	1.93nM
105057	Glycosylated IgA2m2 Dimer (NL387.389.264)	2.19e5	2.70e-3	135.5	12.31nM	3.34(1)e5	0.00464(1)	110.0(2)	13.91nM
119240	Glycosylated IgA2m2 Monomer (NL387.389)			2.368	NB			1.924	NB
136845	Glycosylated IgG1-full length-LeuIgA2m1.P221R Fc only + J-chain-Tetramer (NL978.389.265)	4.87e5	7.68e-4	54.24	1.58nM	6.12(2)e5	0.001119(6)	53.49(6)	1.83nM
136929	Glycosylated IgG1-full length-LeuIgA2m1.P221R Fc only + J-chain-Dimer (NL978.389.265)	4.61e5	0.00226	44.06	4.92nM	5.02(3)e5	0.00371(2)	32.06(8)	7.40nM
136844	Glycosylated IgG1-full length-LeuIgA2m1.P221 Fc only.No tail-Monomer (NL984.389)			1.972	NB			0.7482	NB
119385	Glycosylated IgA2m2.Fc only Tetramer	4.02e5	3.17e-4	243.8	790pM	4.80(1)e5	4.69(6)e-4	237.1(3)	980pM
102746	Glycosylated IgA2m1.P221R Dimer (NL439.389.265)	3.39e5	0.00255	58.3	7.53nM	3.91(3)e5	0.00604(2)	43.6(1)	15.5nM
119388	Glycosylated IgA2m1.P221R Monomer (NL439.389)			3.771	NB			5.3(9)	NB
119387	Glycosylated IgA1.Fc only Dimer	3.18e5	0.00356	52.7	11.20nM	5.01(3)e5	0.00552(2)	40.9(1)	11.02nM
108555	Glycosylated IgG1 Monomer		0		NB			0.1033	NB

Figure 33C

	T _{m1} (°C)	T _{m2} (°C)	T _{m3} (°C)
Aglycosylated IgA2m2 + Glycosylated J-chain-Tetramer (NL1011.389.265)	70.13	77.60	
Glycosylated IgA2m2 Tetramer (NL387.389.265)	69.80		
Glycosylated IgA2m2 Dimer (NL387.389.265)	71.20		
Glycosylated IgA2m2 Monomer (NL387.389)	70.80		
Glycosylated IgG1-full length.Leu:IgA2m1.P221R Fc only + J-chain-Tetramer (NL978.389.265)	63.3	67.3	77.2
Glycosylated IgG1-full length.Leu:IgA2m1.P221R Fc only + J-chain-Dimer (NL978.389.265)	63.5	67.2	72.0
Glycosylated IgG1-full length.Leu:IgA2m1.P221R Fc only.No tail-Monomer (NL984.389)	67.20		
Glycosylated IgA2m2.Fc only Tetramer	76.93		
Glycosylated IgA2m1.P221R Dimer (NL439.389.265)	69.93		
Glycosylated IgA2m1.P221R Monomer (NL439.389)	70.20		
Glycosylated IgA1.Fc only Dimer	67.07		

Figure 33D

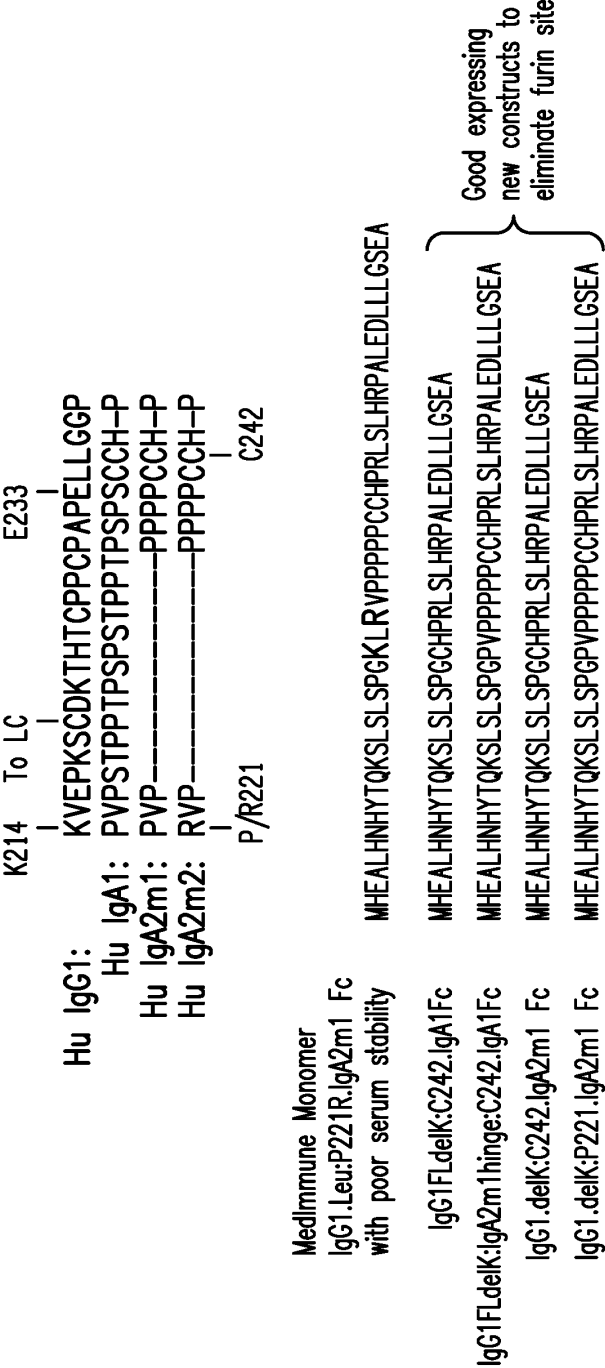


Figure 34A

Constructs with expression
>/= hlgG1 were considered

	# Constructs	Expression (OD _{280nm} ≥0.8)
lgG1 full-length: lgA1 Fc	12	7
lgG1 full-length: lgA2m1 Fc	10	4
lgG1 full-length: lgA2m2 Fc	10	0

IgA2m2 Fc fusions expressed
not as well as Fc fusions with
lgA1 and IgA2m1

xIL13.NL1147.lgG1.delK_C
242.lgA1.Fc dimer

Name	Avg. OD280nm	SDDev	Use?
lgG1FLdelK_P221toP227.lgA2m1hinge_C242.lgA1Fc	1.33	0.07	Yes
lgG1FLdelK_V222toP227.lgA2hinge_C242.lgA1Fc	1.23	0.10	No (similar to first one)
lgG1FL_C241.lgA1Fc	1.18	0.03	No (not delK)
lgG1FL_C241.lgA2m1Fc	1.12	0.14	No (not delK)
lgG1FLdelK_V222toP227.lgA2hinge_C241.lgA1Fc	1.08	0.04	No (C241)
lgG1FLdelK_C242.lgA2m1Fc	1.02	0.23	Yes
lgG1FLdelK_C242.lgA1Fc	0.96	0.02	Yes
lgG1FLdelK_P221.lgA2m1Fc	0.88	0.06	Yes
huIgG1 (Control)	0.88	0.04	Control, yes
lgG1FLLeu.P221R.lgA2m1Fc (Serum unstable original construct)	0.87	0.02	Control, yes
lgG1FLdelK_R221toP227.lgA2m2hinge_C242.lgA1Fc	0.84	0.03	
lgG1FLdelK_Leu.P221R.lgA2m1Fc	0.79	0.02	
lgG1FLdelK_P223toP227.lgA2hinge_C242.lgA1Fc	0.79	0.05	
lgG1FLdelK_P223toP227.lgA2hinge_C241.lgA1Fc	0.69	0.02	
lgG1FLdelK_V222.lgA2m1Fc	0.61	0.01	
lgG1FLdelK_C241.lgA2m1Fc	0.54	0.06	
lgG1FLdelK_R221.lgA2m2Fc	0.49	0.06	
lgG1FLdelK_P223.lgA2m2Fc	0.48	0.04	

Figure 34B

Constructs with expression
>/= hlgG1 were considered

	# Constructs	Expression (OD _{280nm} ≥ 0.8)
IgG1 full-length: IgA1 Fc	12	7
IgG1 full-length: IgA2m1 Fc	10	4
IgG1 full-length: IgA2m2 Fc	10	0

IgA2m2 Fc fusions expressed
not as well as Fc fusions with
IgA1 and IgA2m1

xIL13.NL1147.IgG1.delK_C
242.IgA1.Fc dimer

Name	Avg. OD _{280nm}	SDDev	Use?
IgG1FLdelK_P223.IgA2m1Fc	0.48	0.03	
IgG1FL_C242.IgA2m1Fc	0.46	0.01	
IgG1FLdelK_P221R.IgA2m1Fc	0.44	0.02	
IgG1FLdelK_Leu.R221P.IgA2m2Fc	0.43	0.04	
IgG1FLdelK_P221toP227.IgA2m1hinge_C241.IgA1Fc	0.40	0.03	
IgG1FLdelK_C242.IgA2m2Fc	0.37	0.03	
IgG1FL_C242.IgA1Fc	0.33	0.00	
IgG1FLdelK_C241.IgA1Fc	0.32	0.02	
IgG1FLdelK_Leu.P221.IgA2m1Fc	0.30	0.05	
IgG1FL_C242.IgA2m2Fc	0.30	0.02	
IgG1FLdelK_C241.IgA2m2Fc	0.23	0.01	
IgG1FLdelK_R221toP227.IgA2m2hinge_C241.IgA1Fc	0.21	0.01	
IgG1FLdelK_R221P.IgA2m2Fc	0.17	0.02	
IgG1FLdelK_Leu.R221.IgA2m2Fc	0.03	0.05	
IgG1FLdelK_V222.IgA2m2Fc	0.02	0.01	
IgG1FL_C241.IgA2m2Fc	0.02	0.01	

Figure 34B continued

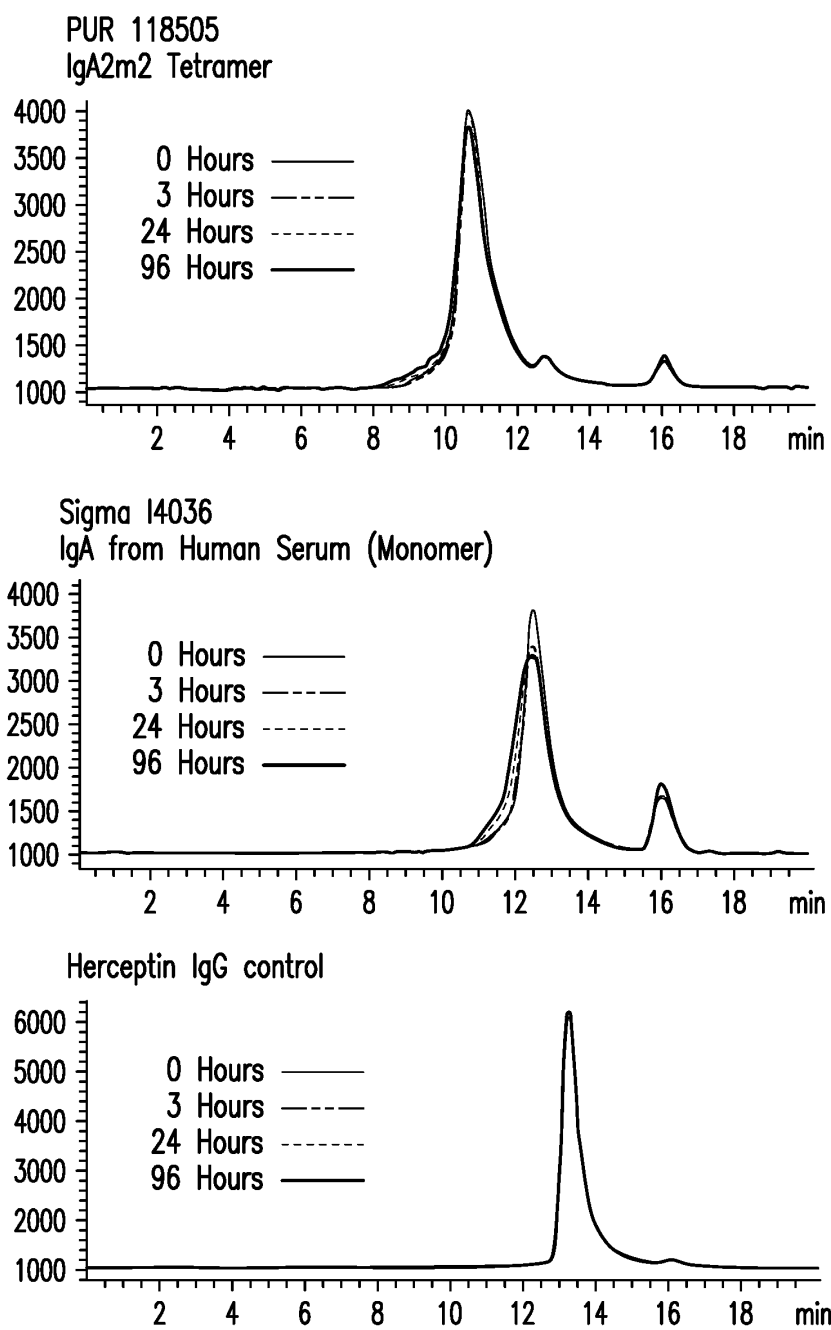
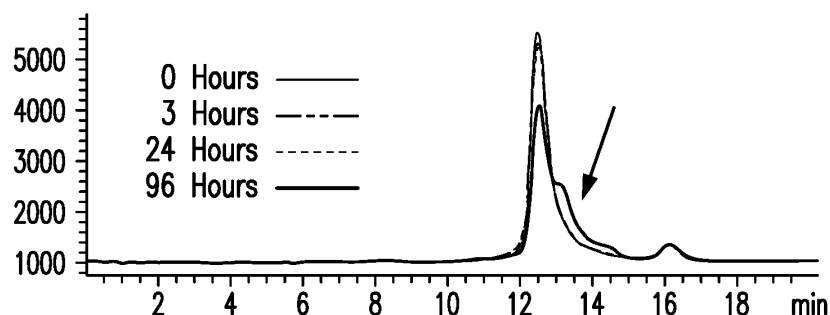


Figure 34C

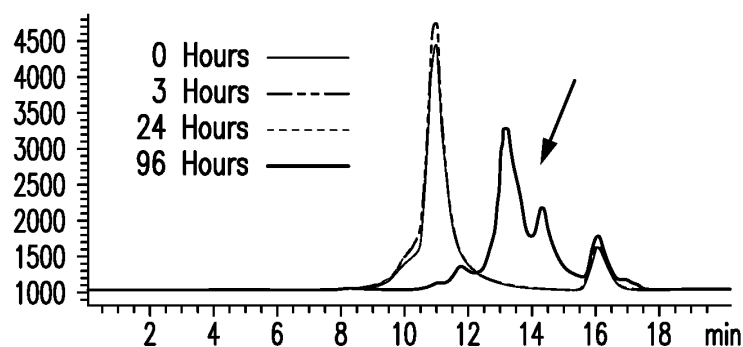
PUR 136844

IgG full-length:P221R.IgA2m1 Fc only No tail Monomer



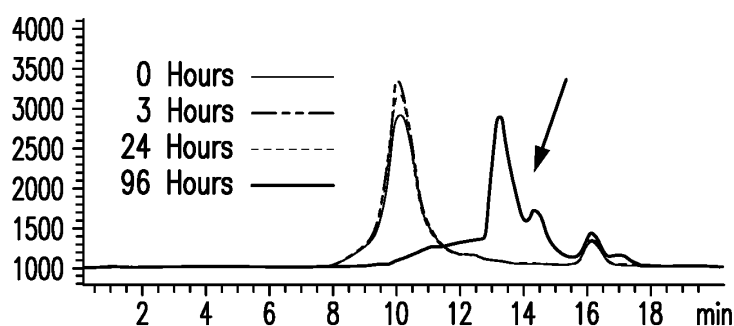
PUR 136929

IgG full-length:P221R.IgA2m1 Fc only Dimer



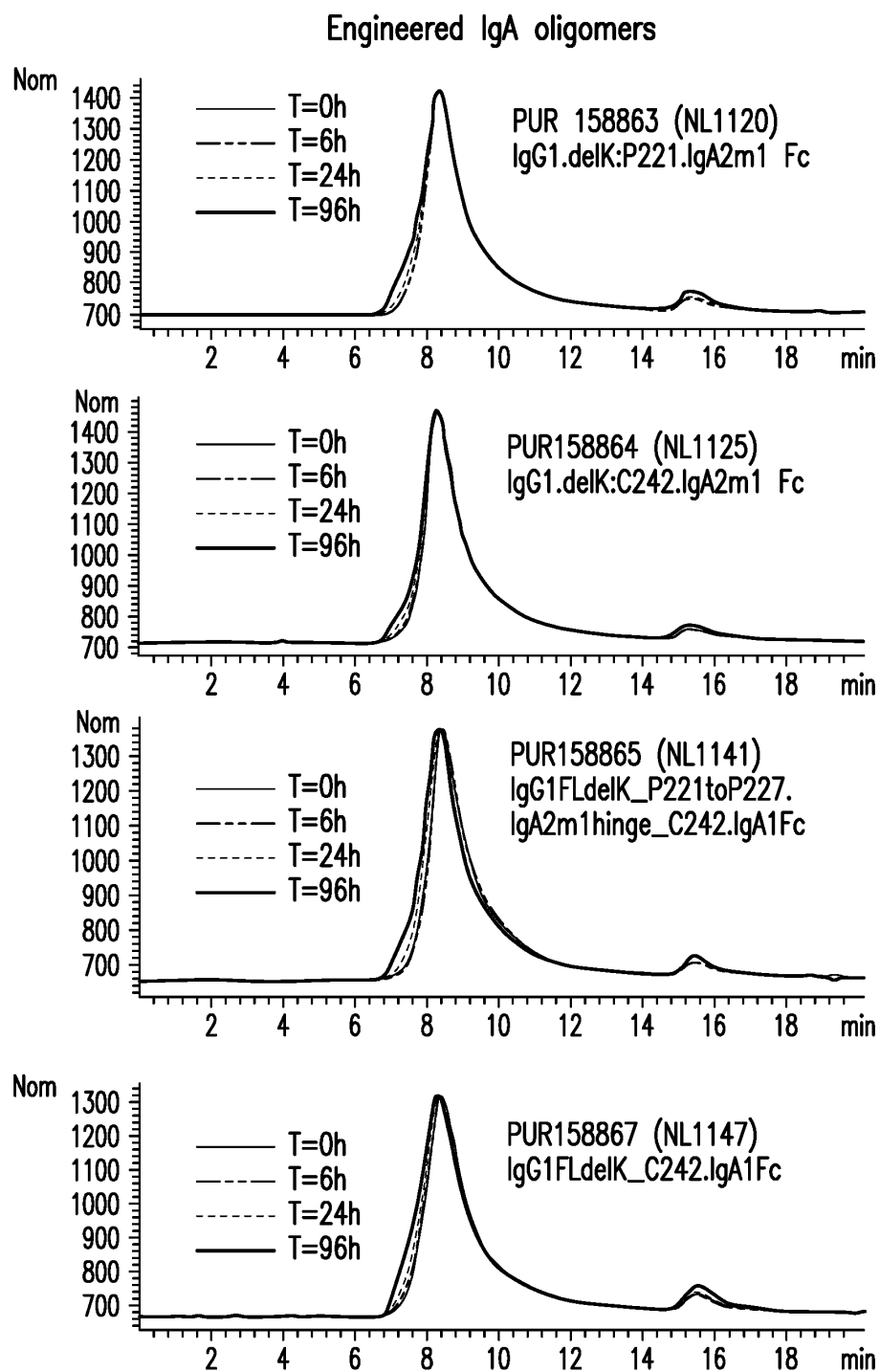
PUR 136845

IgG full-length:P221R.IgA2m1 Fc only Tetramer



All antibodies were ^{131}I labeled. 5 uCi of each antibody was spiked into 100 ul of mouse plasma and incubated at 37 C for up to 96 hours. Antibodies were frozen using dry ice and stored at -80 C at 0, 3 or 6, 24, and 96 hours to stop any further degradation. Antibodies were subjected to HPLC analysis and traces of retention time of radiolabel are shown.

Figure 34C (continued)



PUR158867 was dropped due to stickiness
to concentrator

Figure 34C (continued)

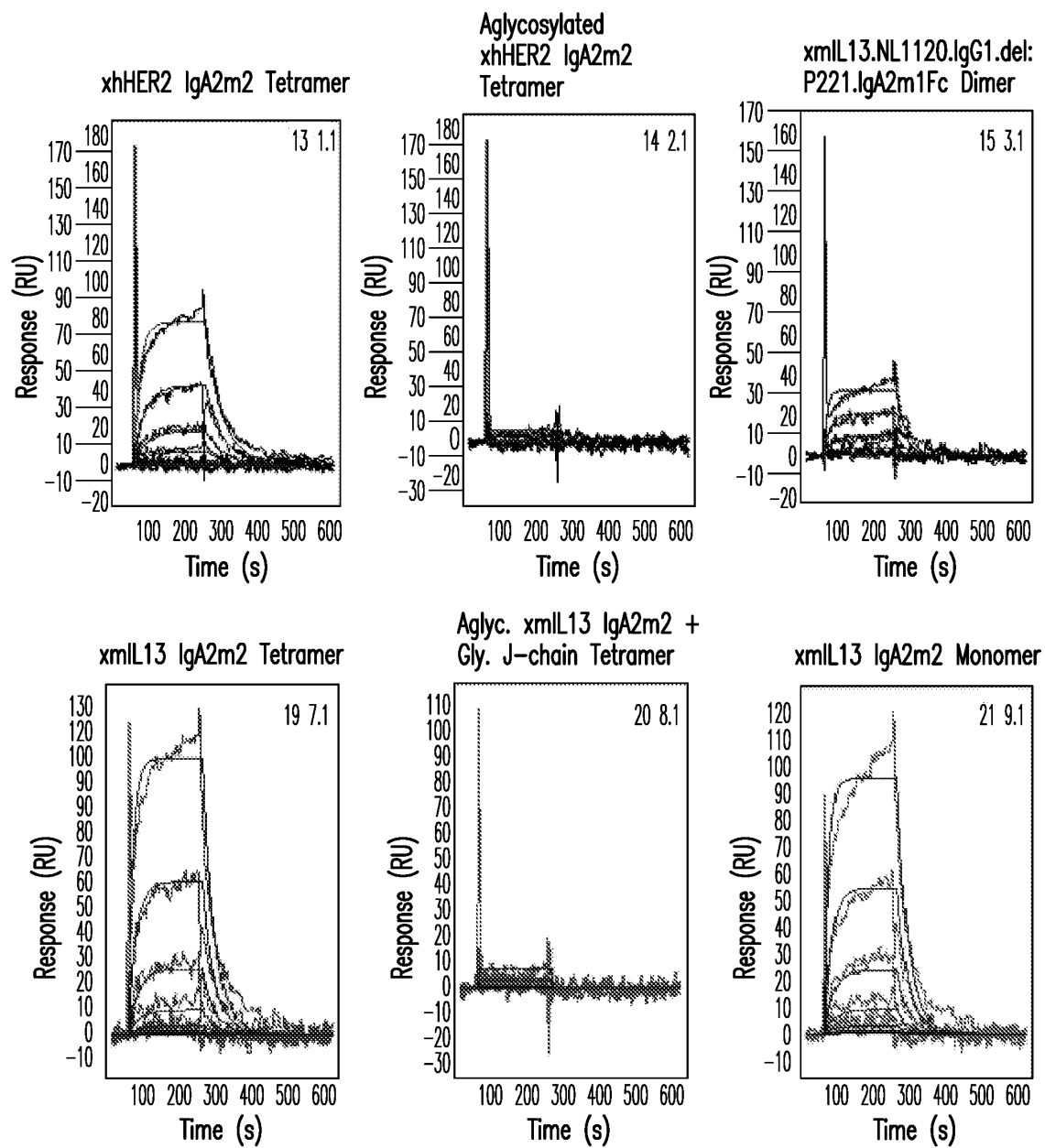
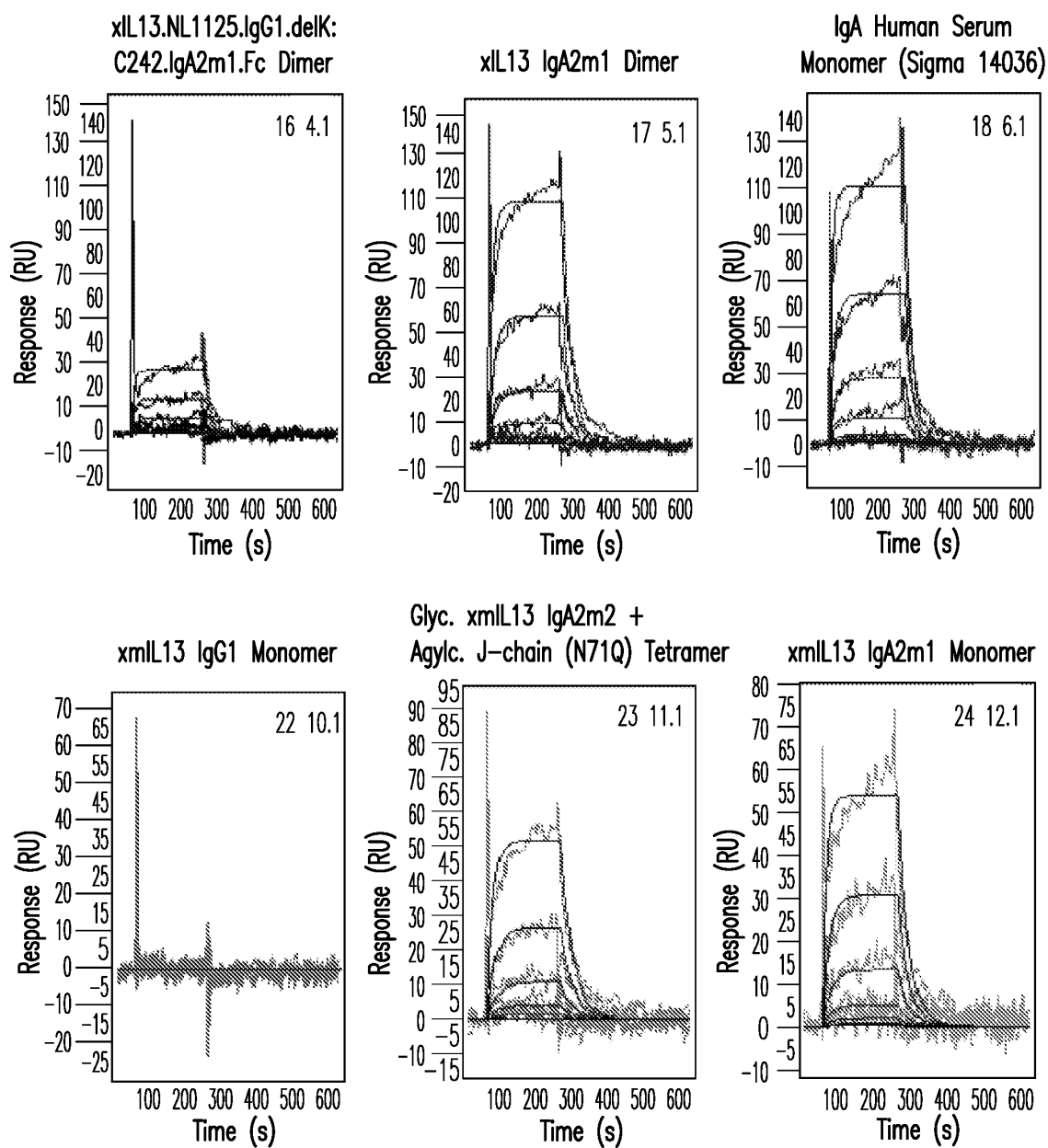


Figure 35A



Wasatch Binding Kinetics

Figure 35A continued

	ka	ka st dev	kd	kd st dev	KD	KD St Dev
Glycosylated xhHer2.IgA2m2 Tetramer	1.37E+04	3.89E+03	2.20E-02	7.82E-03	1.59E-06	2.00E-07
Aglycosylated xhHer2.IgA2m2 Tetramer	No Binding					
xmlL-13.IgA2m1 Dimer	3.51E+04	2.07E+03	3.71E-02	8.06E-04	1.06E-06	5.10E-08
xlL13.IgG1 Monomer	No Binding					
Glycosylated IgA Human Serum Monomer (Sigma I4036)	2.76E+04	1.27E+03	4.82E-02	3.79E-04	1.75E-06	9.29E-08
Glyc. xmlL13 IgA2m2 + Aglyc. J chain (N71Q) Oligomer mix	1.54E+04	1.89E+03	2.71E-02	2.78E-03	1.77E-06	2.14E-07
Aglyc. xmlL13 IgA2m2 + Glyc. J chain Tetramer	No Binding					

Figure 35B

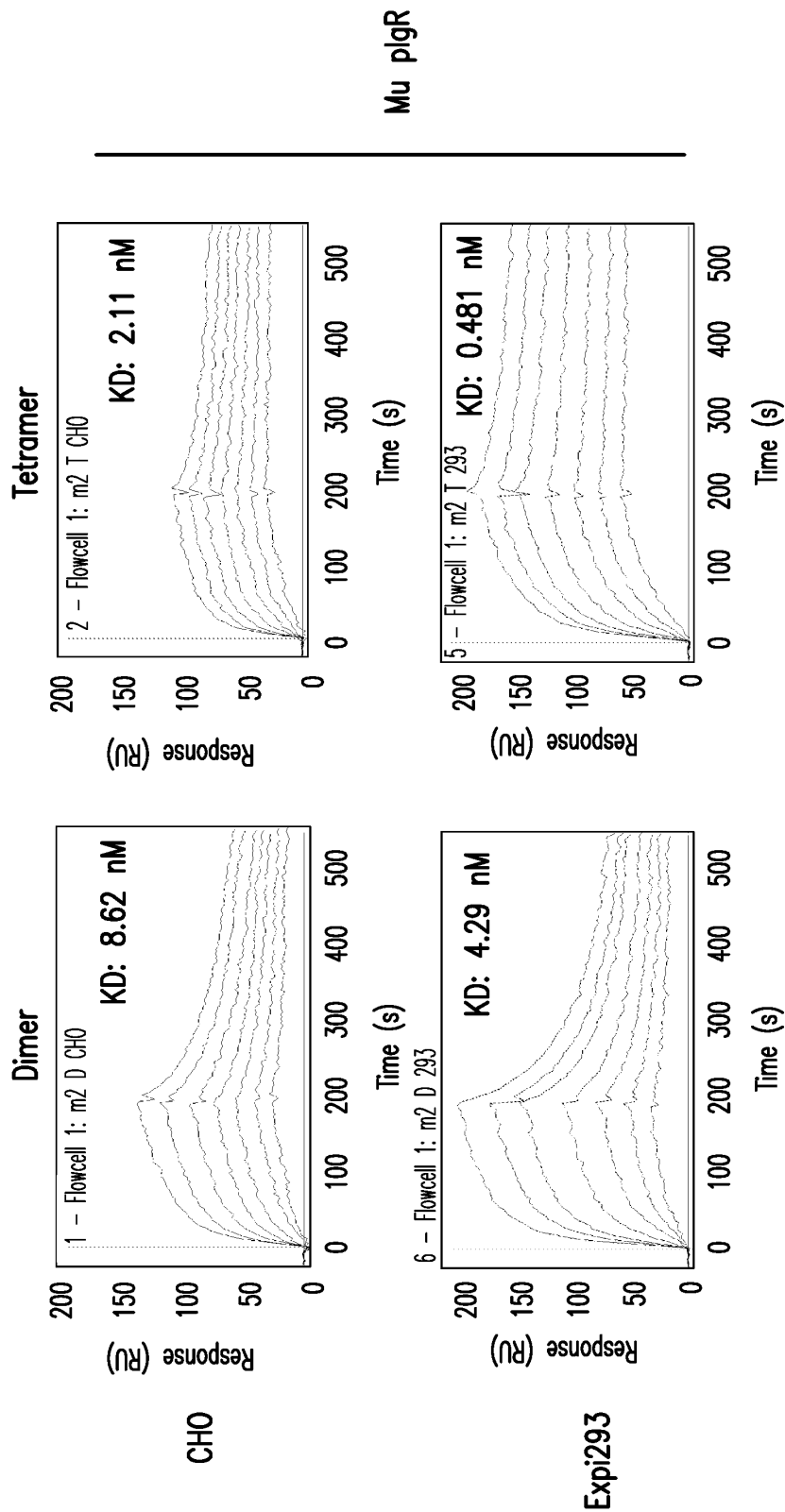


Figure 36A

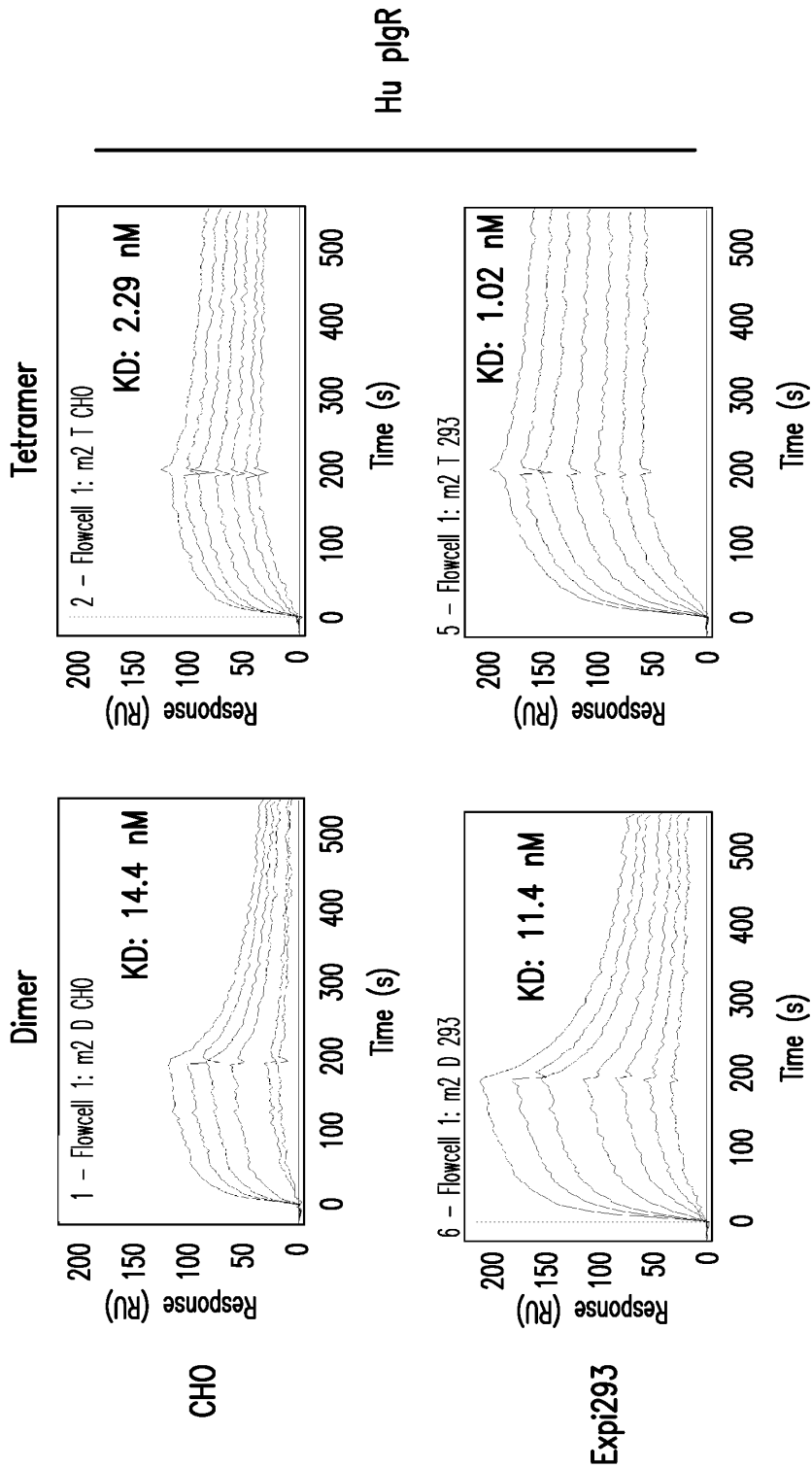
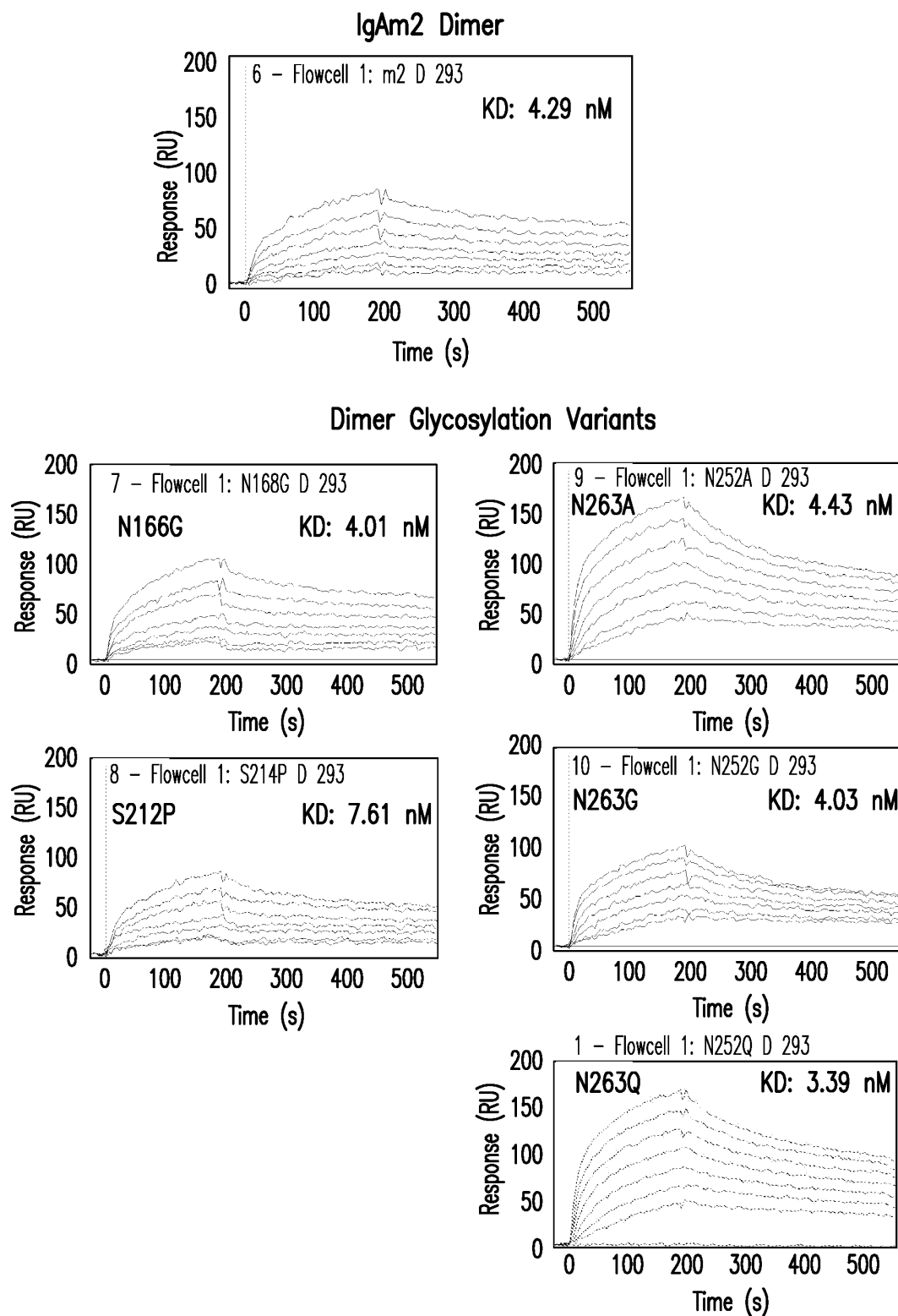
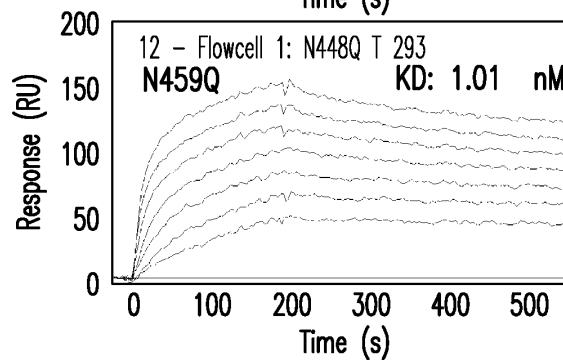
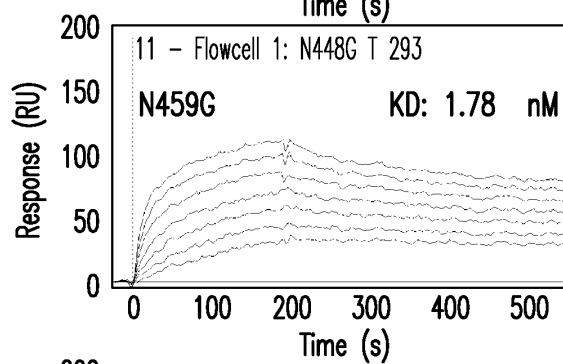
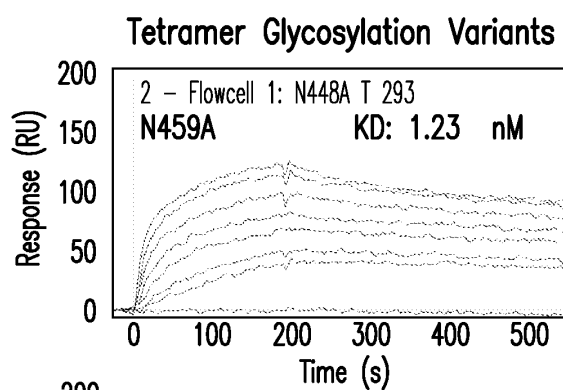
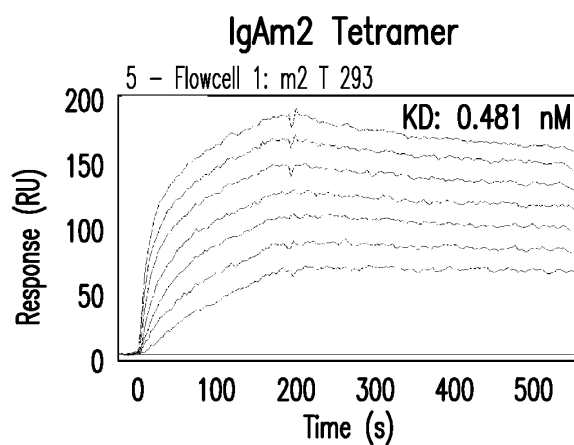
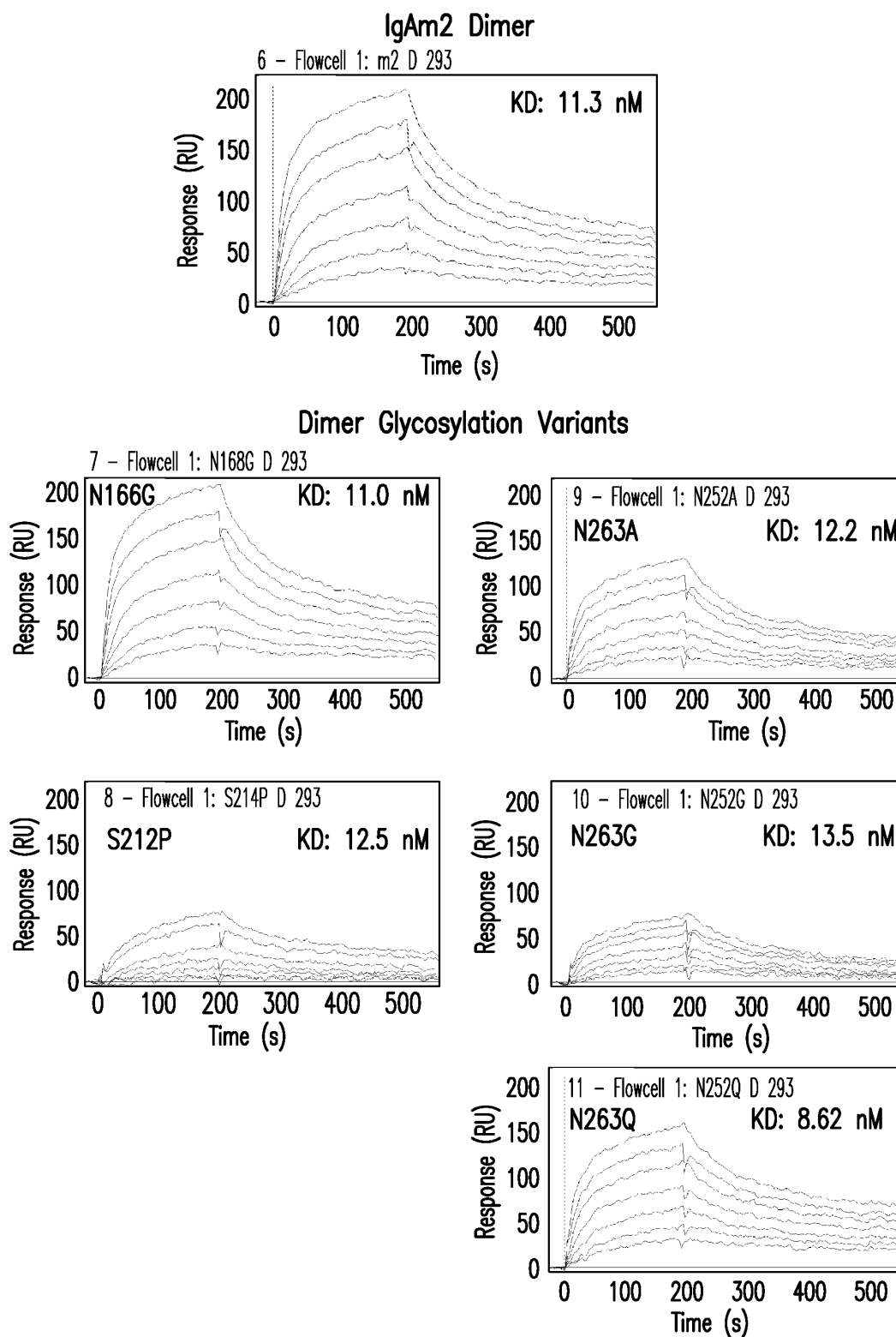


Figure 36A continued

**Figure 36B**

**Figure 36B continued**

**Figure 36C**

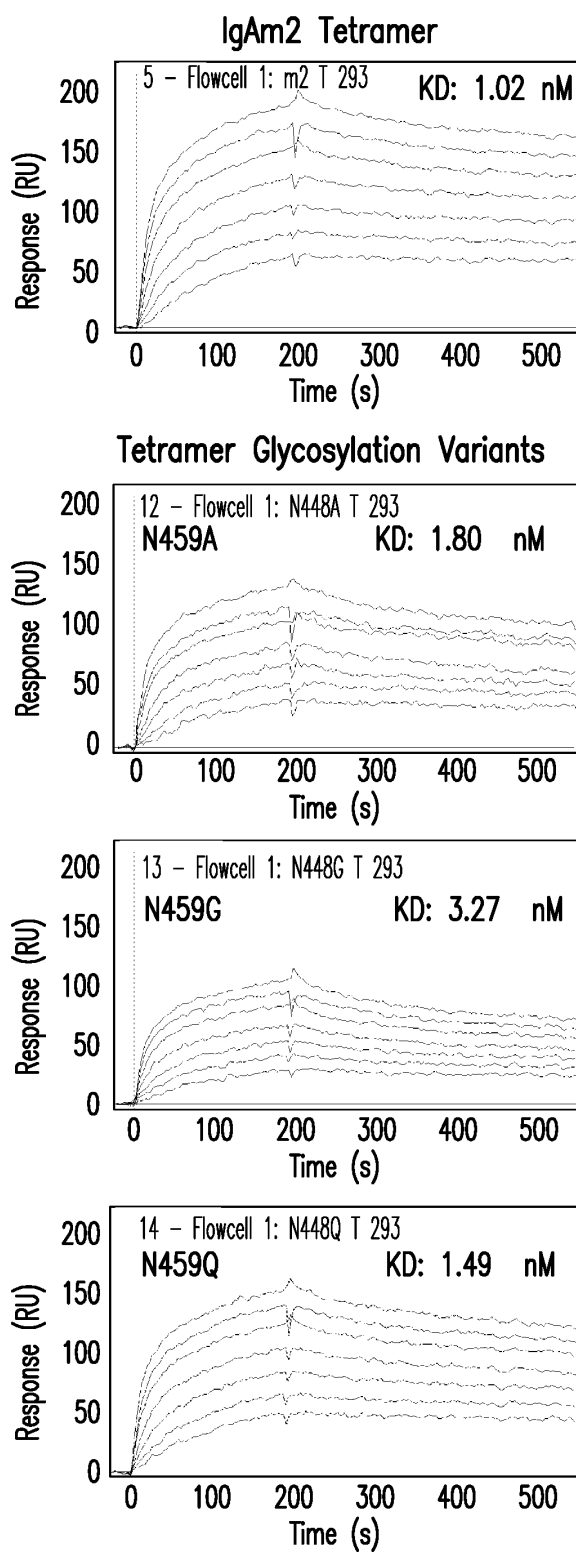


Figure 36C continued

Mu plgR Binding						Hu plgR Binding					
	ka	kd	Rmax	KD	Res sd		ka	kd	Rmax	KD	Res sd
m2 D CHO	2.954(9)e5	0.002546(7)	121.4(1)	8.62(4)nM	12.038	m2 D CHO	3.02(1)e5	0.004362(9)	114.6(1)	14.44(5)nM	9.875
m2 T CHO	4.098(9)e5	8.63(4)e-4	87.57(7)	2.11(1)nM	7.555	m2 T CHO	4.080(9)e5	9.33(4)e-4	97.71(8)	2.29(1)nM	9.039
m2 M CHO	41930.5	3.89E-214	1.658	0	2.672	m2 M CHO	2.32E+05	6.79E-261	2.275	0(0.000004)	3.533
hlgG1 M CHO	3.44E+06	8.75E-101	0.3661	0	2.53	hlgG1 M CHO	0(9e19)	0(1e1)	0(2e-2)	0(0.000001)	4.101
m2 T 293	4.93(1)e5	2.37(4)e-4	159.2(1)	481(9)pM	17.41	m2 T 293	3.966(9)e5	4.05(4)e-4	164.8(1)	1.02(1)nM	16.861
m2 D 293	3.586(9)e5	0.001538(5)	216.9(2)	4.29(2)nM	19.164	m2 D 293	3.040(7)e5	0.003474(6)	176.9(2)	11.43(3)nM	12.819
N168G D 293	3.742(8)e5	0.001501(4)	224.3(2)	4.01(1)nM	17.433	N168G D 293	3.051(7)e5	0.003359(6)	186.6(2)	11.01(3)nM	12.742
S214P D 293	1.678(6)e5	0.001276(8)	56.9(1)	7.61(6)nM	7.394	S214P D 293	1.225(5)e5	0.001532(8)	54.1(1)	12.51(8)nM	6.451
N252A D 293	3.65(1)e5	0.001616(6)	135.2(2)	4.43(2)nM	15.224	N252A D 293	2.937(9)e5	0.003572(8)	116.9(1)	12.16(5)nM	10.235
N252G D 293	3.95(1)e5	0.001591(8)	70.2(1)	4.03(2)nM	9.817	N252G D 293	2.618(9)e5	0.00352(9)	60.3(8)	13.45(6)nM	5.794
N252Q D 293	3.863(9)e5	0.001309(5)	145.4(1)	3.39(1)nM	12.589	N252Q D 293	3.177(7)e5	0.002740(5)	123.0(1)	8.62(3)nM	9.17
N448A T 293	4.489(9)e5	5.51(4)e-4	104.66(8)	1.227(9)nM	9.246	N448A T 293	3.833(8)e5	6.91(4)e-4	107.18(9)	1.80(1)nM	10.072
N448G T 293	3.90(1)e5	6.93(6)e-4	115.3(1)	1.78(2)nM	14.346	N448G T 293	3.39(1)e5	0.001108(6)	111.8(1)	3.27(2)nM	13.003
N448Q T 293	4.246(9)e5	4.27(4)e-4	135.4(1)	1.01(0)nM	12.7	N448Q T 293	4.011(8)e5	5.97(4)e-4	139.1(1)	1.49(1)nM	12.884
JN71Q T 293	4.72(1)e5	2.87(5)e-4	146.1(1)	610(10)pM	17.218	JN71Q T 293	4.12(1)e5	3.47(5)e-4	152.5(1)	840(10)pM	17.423

072216 IgA Mu plgR
Included 49-96, 3, 8, 20, 32
Excluded 51, 56, 68, 80, Conc. 1 (500nM), Blank2,

072216 IgA Hu plgR
Include 49-96, 8, 20, 32
Exclude 51, 56, 68, 80, conc. 1 (500nM)

Figure 36D

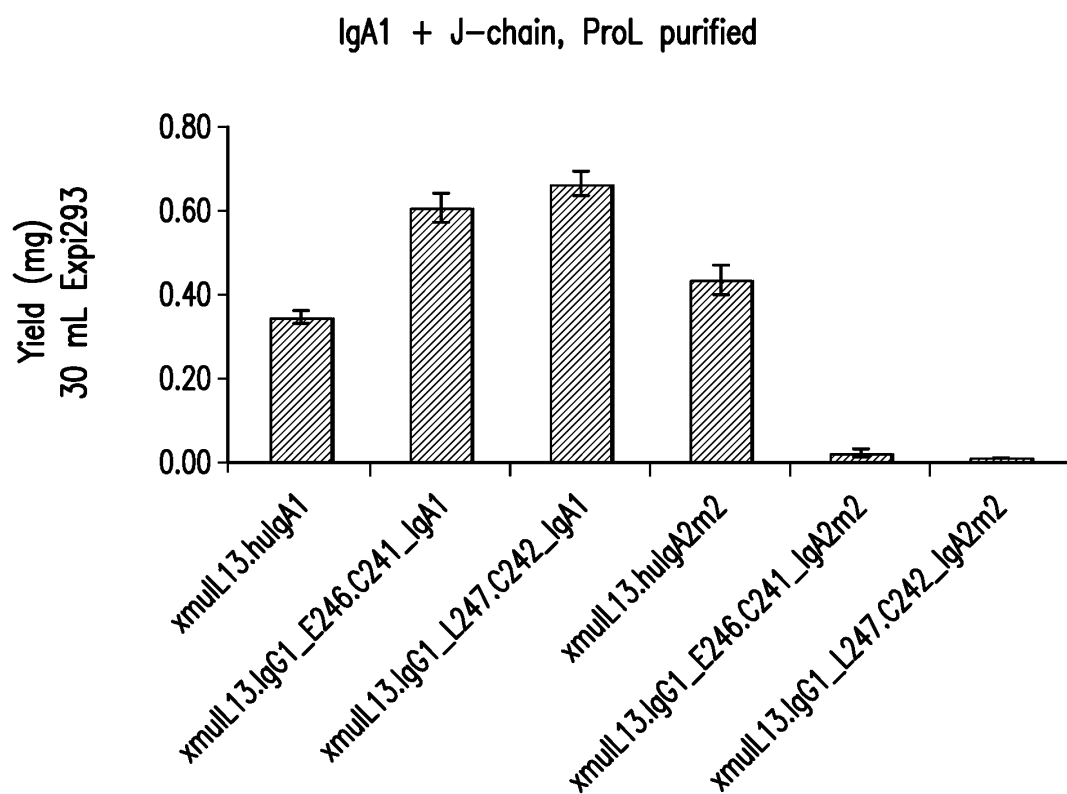


Figure 37A

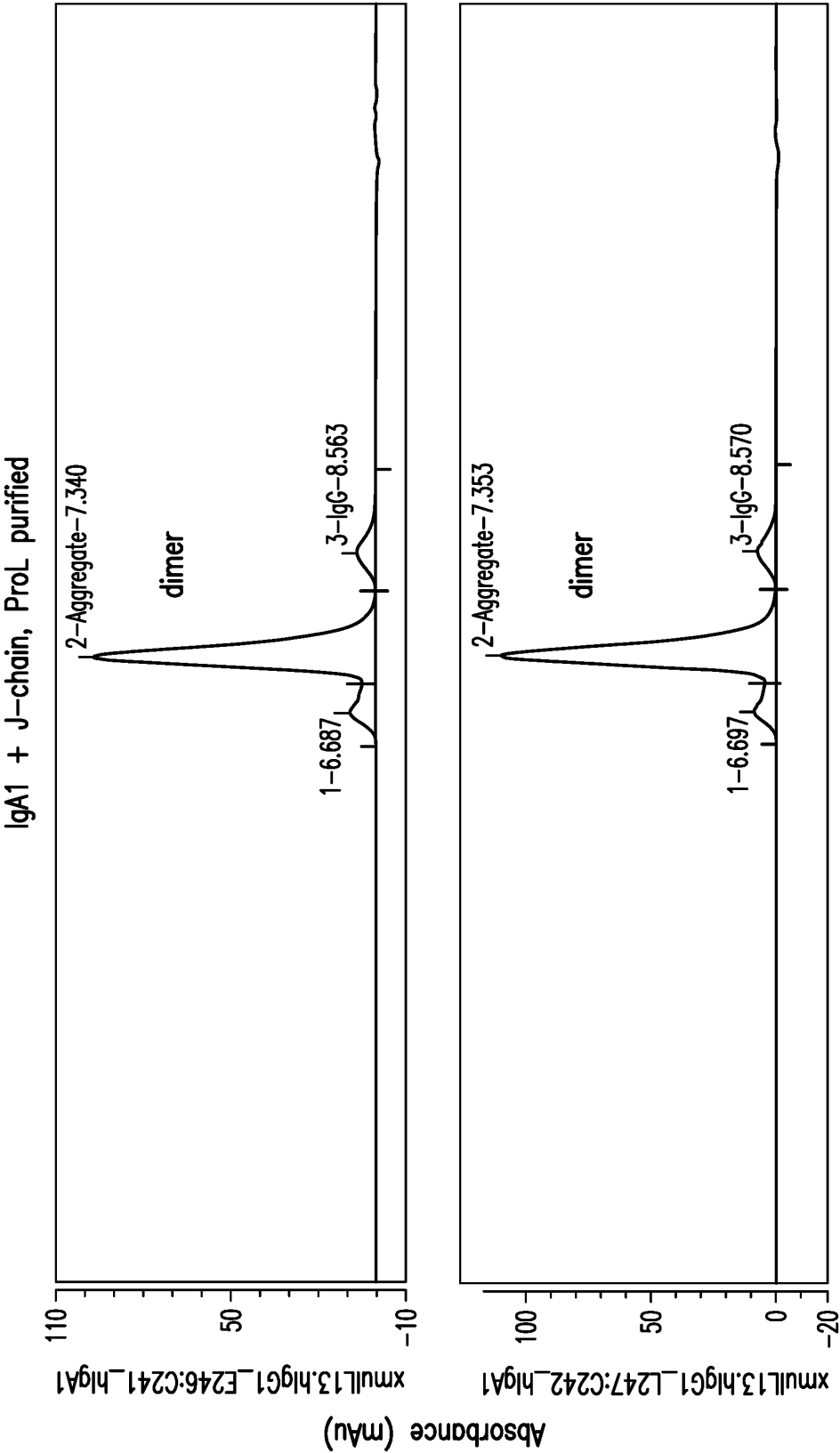


Figure 37B

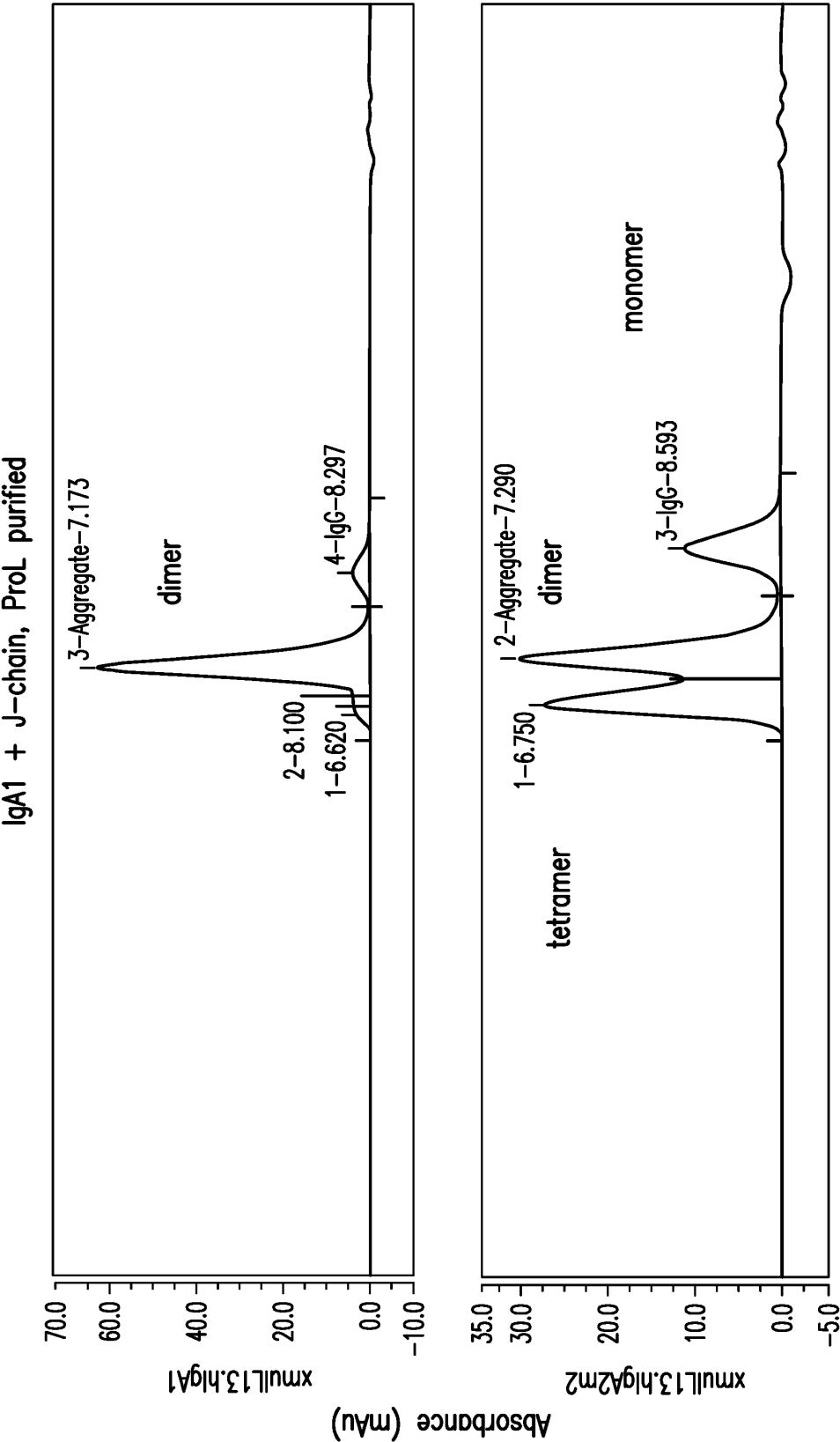


Figure 37B continued

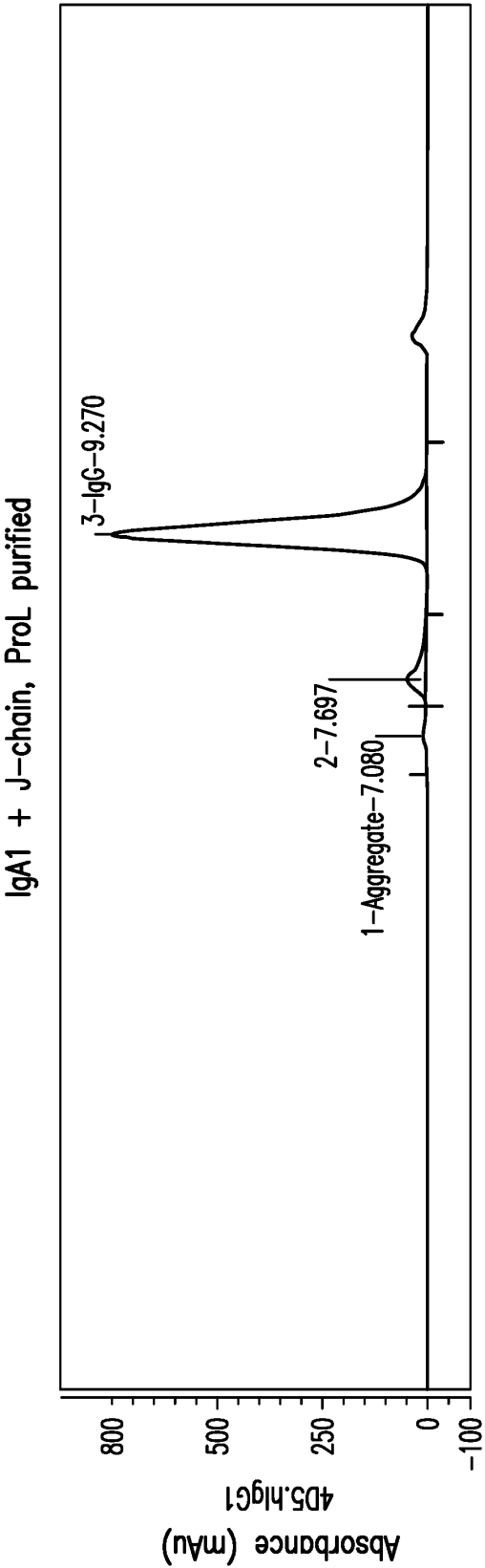


Figure 37B continued

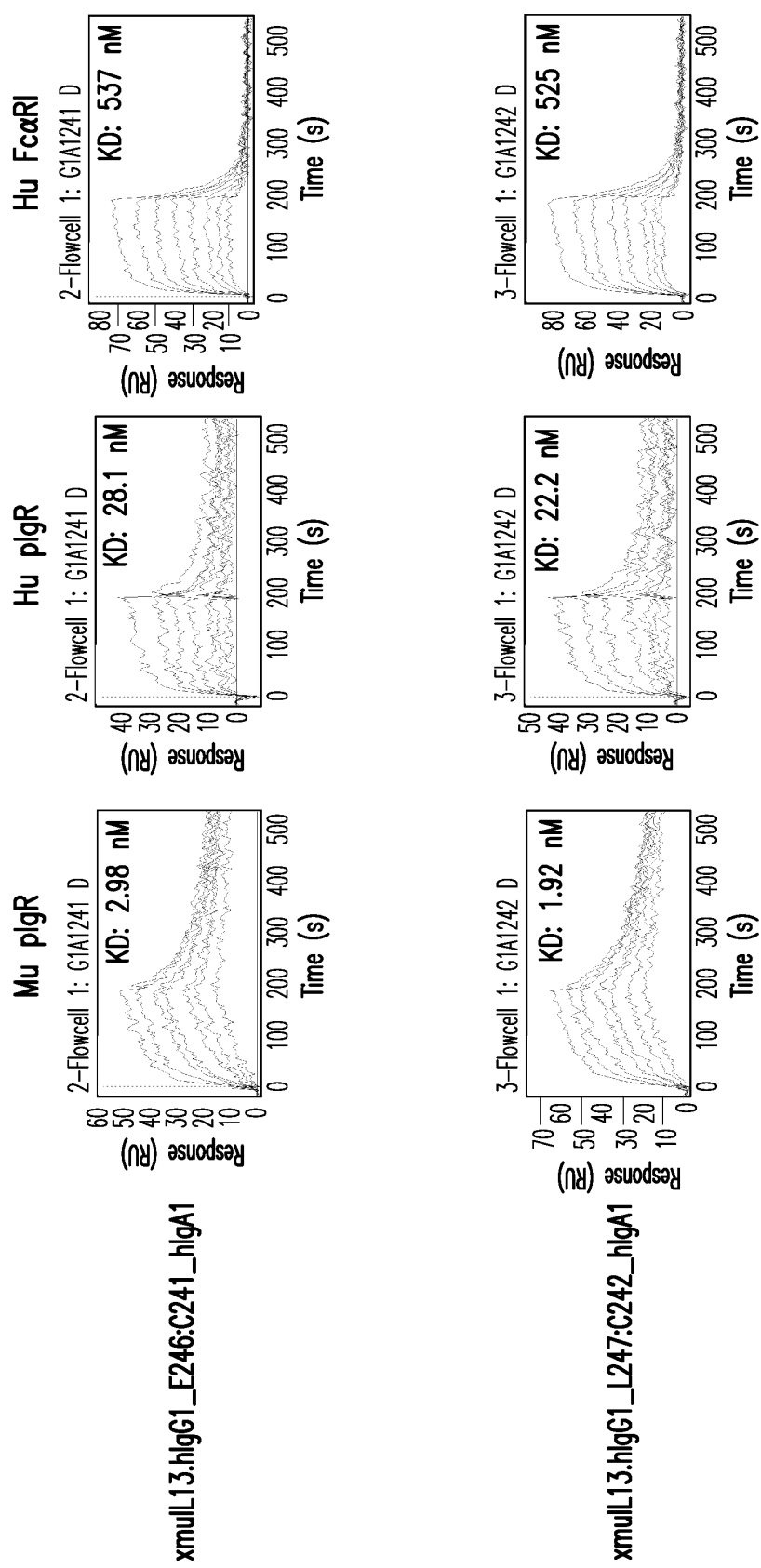


Figure 37C

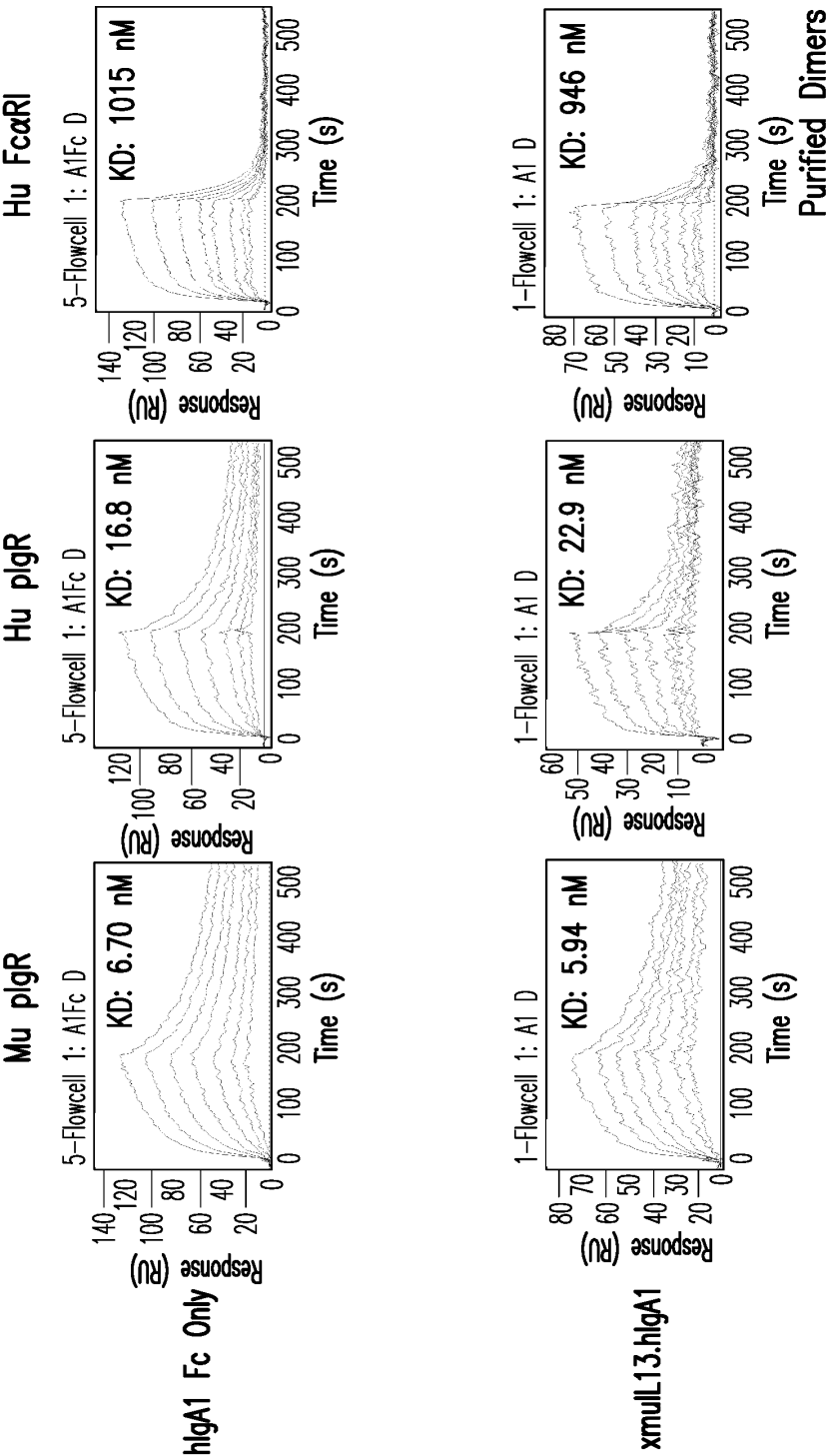
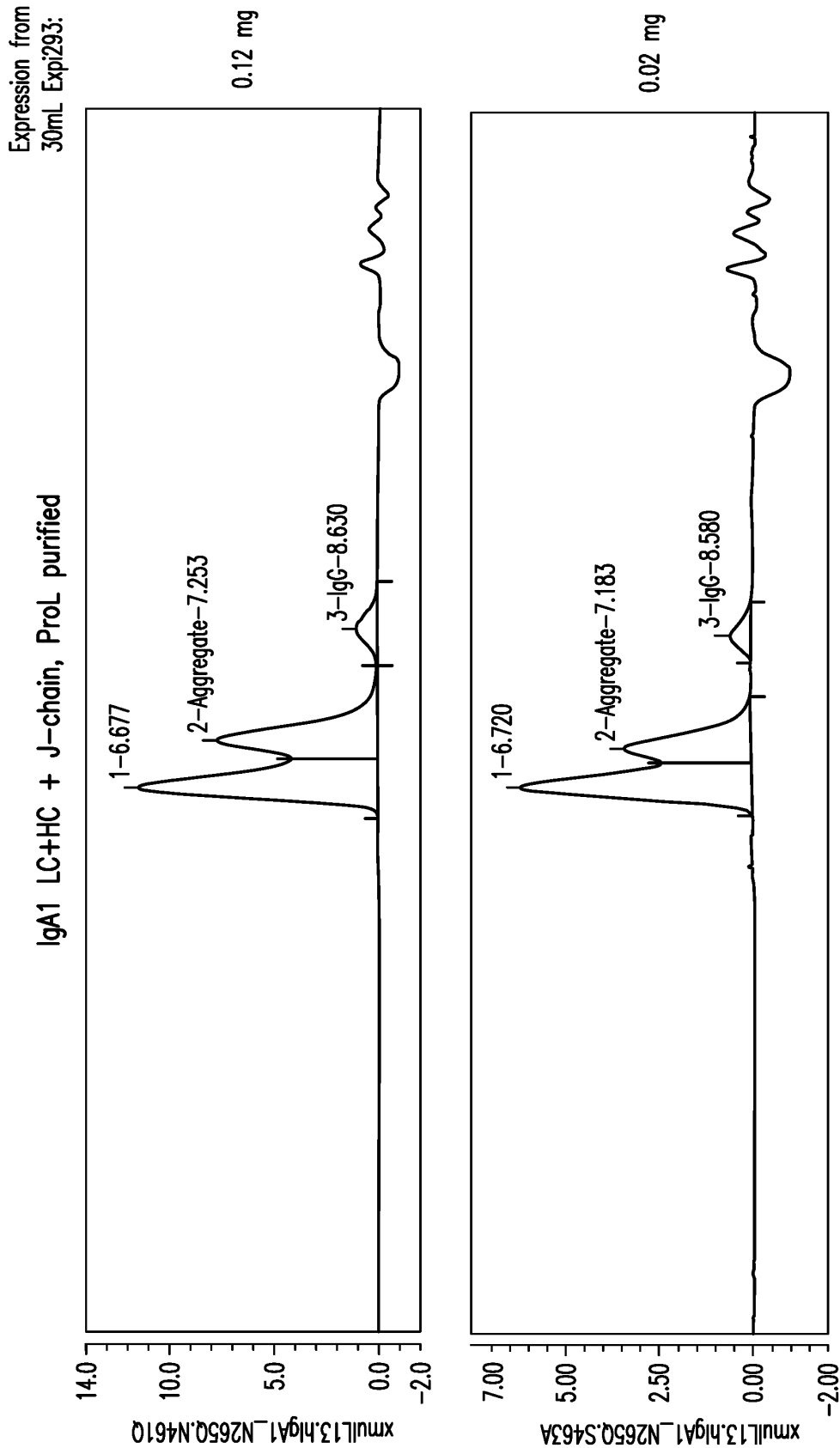
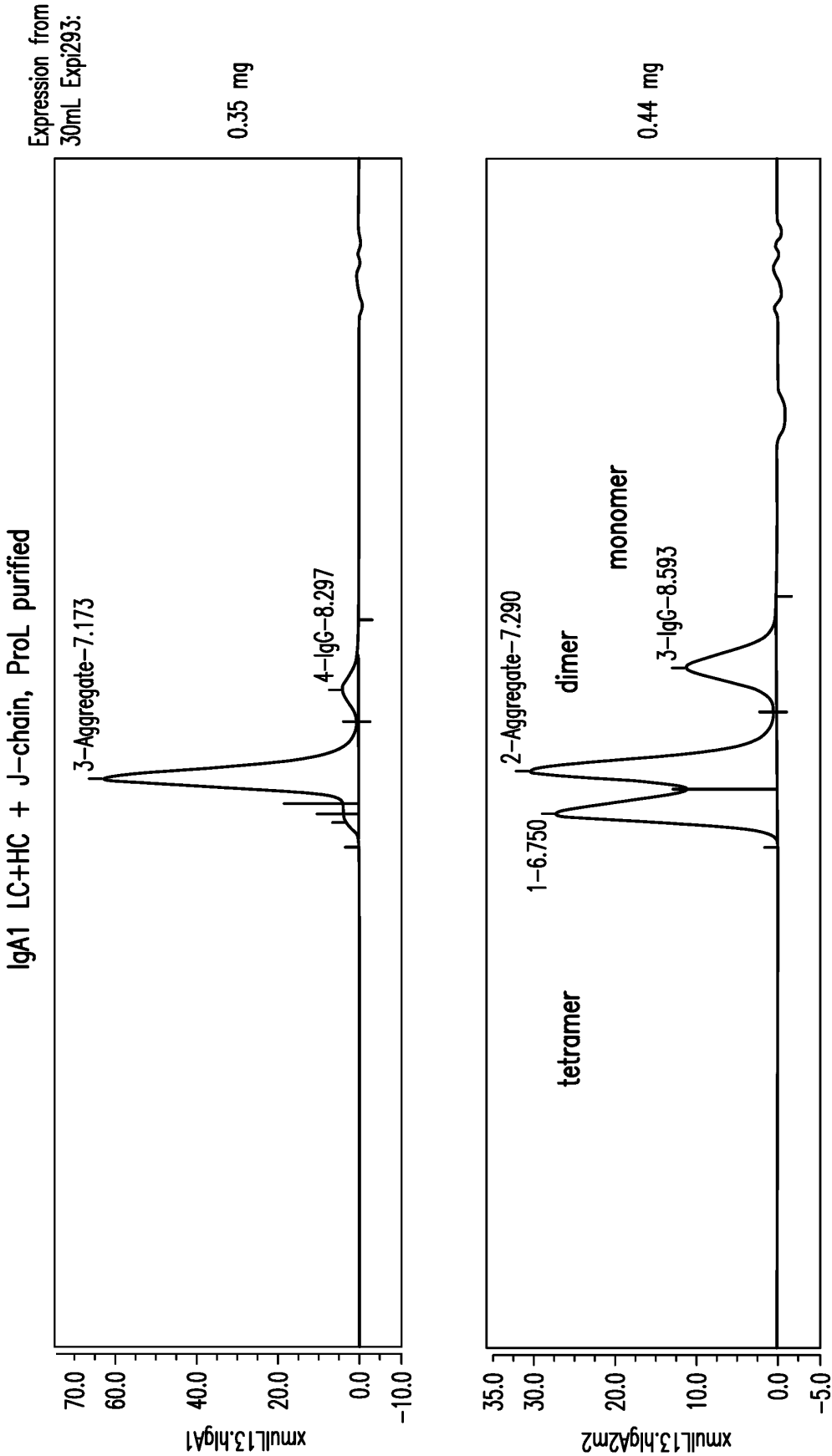


Figure 37C continued



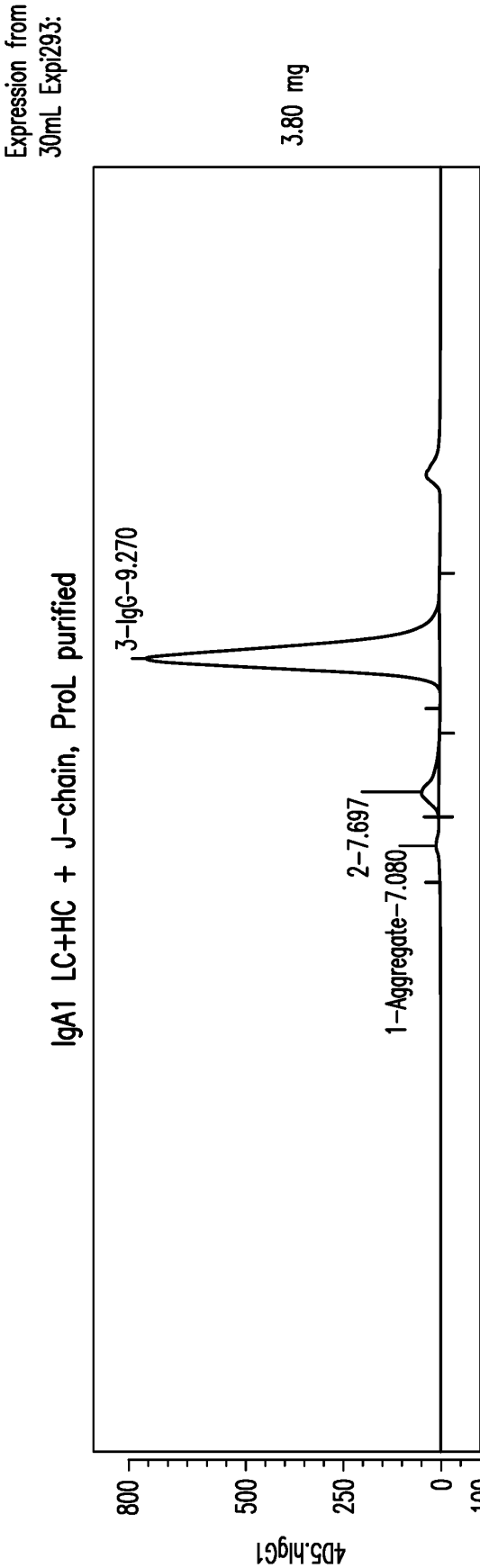
AP00043342

Figure 38



AP00043342

Figure 38 continued



AP00043342

Figure 38 continued

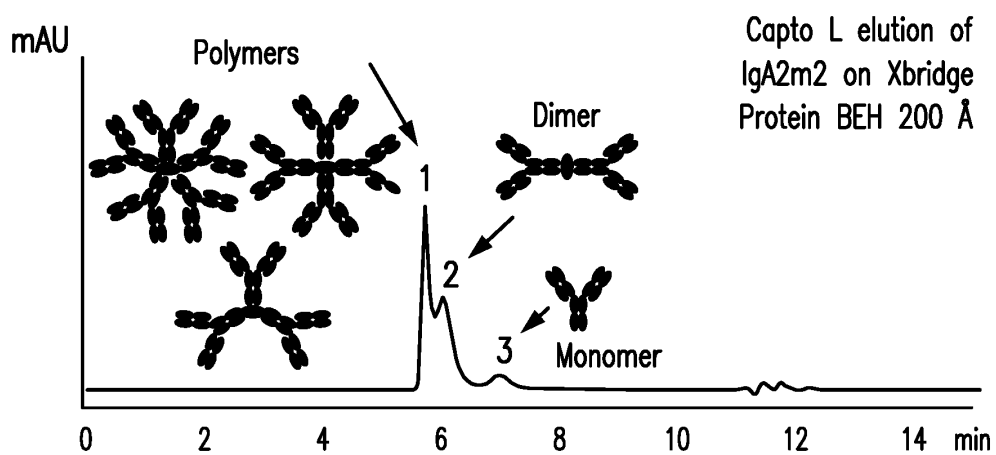


Figure 39

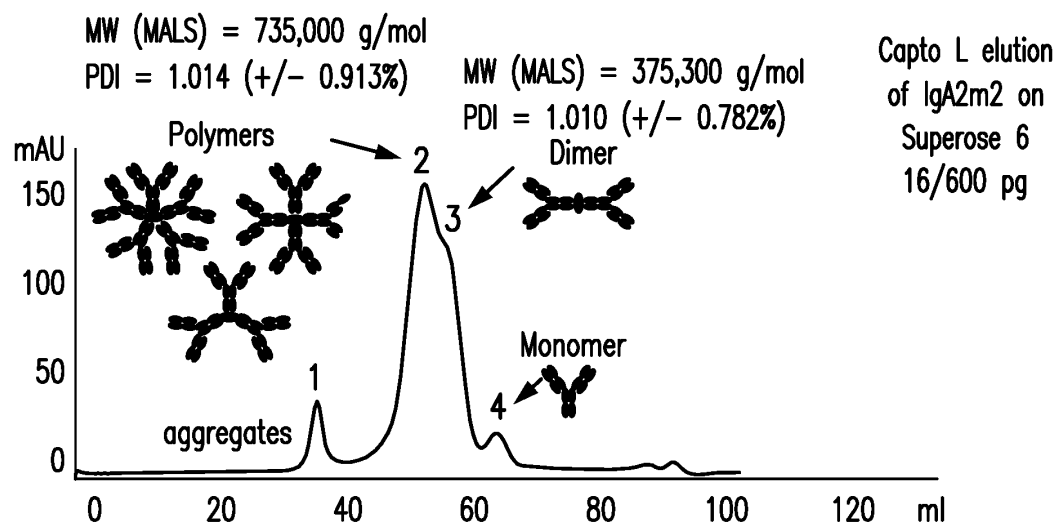


Figure 40

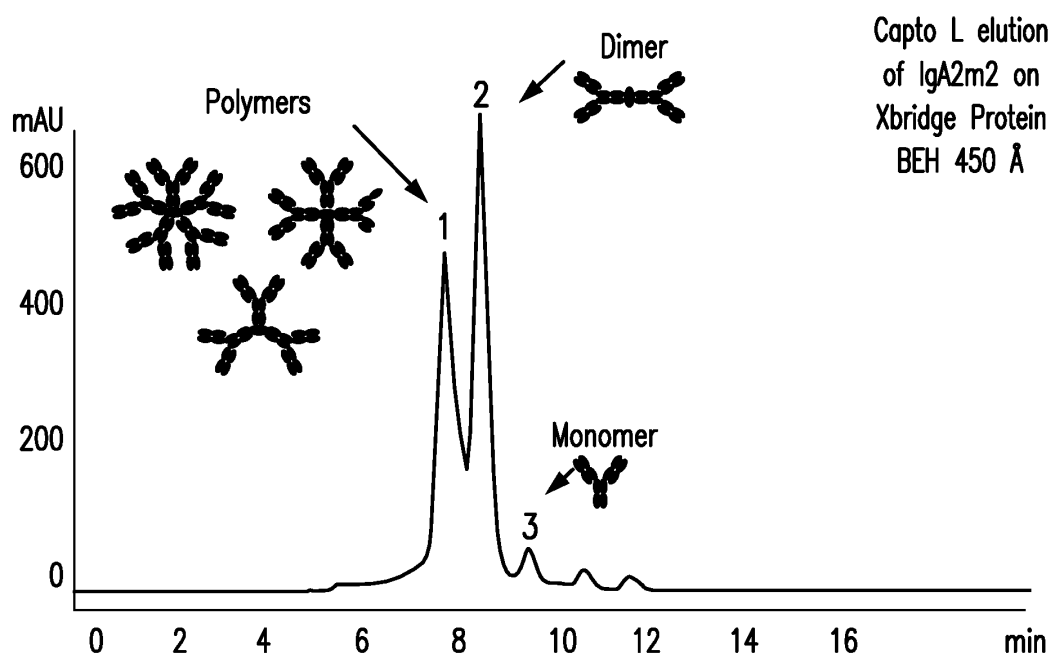


Figure 41

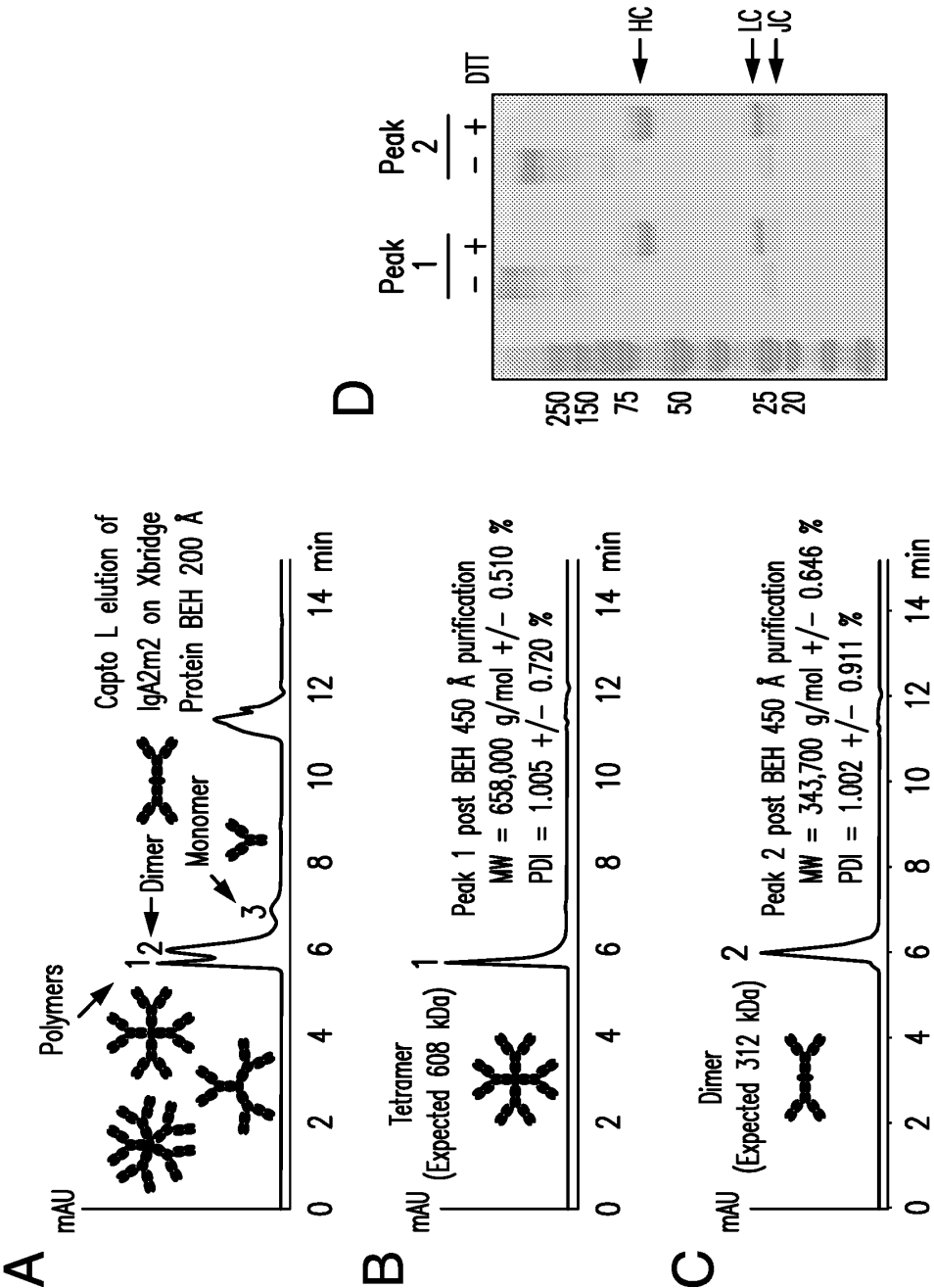


Figure 42A-42D

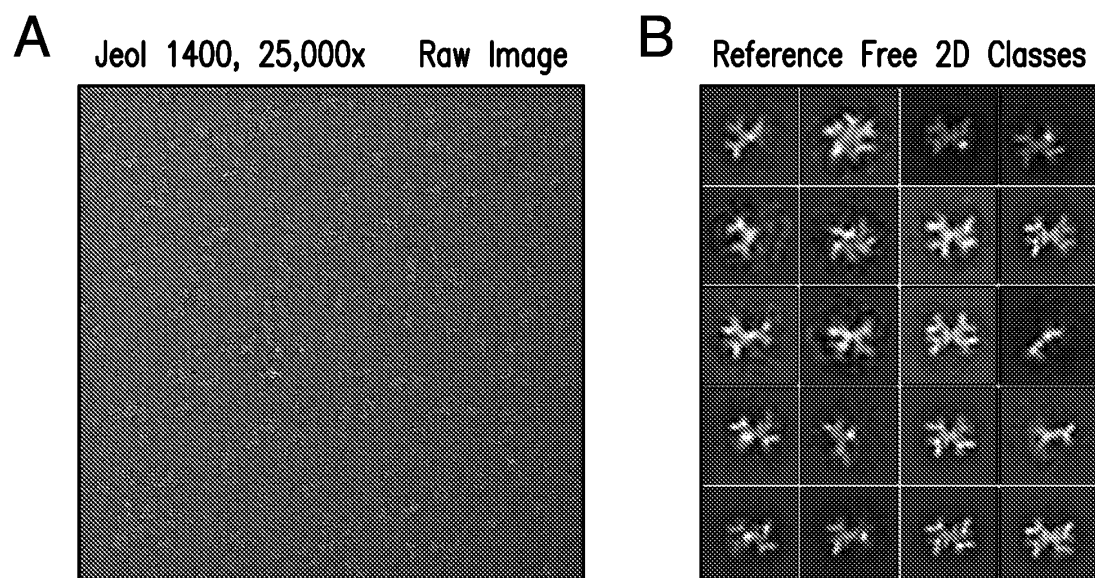


Figure 43A-43B

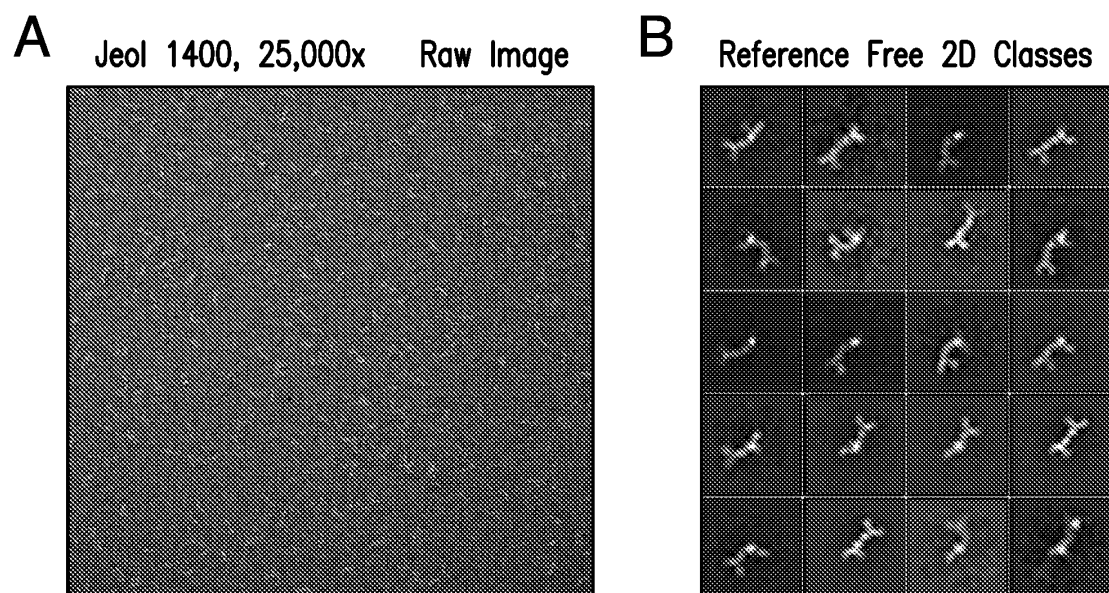


Figure 44A-44B

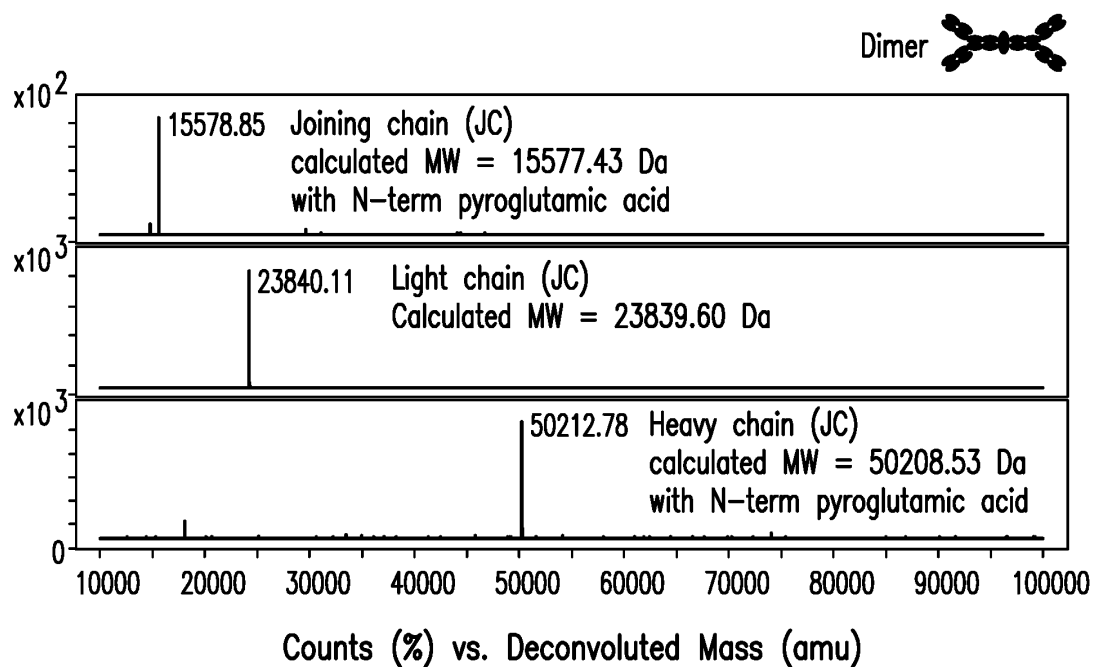


Figure 45

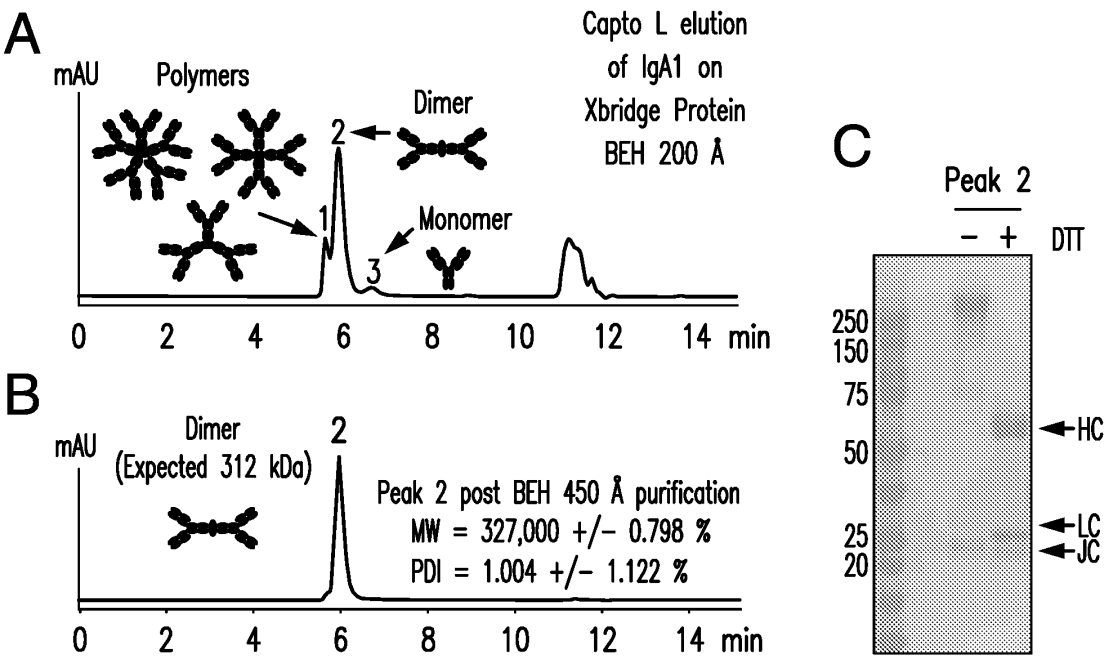


Figure 46A-46C

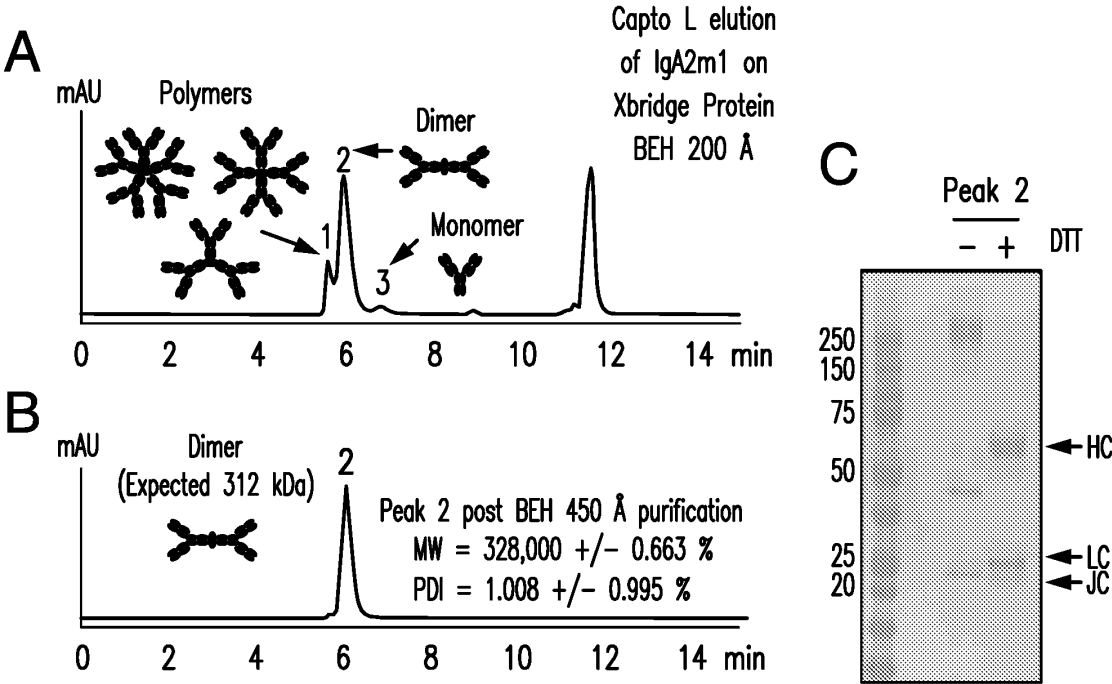


Figure 47A-47C

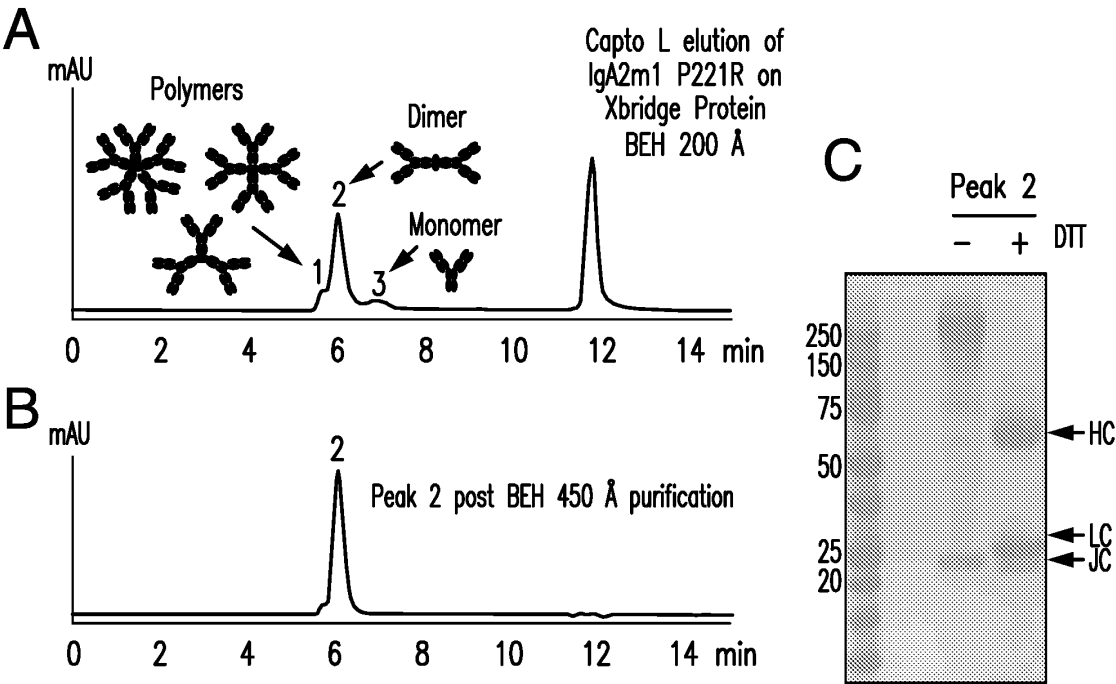
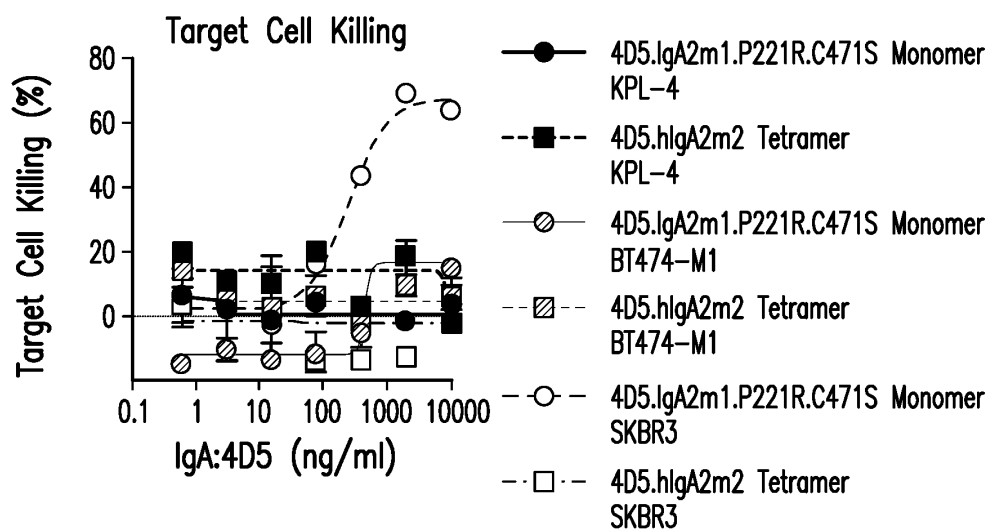
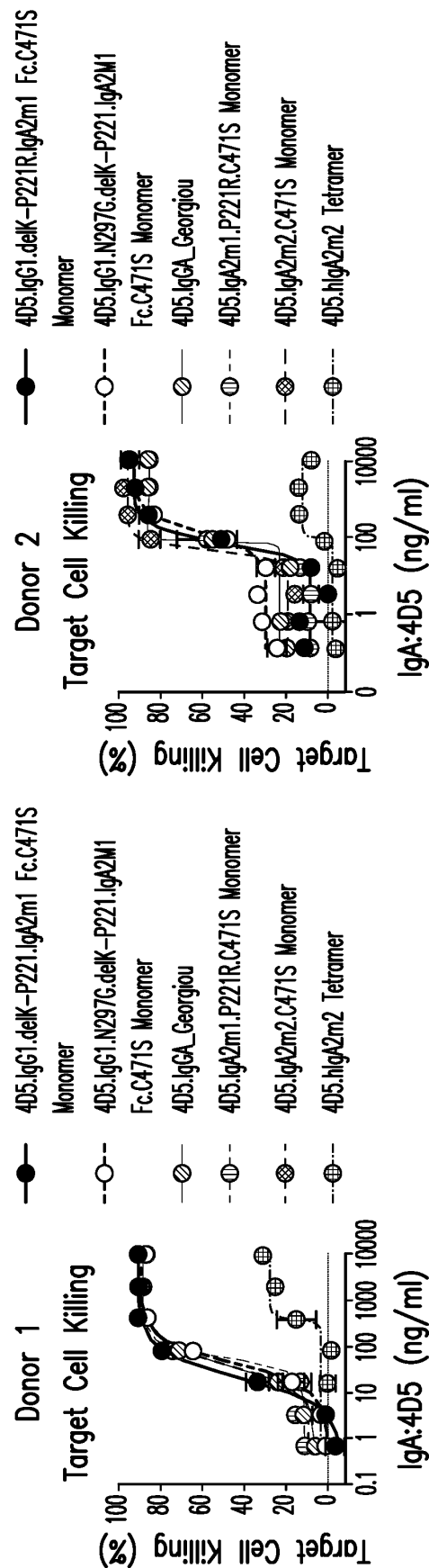


Figure 48A-48C



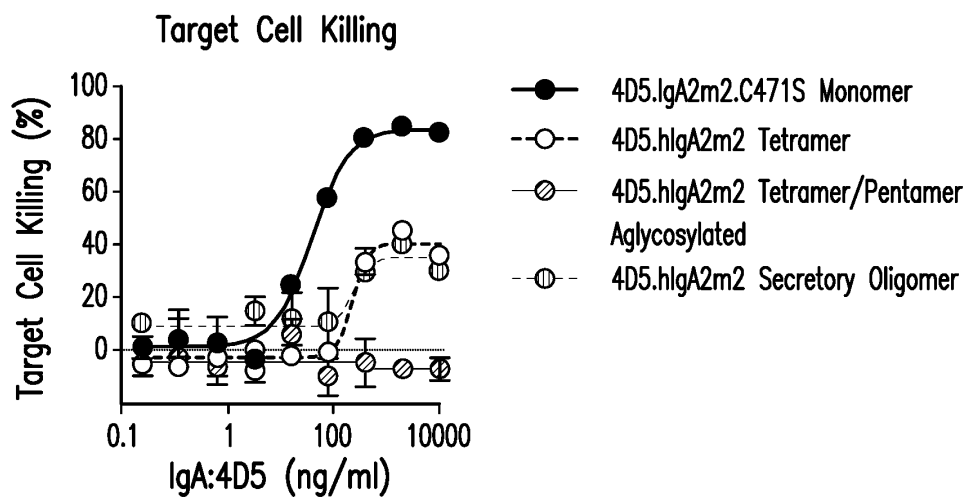
- Effector cells: human neutrophils from blood
- Target cells: KPL-4, BT474-M1 or SKBR3
- E:T ratio = 20:1
- Time: 48 hours
- Killing assay: Cell Titer Glo

Figure 49



- Effector cells: human neutrophils from blood
- Target cells: SKBR3
- E:1 ratio = 20:1
- Time: 48 hours
- Killing assay: Cell Titer Glo

Figure 50



- Effector cells: human neutrophils from blood
- Target cells: SKBR3
- E:T ratio = 20:1
- Time: 48 hours
- Killing assay: Cell Titer Glo

Figure 51

Anti-mouse IL-13 hlgA2m2 Oligomer

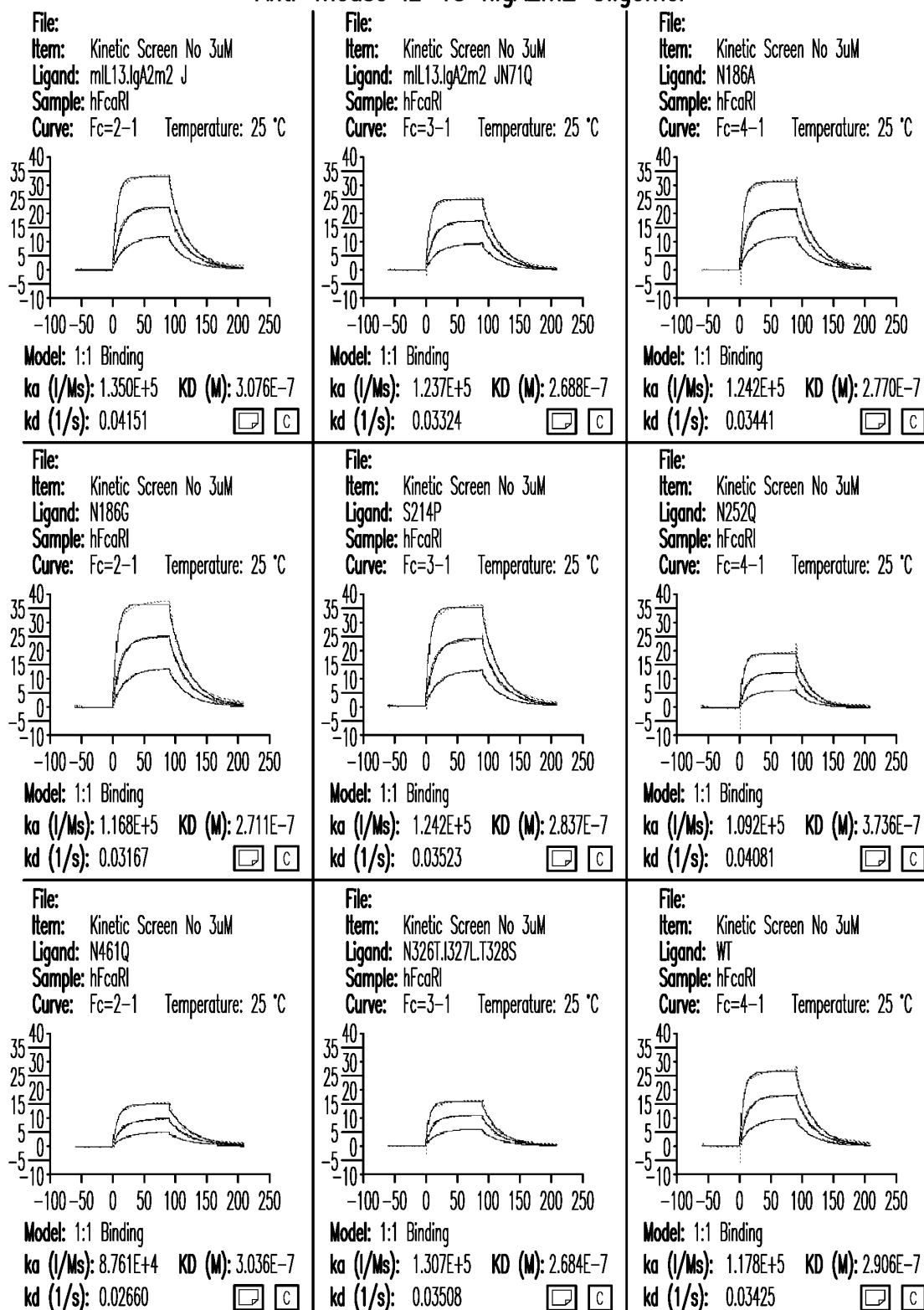
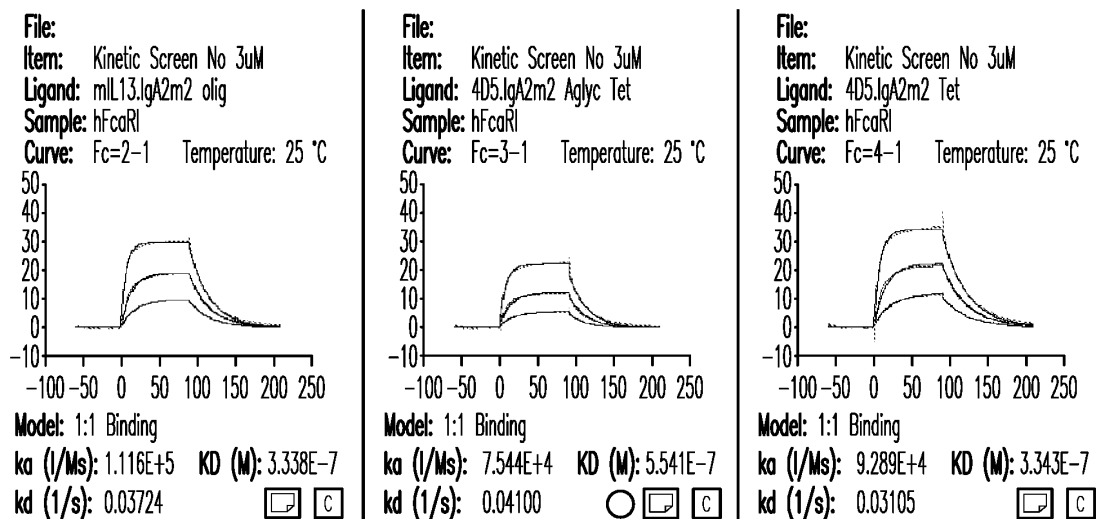


Figure 52A

Anti-HER2.4D5 hlgA2m2 Tetramer



25C, T200, anti-Fab capture IgA Abs
hFcaRI conc. series: 1000, 333, 111 nM
120319, 120419

Figure 52A continued

Sample	k_a (1/Ms)	k_d (1/s)	KD (M)	Rmax(RU)	χ^2 (RU ²)	Ligand	Ligand Level (RU)	Mode	Curve
hFcαRI	1.35E+05	4.15E-02	3.08E-07	40.9	0.249	xmlL13.IgA2m2 Oligomer WT	364.9	1:1 Binding	Fc=2-1
hFcαRI	1.24E+05	3.32E-02	2.69E-07	29.3	0.599	WT, J-N71Q	267.9	1:1 Binding	Fc=3-1
hFcαRI	1.24E+05	3.44E-02	2.77E-07	37.9	0.878	N186A, J-N71Q	336.5	1:1 Binding	Fc=4-1
hFcαRI	1.17E+05	3.17E-02	2.71E-07	43.2	0.382	N186G, J-N71Q	388.4	1:1 Binding	Fc=2-1
hFcαRI	1.24E+05	3.52E-02	2.84E-07	41.6	0.781	S214P, J-N71Q	400.3	1:1 Binding	Fc=3-1
hFcαRI	1.09E+05	4.08E-02	3.74E-07	25.2	0.838	N252Q, J-N71Q	295.4	1:1 Binding	Fc=4-1
hFcαRI	1.31E+05	3.51E-02	2.68E-07	18.2	0.515	N326T.I327L.T328S, J-N71Q	155	1:1 Binding	Fc=3-1
hFcαRI	8.76E+04	2.66E-02	3.04E-07	17.1	0.128	N461Q, J-N71Q	255.2	1:1 Binding	Fc=2-1
hFcαRI	9.29E+04	3.11E-02	3.34E-07	43.4	0.823	xHER2 4D5.IgA2m2 Tetramer WT	762.7	1:1 Binding	Fc=4-1
hFcαRI	7.54E+04	4.18E-02	5.54E-07	29.3	0.429	xHER2 4D5.IgA2m2 Tetramer N168A.S214P.N252Q. N326T.I327L.T328S.N461Q, J-N71Q	1085.7	1:1 Binding	Fc=3-1

Figure 52B