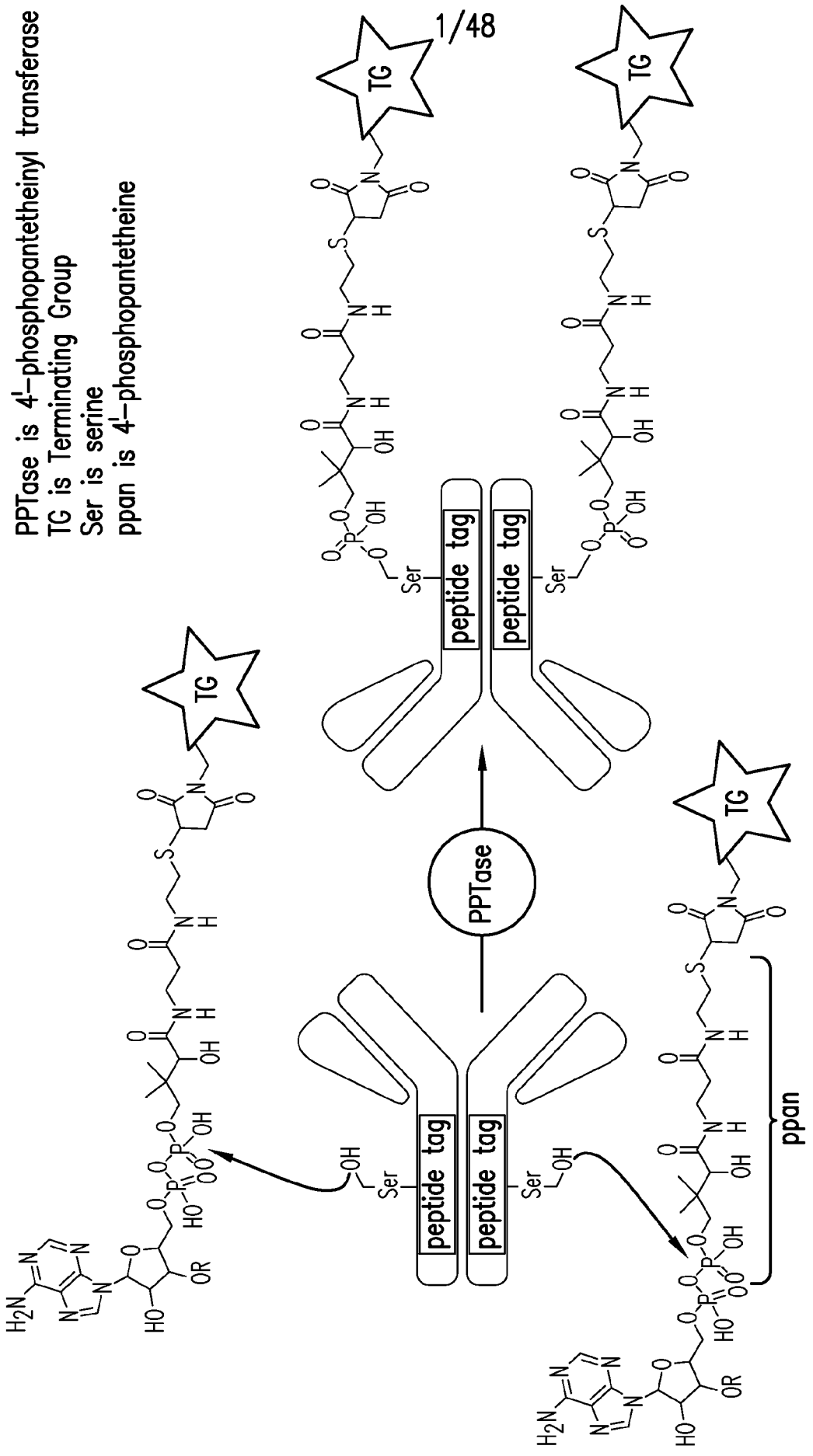
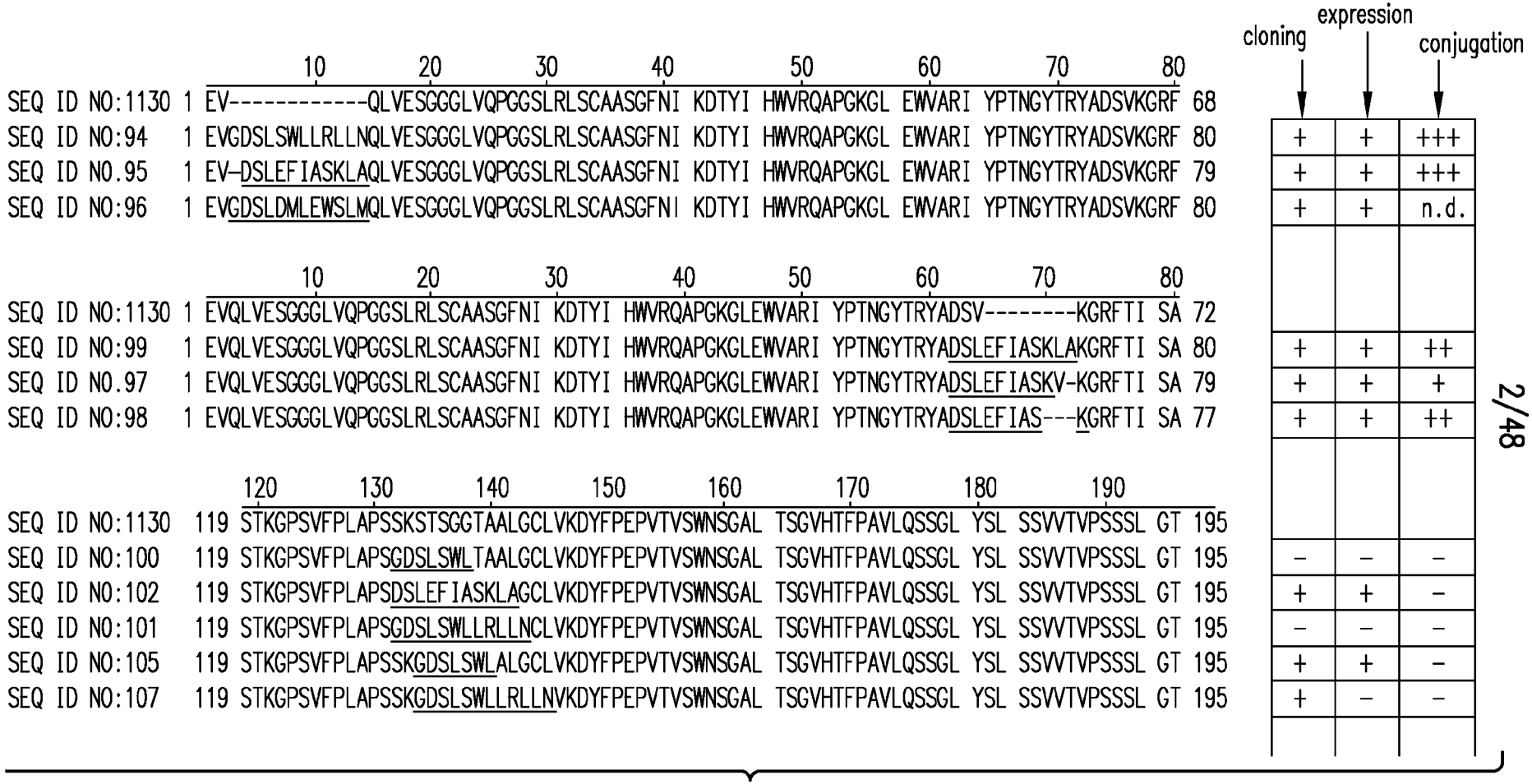

PPTase is 4'–phosphopantetheinyl transferase
TG is Terminating Group
Ser is serine
ppan is 4'–phosphopantetheine
TG
peptide tag
Ser
PPTase
ppan

FIG. 1



cont'd on Fig.2A-1

FIG.2A

cont'd from Fig.2A

		120	130	140	150	160	170	180	190				
SEQ ID NO:1130	119	STKGPSVFPLAPSSKSTSGG--------TAALGCL	VKDYFPEPVTVSWNSGAL	TSGVHTFPAVLQSSGL	YSLSSVVT	187							
SEQ ID NO:108	119	STKGPSVFPLAPSSKSGDSLSWLLRLLN-AALGCL	VKDYFPEPVTVSWNSGAL	TSGVHTFPAVLQSSGL	YSLSSVVT	194				–	–	–	
SEQ ID NO:103	119	STKGPSVFPLAPSSGDSLSWLLRLLN--TAALGCL	VKDYFPEPVTVSWNSGAL	TSGVHTFPAVLQSSGL	YSLSSVVT	193				+	+	+	
SEQ ID NO:106	119	STKGPSVFPLAPSSKGDSLSWLLRLLN---ALGCL	VKDYFPEPVTVSWNSGAL	TSGVHTFPAVLQSSGL	YSLSSVVT	192				+	+	–	

		170	180	190	200	210	220	230				
SEQ ID NO:1130	161	GAL TSGVHTFPAVLQSSGL	YSL SSVVTVPSSS---------LGTQTYI	CNVNHKPSNTKVDKKVEPKSCDKTHTCPP	228							
SEQ ID NO:118	161	GAL TSGVHTFPAVLQSSGL	YSL SSVVTVPSSSGDSLSWLLRLLN----	CNVNHKPSNTKVDKKVEPKSCDKTHTCPP	233				–	–	–	
SEQ ID NO:110	161	GAL TSGVHTFPAVLQSSGL	YSL SSVVTVGDSLSWLLRLLN----QTYI	CNVNHKPSNTKVDKKVEPKSCDKTHTCPP	233				+	+	–	
SEQ ID NO:113	161	GAL TSGVHTFPAVLQSSGL	YSL SSVVTVPGDSLSWLLRLLN-----YI	CNVNHKPSNTKVDKKVEPKSCDKTHTCPP	232				+	+	++	

		170	180	190	200	210	220	230				
SEQ ID NO:1130	161	GAL TSGVHTFPAVLQSSGL	YSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GG						237				
SEQ ID NO:119	161	GAL TSGVHTFPAVLQSSGL	YSL SSVVTVPSSSGDSLSWLLRLLNKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GG						237	+	–	–	
SEQ ID NO:109	161	GAL TSGVHTFPAVLQSSGL	YSL SSVVTVGDSLSWLQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GG						237	+	+	+	
SEQ ID NO:112	161	GAL TSGVHTFPAVLQSSGL	YSL SSVVTVDSLEFIASKLACNVNHKPSNTKVPKKVEPKSCPKTHTCPPCPAPELL GG						237	+	+	–	
SEQ ID NO:111	161	GAL TSGVHTFPAVLQSSGL	YSL SSVVTVGDSLSWLLRLLNNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GG						237	+	–	–	
SEQ ID NO:114	161	GAL TSGVHTFPAVLQSSGL	YSL SSVVTVPDSLEFIASKLCNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GG						237	+	+	–	
SEQ ID NO:115	161	GAL TSGVHTFPAVLQSSGL	YSL SSVVTVPDSLEFIASKLANVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GG						237	+	–	–	
SEQ ID NO:116	161	GAL TSGVHTFPAVLQSSGL	YSL SSVVTVPSDSLEFIASKCNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GG						237	+	+	–	
SEQ ID NO:117	161	GAL TSGVHTFPAVLQSSGL	YSL SSVVTVPSSSGDSLSWLCNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GG						237	+	+	–	

cont'd on Fig.2A-2

FIG.2A-1

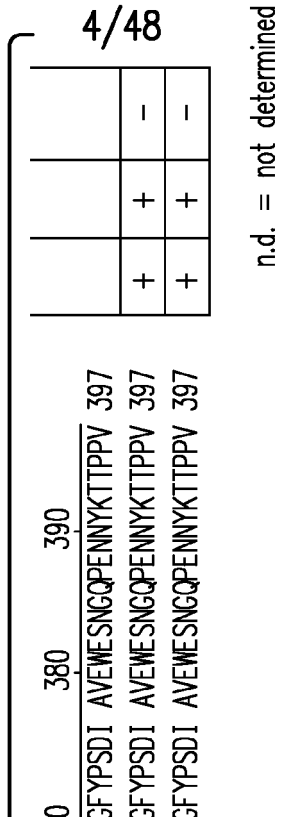

cont'd from Fig.2A-1
330 340 350 360 370 380 390
SEQ ID NO:1130 321 CKVSNKALPAPI EKTI SKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDI AVEWESNGQPENNYKTTPPV 397
SEQ ID NO:123 321 CKVSNKALPAPI EKTI SKAKGQPREPQVYTLPPSREEMGDSLSWL TCLVKGFYPSDI AVEWESNGQPENNYKTTPPV 397
SEQ ID NO:120 321 CKVSNKALPAPI EKTI SKAKGQPREPQVYTLPPSREGDSLSWLSL TCLVKGFYPSDI AVEWESNGQPENNYKTTPPV 397
+ + -
+ + -
n.d. = not determined

FIG.2A-2

```
                                330       340       350       360       370       380       390
SEQ ID NO:1130          321 CKVSNKALPAPI EKTI SKAKGQPREPQVYTLPPSREEMT----------KNQVSLTCLVKGFYPSDI AVEWESNG 385
SEQ ID NO:122           321 CKVSNKALPAPI EKTI SKAKGQPREPQVYTLPPSREEMT-DSLEFIASKLAKNQVSLTCLVKGFYPSDI AVEWESNG 396
SEQ ID NO:121           321 CKVSNKALPAPI EKTI SKAKGQPREPQVYTLPPSREEMTGDSLSWLLRLLNKNQVSLTCLVKGFYPSDI AVEWESNG 397

                                370       380       390       400       410       420       430       440
SEQ ID NO:1130          370 KGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSL SPG 446
SEQ ID NO:124           370 KGFYPSDI AVEWDSLSWLLNNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSL SPG 446
SEQ ID NO:125           370 KGFYPSDI AVEWDSLEFIANNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSL SPG 446
SEQ ID NO:135           370 KGFYPSDI AVEWESNGQPENNYKTTPPVDSLEFIASKLAKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSL SPG 446
SEQ ID NO:137           370 KGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDSLSWLLRLLFSCSVMHEAL HNHYTQKSLSL SPG 446
SEQ ID NO:138           370 KGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDSLEFIASKLFSCSVMHEAL HNHYTQKSLSL SPG 446

                                370       380       390       400       410       420       430       440
SEQ ID NO:1130          370 KGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSF-----FLYSKL TVDKSRWQQGNVFSCSVMHEAL HNHYTQKSL 441
SEQ ID NO:134           370 KGFYPSDI AVEWESNGQPENNYKTTPPVGDSLSWLLRLLNFLYSKL TVDKSRWQQGNVFSCSVMHEAL HNHYTQKSL 446
```

+	+	++	
+	+	+++	
+	+	-	
+	+	-	
+	+	-	
+	-	-	
+	+	-	
+	-	-	

cont'd on Fig.2B-1

FIG.2B

cont'd from Fig.2B

	390 400 410 420 430 440 450 460 470			
SEQ ID NO:1130	387 PE------------NNYKTTPPVLDS------------DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG 446			
SEQ ID NO:127	387 PEGDSLSWLLRLLNNNYKTTPPVLDS------------DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG 458	+	+	+++
SEQ ID NO:126	387 PEGDSLSWL-----NNYKTTPPVLDS------------DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG 453	+	+	–
SEQ ID NO:129	387 PE-DSLEFIASKLANNYKTTPPVLDS------------DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG 457	+	+	+++
SEQ ID NO:131	387 PE-DSLEFIASK--NNYKTTPPVLDS------------DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG 455	+	+	++
SEQ ID NO:130	387 PE-DSLEFIASKL-NNYKTTPPVLDS------------DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG 456	+	+	+++
SEQ ID NO:132	387 PEGDSLDMLEWSLMNNYKTTPPVLDS------------DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG 458	+	+	n.d.
SEQ ID NO:136	387 PE------------NNYKTTPPVLDSGDSLSWLLRLLNDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG 458	+	–	–

	380 390 400 410 420 430 440 450 460			
SEQ ID NO:1130	380 EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG-------------- 446			
SEQ ID NO:140	380 EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGDSLSWLLRLLN- 459	–	–	–
SEQ ID NO:139	380 EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG-GDSLSWLLRLLN- 458	–	–	–
SEQ ID NO:141	380 EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK-DSLEFIASKLA- 458	+	+	++

cont'd on Fig.2B–2

FIG.2B-1

cont'd from Fig.2B-1

		10 20 30 40 50 60 70 80				
SEQ ID NO:1131	1	DI------------QMTQSPSSLSASVGDRVTI TCRASQDVNTAVAWYQQKPGKAPKLLI YSASFLYSGVPSRFSGSRSG	68			
SEQ ID NO:26	1	DIGDSLSWLLRLLNQMTQSPSSLSASVGDRVTI TCRASQDVNTAVAWYQQKPGKAPKLLI YSASFLYSGVPSRFSGSRSG	80	+	−	−
SEQ ID NO:27	1	DI-DSLEFIASKLAQMTQSPSSLSASVGDRVTI TCRASQDVNTAVAWYQQKPGKAPKLLI YSASFLYSGVPSRFSGSRSG	79	+	+	++
		80 90 100 110 120 130 140 150 160				
SEQ ID NO:1131	76	SSL--------QPEDFATYYCQQHYTTPPTFGQGTKVEI KRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN	152			
SEQ ID NO:30	76	DSLEFIASKLAQPEDFATYYCQQHYTTPPTFGQGTKVEI KRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN	160	+	+	−
		150 160 170 180 190 200 210 220				
SEQ ID NO:1131	150	VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC------------	214			
SEQ ID NO:29	150	VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC-DSLEFIASKLA	225	+	+	−
SEQ ID NO:28	150	VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGDSLSWLLRLLN	226	+	+	−

FIG.2B-2

119 STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS 177
178 GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKX'1VEPKSCDKTHTCPPCPAPELLGG 237
238 PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN 297
298 STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRX'2E 357
358 X'3TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW 417
418 QQGNVFSCSVMHEX'4LHNHYTQKSLSLSPG 446

FIG.3A

109 TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNX'5LQSGNSQESVTEQDS 168
169 KDSTYSLSSTLTLSKADYEKHKX'6YACEVTHQGLSSPVTKSFNRGEC 214

FIG.3B

trastuzumab, Ig gamma-1 chain C region 119 STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT 197
P01857[1-330], Ig gamma-1 chain C region, Homo sapiens 119 STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT 197
P01859[1-326], Ig gamma-2 chain C region, Homo sapiens 119 STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQT 197
P01860[1-377], Ig gamma-3 chain C region, Homo sapiens 119 STKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT 197
P01861[1-327], Ig gamma-4 chain C region, Homo sapiens 119 STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT 197
trastuzumab, Ig gamma-1 chain C region 198 YTCNVNHKPSNTKVDKKVE---------- PKSCDKTHTCPPCP 230
P01857[1-330], Ig gamma-1 chain C region, Homo sapiens 198 YTCNVNHKPSNTKVDKRVE---------- PKSCDKTHTCPPCP 230
P01859[1-326], Ig gamma-2 chain C region, Homo sapiens 198 YTCNVDHKPSNTKVDKTVER---------- KCCVECPPCP 227
P01860[1-377], Ig gamma-3 chain C region, Homo sapiens 198 YTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCP 277
P01861[1-327], Ig gamma-4 chain C region, Homo sapiens 198 YTCNVDHKPSNTKVDKRVES---------- KYGPPCPSCP 227
trastuzumab, Ig gamma-1 chain C region 231 APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH 310
P01857[1-330], Ig gamma-1 chain C region, Homo sapiens 231 APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH 310
P01859[1-326], Ig gamma-2 chain C region, Homo sapiens 228 APPVAG-PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVH 306
P01860[1-377], Ig gamma-3 chain C region, Homo sapiens 278 APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLH 357
P01861[1-327], Ig gamma-4 chain C region, Homo sapiens 228 APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLH 307
cont'd on Fig.4A-1

FIG.4A

cont'd from Fig.4A

```
trastuzumab, Ig gamma-1 chain C region          311 QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN 390
P01857[1-330], Ig gamma-1 chain C region, Homo sapiens 311 QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN 390
P01859[1-326], Ig gamma-2 chain C region, Homo sapiens 307 QDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENN 386
P01860[1-377], Ig gamma-3 chain C region, Homo sapiens 358 QDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENN 437
P01861[1-327], Ig gamma-4 chain C region, Homo sapiens 308 QDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN 387

trastuzumab, Ig gamma-1 chain C region          391 YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG 446
P01857[1-330], Ig gamma-1 chain C region, Homo sapiens 391 YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG 446
P01859[1-326], Ig gamma-2 chain C region, Homo sapiens 387 YKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG 442
P01860[1-377], Ig gamma-3 chain C region, Homo sapiens 438 YNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPG 493
P01861[1-327], Ig gamma-4 chain C region, Homo sapiens 388 YKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG 443
```

FIG.4A-1

```
trastuzumab Ig kappa chain C region          109 TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK 188
P01834[1-106], Ig kappa chain C region, Homo sapiens 109 TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK 188

trastuzumab Ig kappa chain Cregion           189 HKVYACEVTHQGLSSPVTKSFNRGEC 214
P01834[1-106], Ig kappa chain C region, Homo sapiens 189 HKDYACEVTHQGLSSPVTKSFNRGEC 214
```

FIG.4B

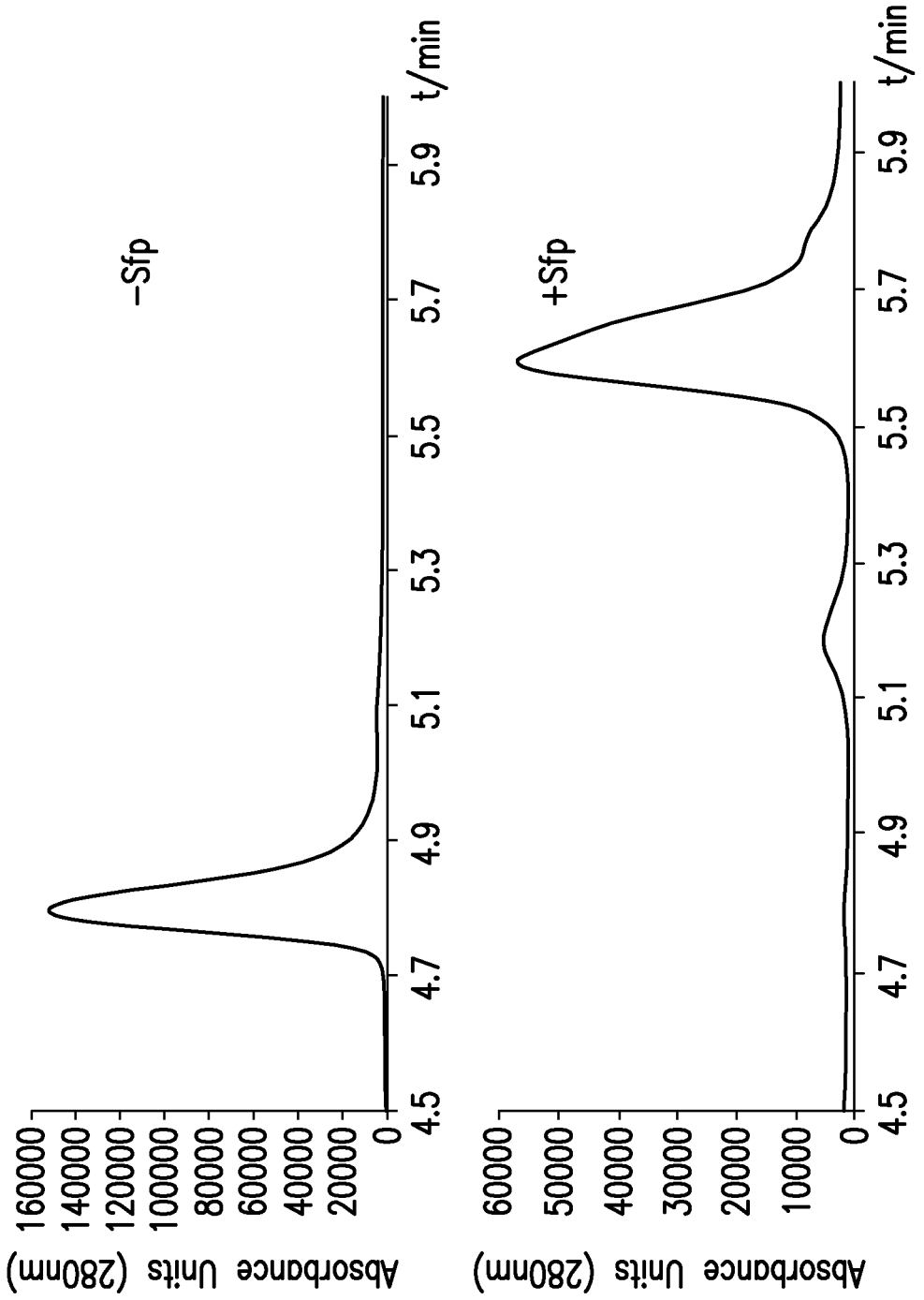

−Sfp
+Sfp
Absorbance Units (280nm)
t/min
160000
140000
120000
100000
80000
60000
40000
20000
0
4.5
4.7
4.9
5.1
5.3
5.5
5.7
5.9
60000
50000
40000
30000
20000
10000

FIG.5A

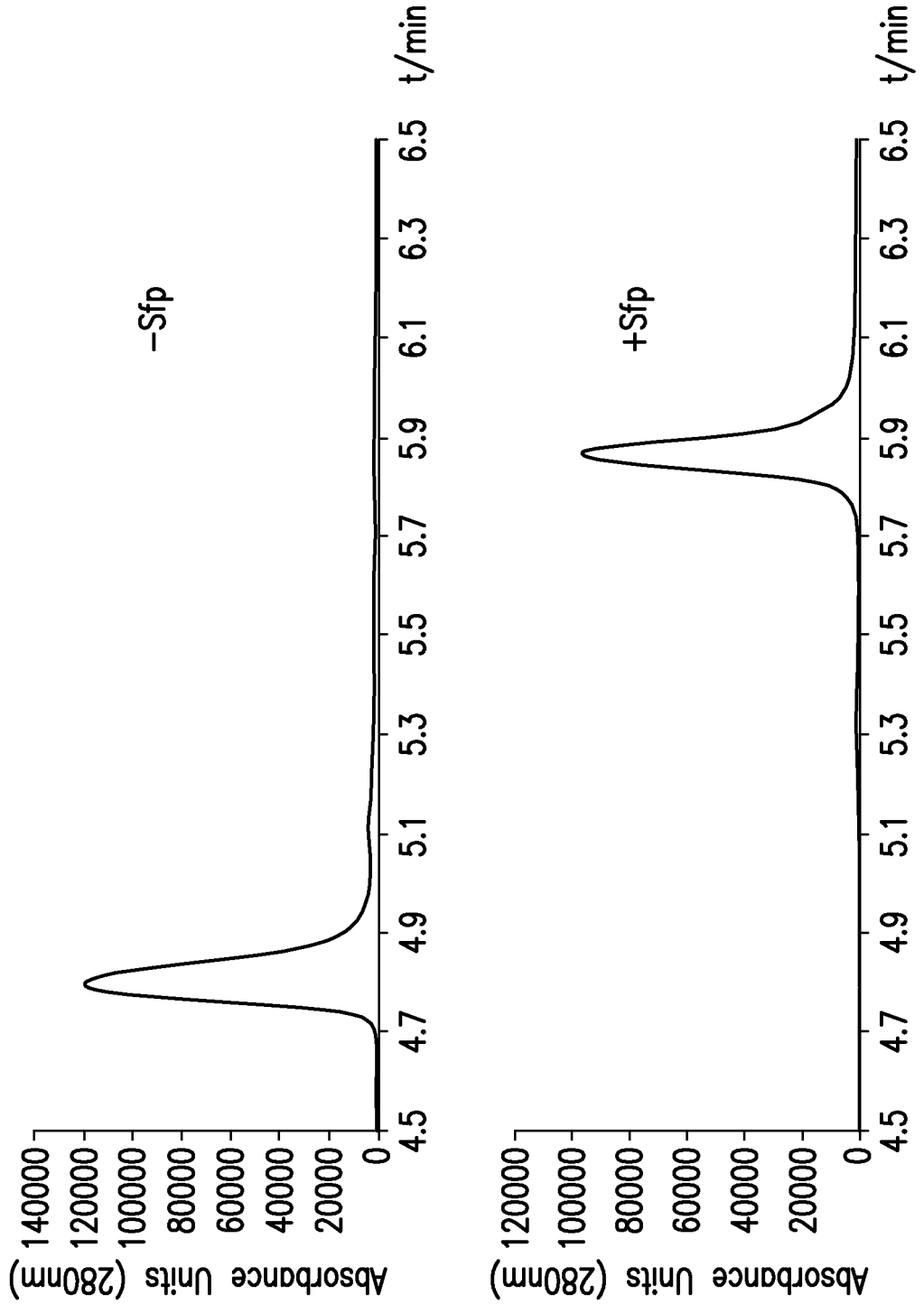

−Sfp
+Sfp
Absorbance Units (280nm)
t/min
0
20000
40000
60000
80000
100000
120000
140000
4.5
4.7
4.9
5.1
5.3
5.5
5.7
5.9
6.1
6.3
6.5

FIG.5B

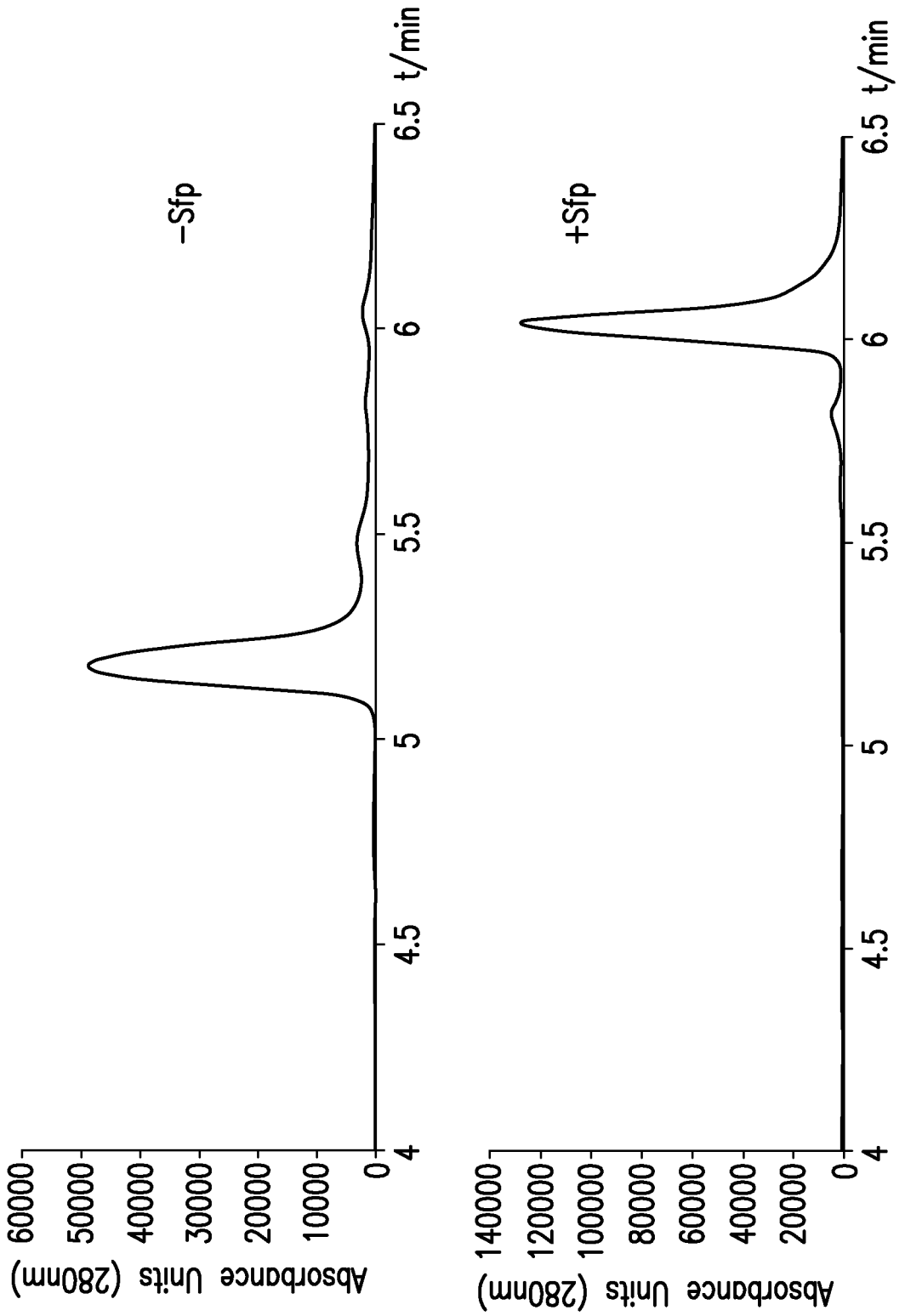

−Sfp
+Sfp
Absorbance Units (280nm)
t/min
0
10000
20000
30000
40000
50000
60000
80000
100000
120000
140000
4
4.5
5
5.5
6
6.5

FIG.5C

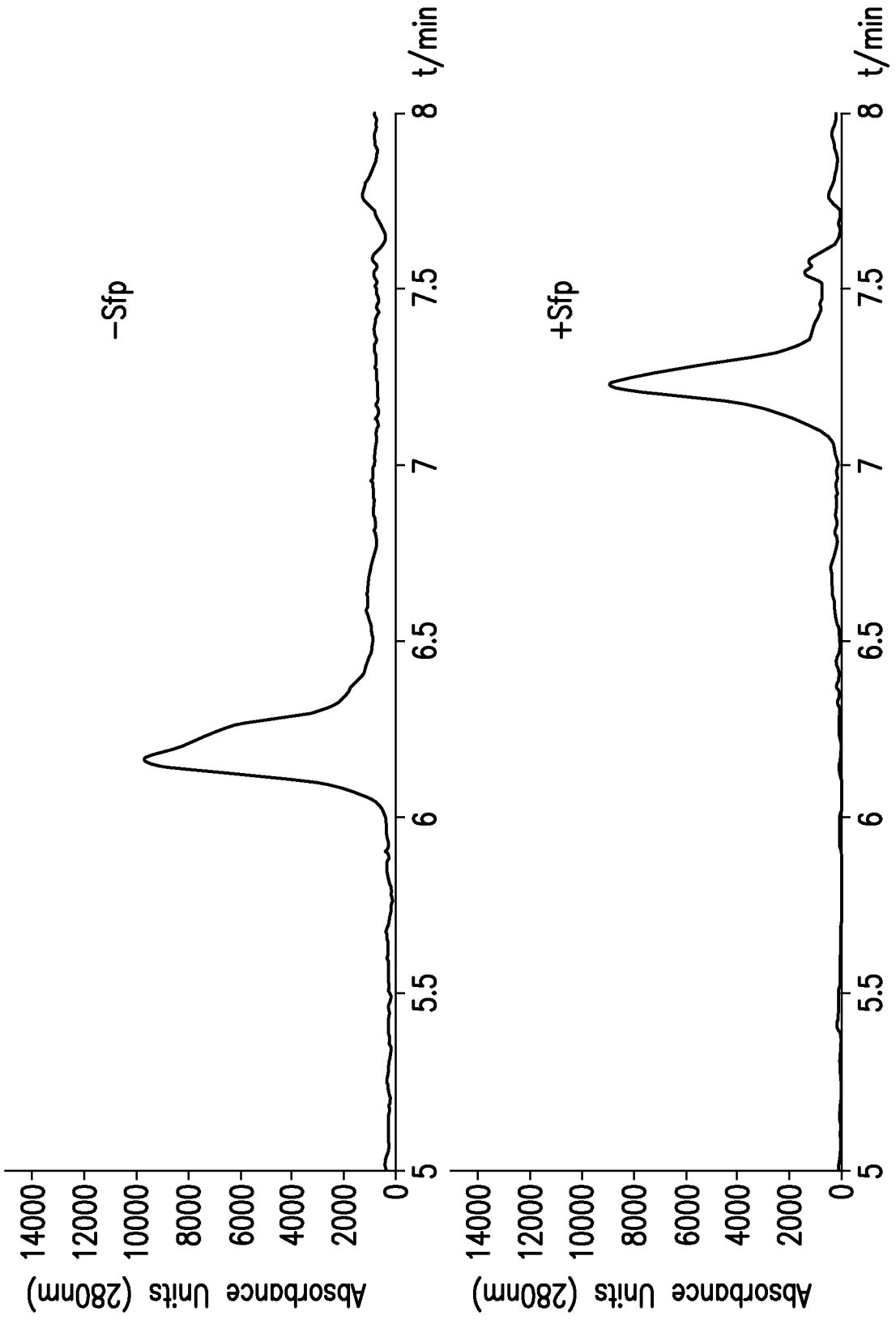

−Sfp
+Sfp
Absorbance Units (280nm)
t/min
0
2000
4000
6000
8000
10000
12000
14000
5
5.5
6
6.5
7
7.5
8

FIG.5D

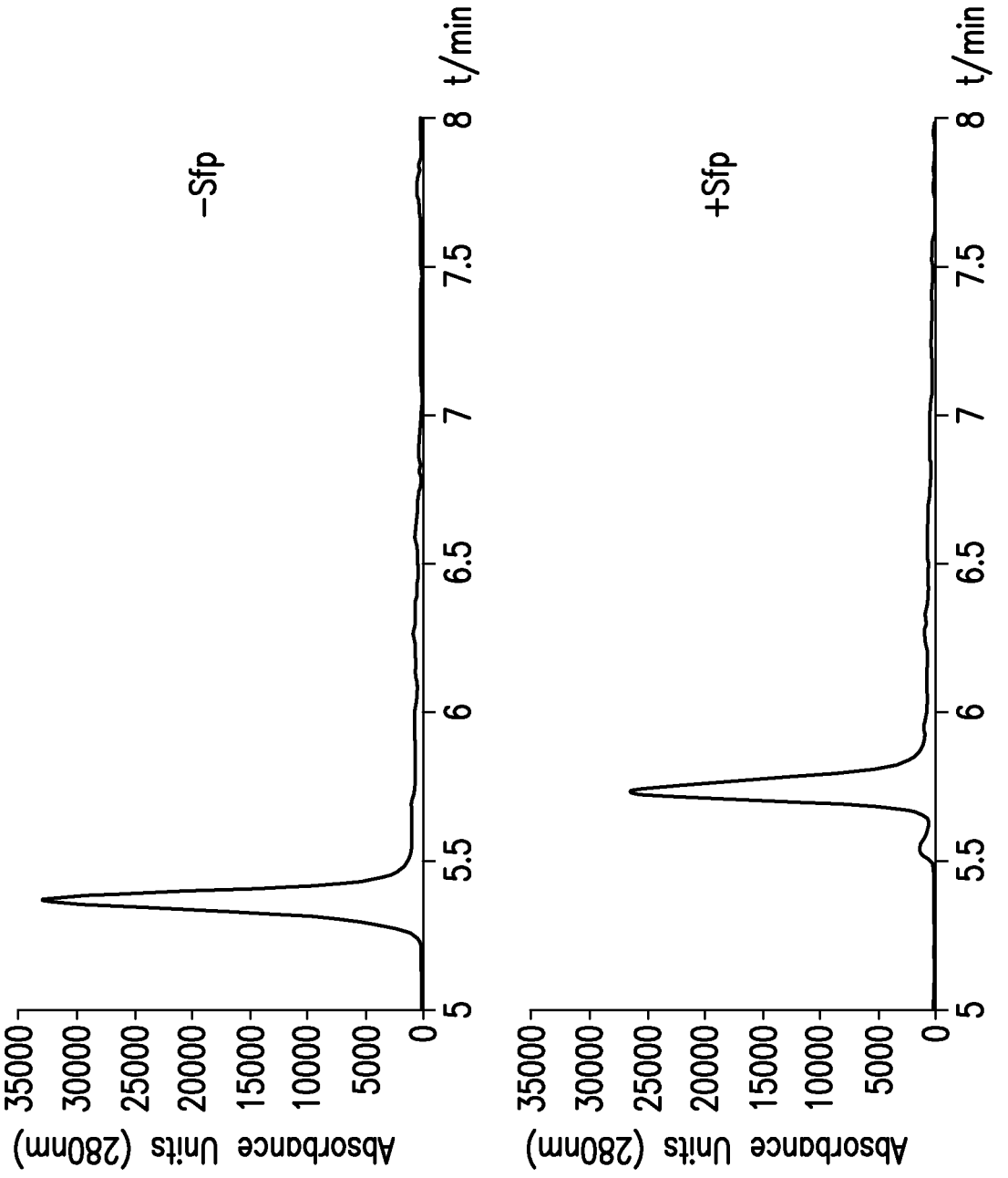

−Sfp
+Sfp
Absorbance Units (280nm)
35000
30000
25000
20000
15000
10000
5000
0
5
5.5
6
6.5
7
7.5
8
t/min

FIG.5E

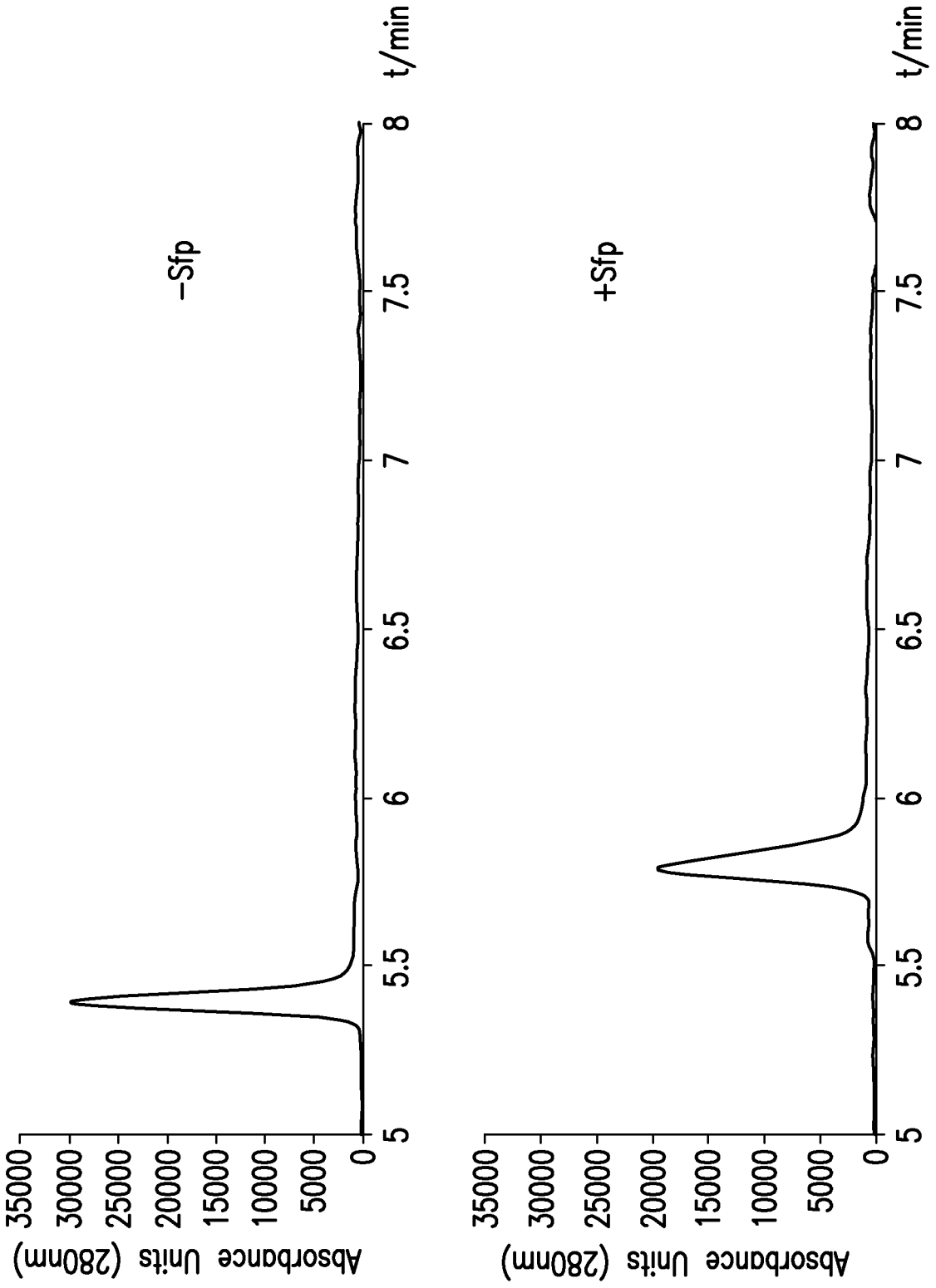

−Sfp
+Sfp
Absorbance Units (280nm)
35000
30000
25000
20000
15000
10000
5000
0
5
5.5
6
6.5
7
7.5
8
t/min

FIG.5F

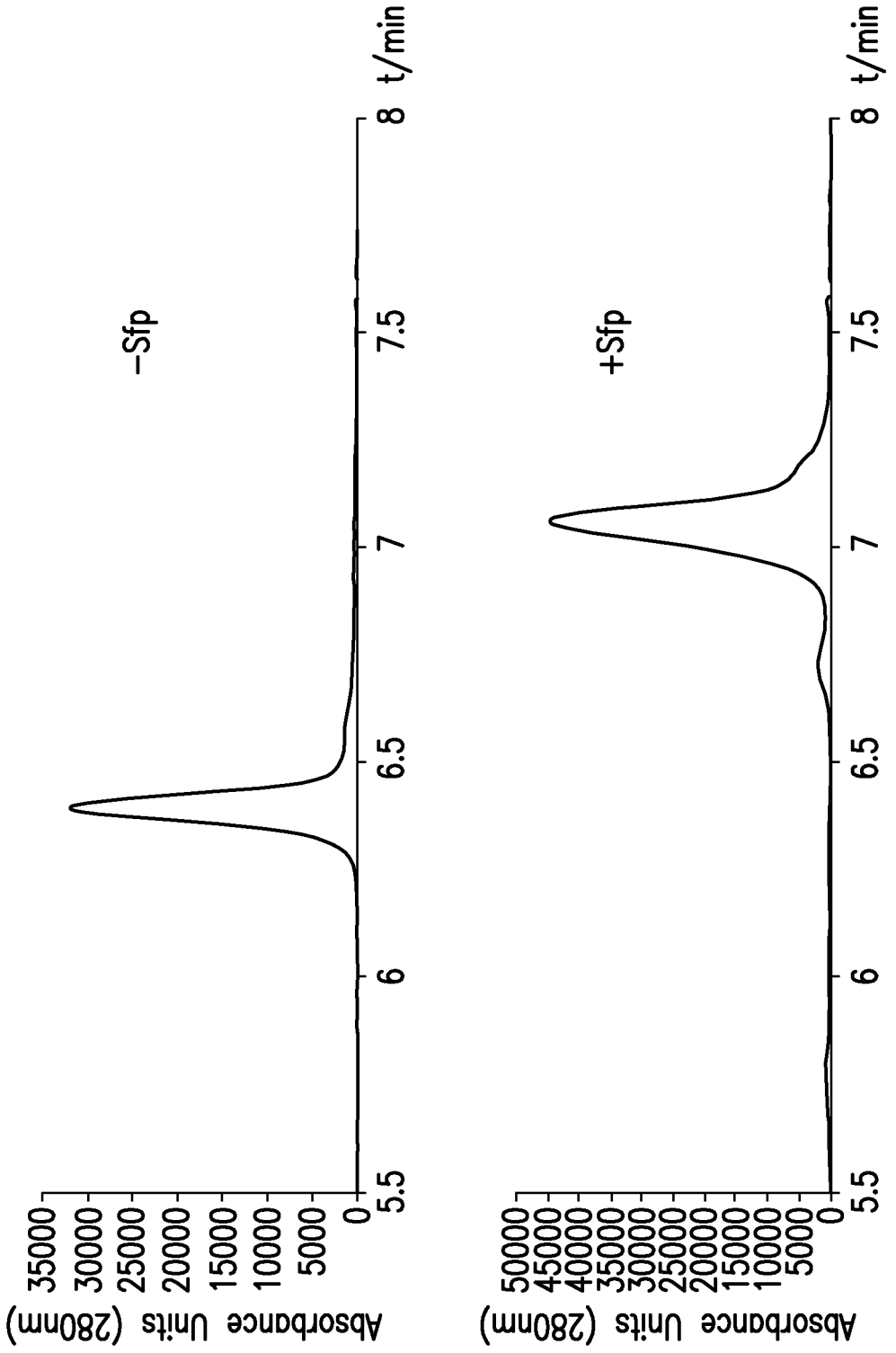

−Sfp
+Sfp
Absorbance Units (280nm)
t/min
0
5000
10000
15000
20000
25000
30000
35000
40000
45000
50000
5.5
6
6.5
7
7.5
8

FIG.5G

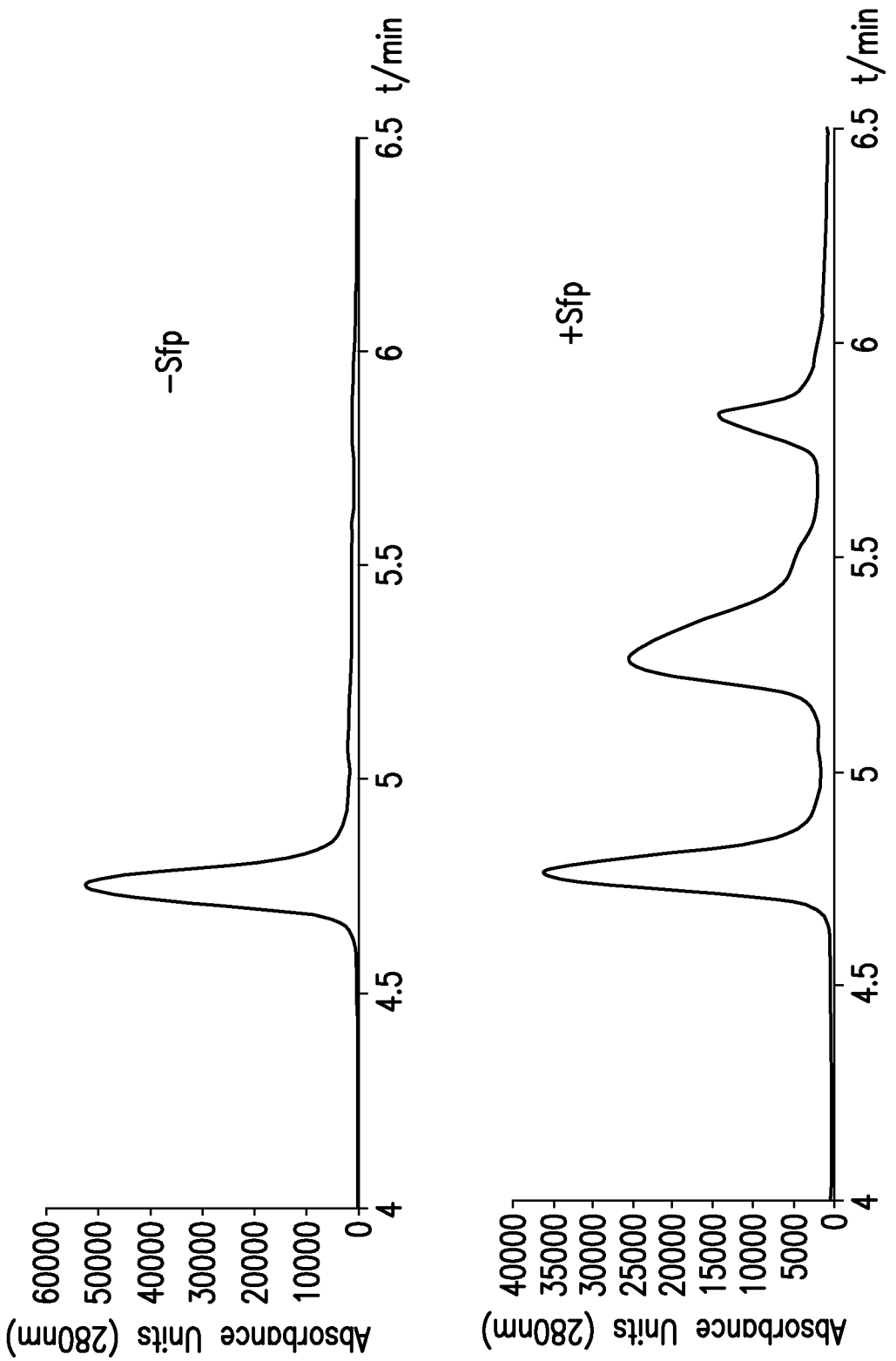

−Sfp
+Sfp
Absorbance Units (280nm)
t/min
0
10000
20000
30000
40000
50000
60000
5000
15000
25000
35000
4
4.5
5
5.5
6
6.5

FIG.5H

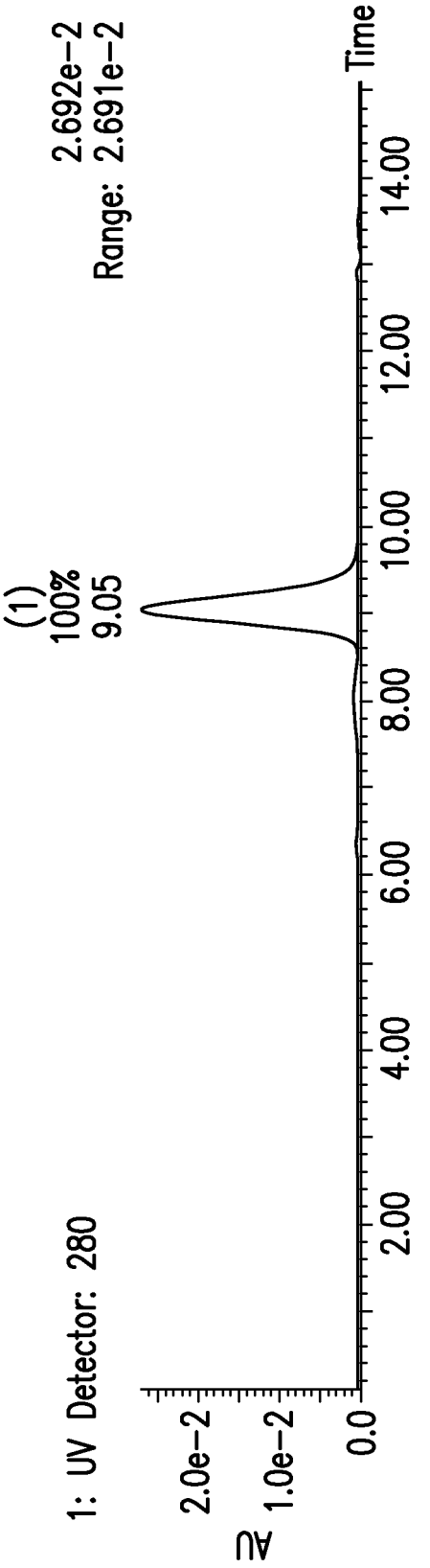

1: UV Detector: 280
AU
2.0e-2
1.0e-2
0.0
2.00
4.00
6.00
8.00
10.00
12.00
14.00
Time
(1)
100%
9.05
2.692e-2
Range: 2.691e-2

FIG.6A

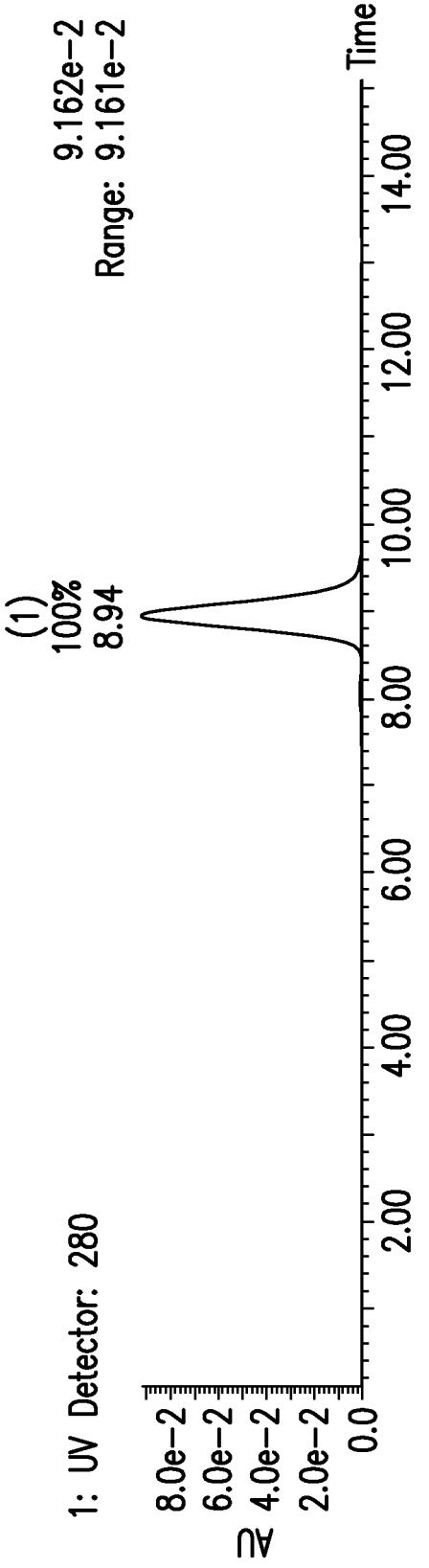

1: UV Detector: 280
AU
8.0e-2
6.0e-2
4.0e-2
2.0e-2
0.0
2.00
4.00
6.00
8.00
10.00
12.00
14.00
Time
(1)
100%
8.94
9.162e-2
Range: 9.161e-2

FIG.6B

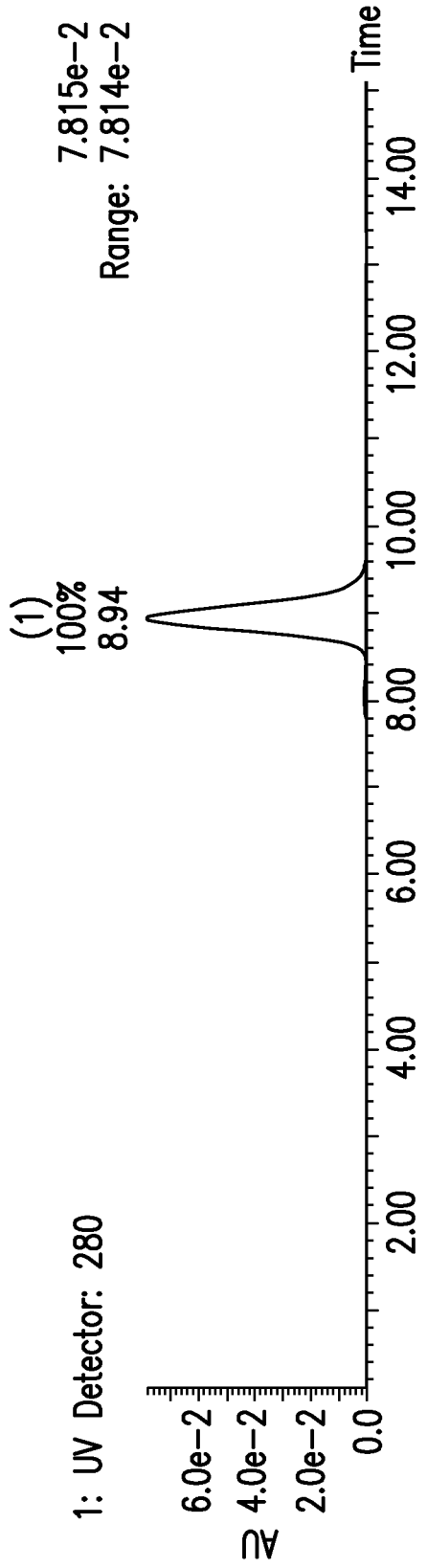

7.815e-2
Range: 7.814e-2
(1)
100%
8.94
Time
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.0
2.0e-2
4.0e-2
6.0e-2
AU
1: UV Detector: 280

FIG.6C

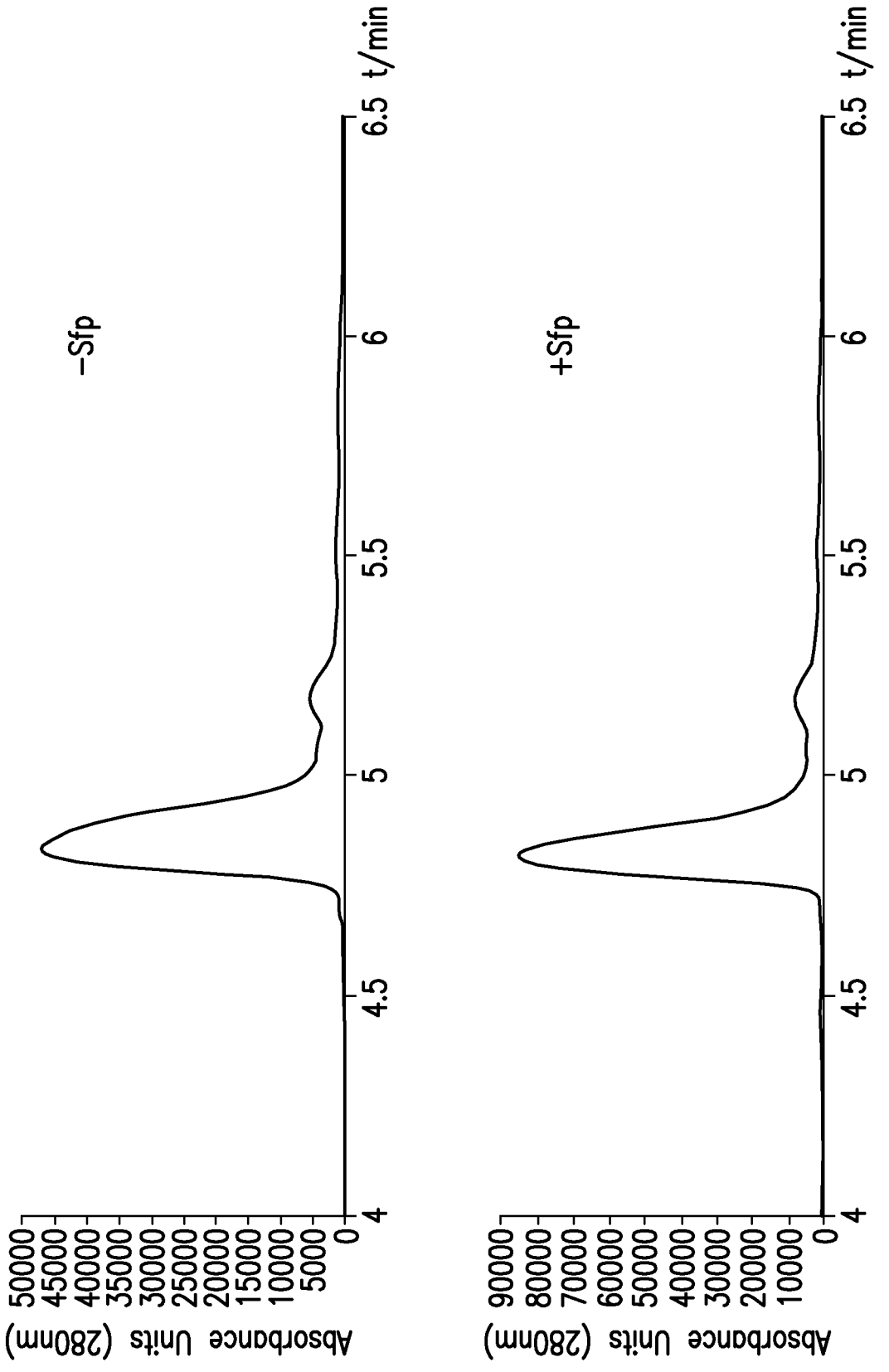

−Sfp
+Sfp
Absorbance Units (280nm)
t/min
0
5000
10000
15000
20000
25000
30000
35000
40000
45000
50000
60000
70000
80000
90000
4
4.5
5
5.5
6
6.5

FIG. 7

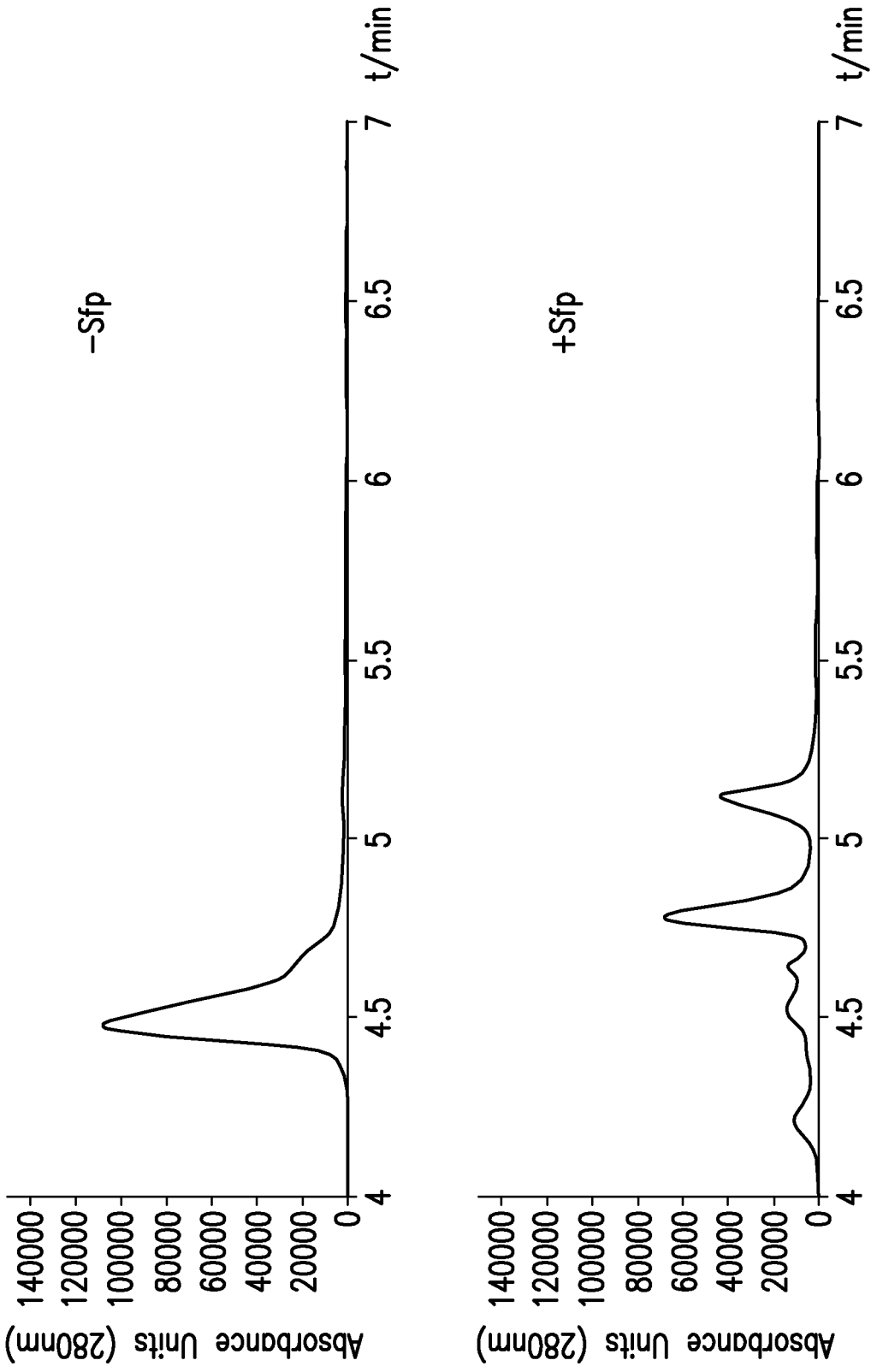

−Sfp
+Sfp
Absorbance Units (280nm)
t/min
0
20000
40000
60000
80000
100000
120000
140000
4
4.5
5
5.5
6
6.5
7

FIG.8A

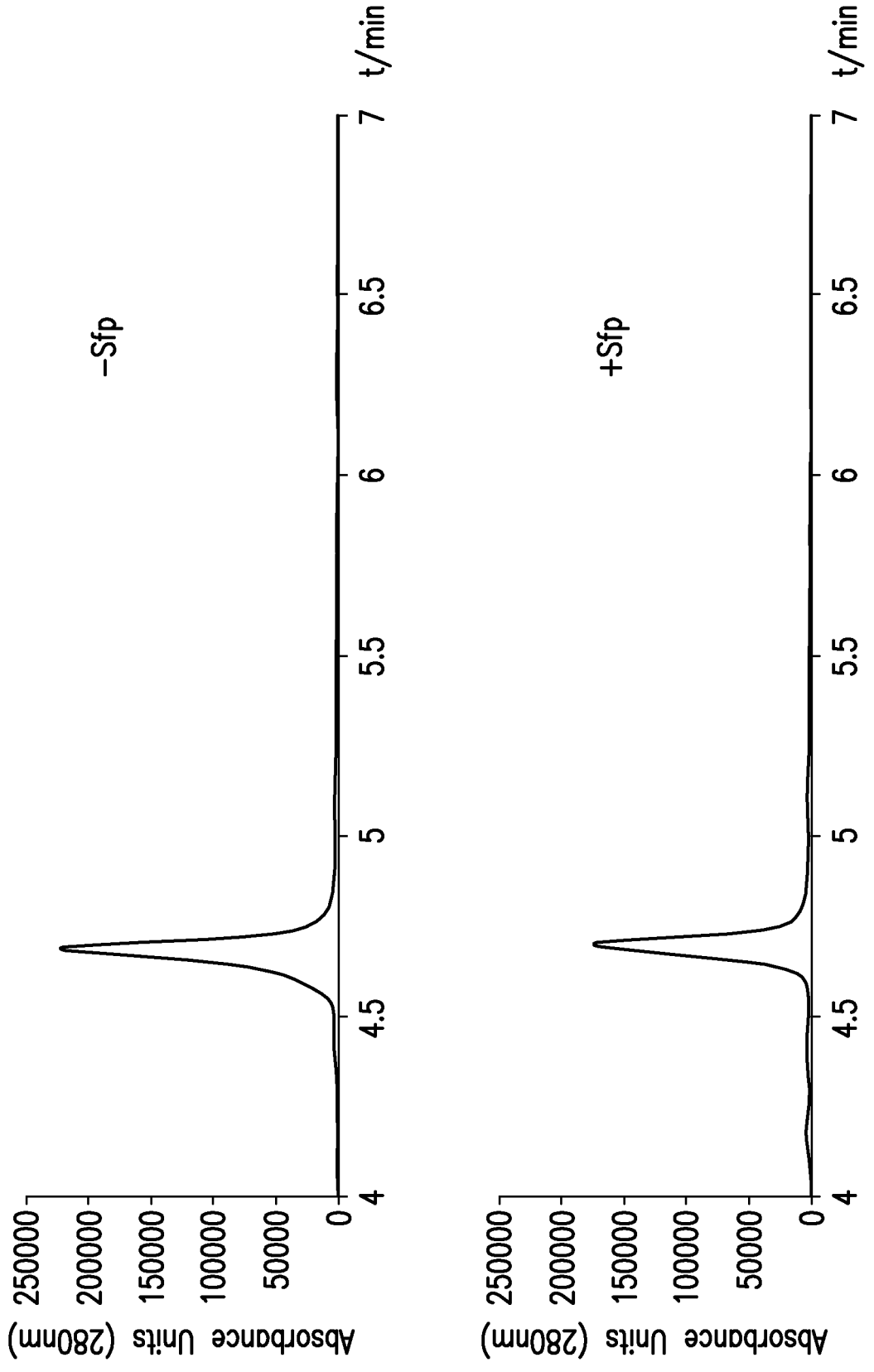

−Sfp
+Sfp
Absorbance Units (280nm)
250000
200000
150000
100000
50000
0
4
4.5
5
5.5
6
6.5
7
t/min

FIG.8B

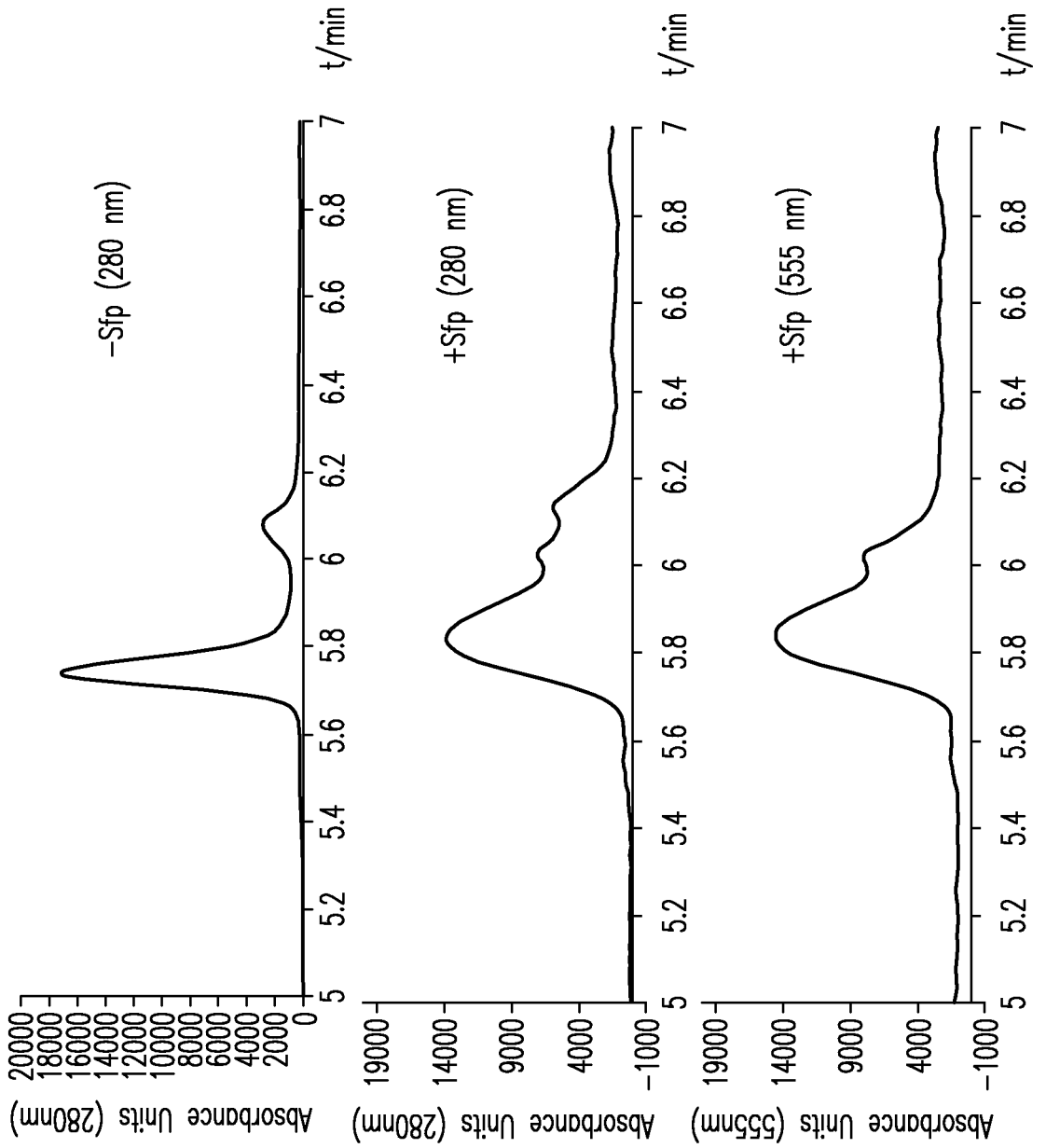

−Sfp (280 nm)
+Sfp (280 nm)
+Sfp (555 nm)
Absorbance Units (280nm)
Absorbance Units (280nm)
Absorbance Units (555nm)
t/min
t/min
t/min
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
19000
14000
9000
4000
−1000
5
5.2
5.4
5.6
5.8
6
6.2
6.4
6.6
6.8
7

FIG.9A

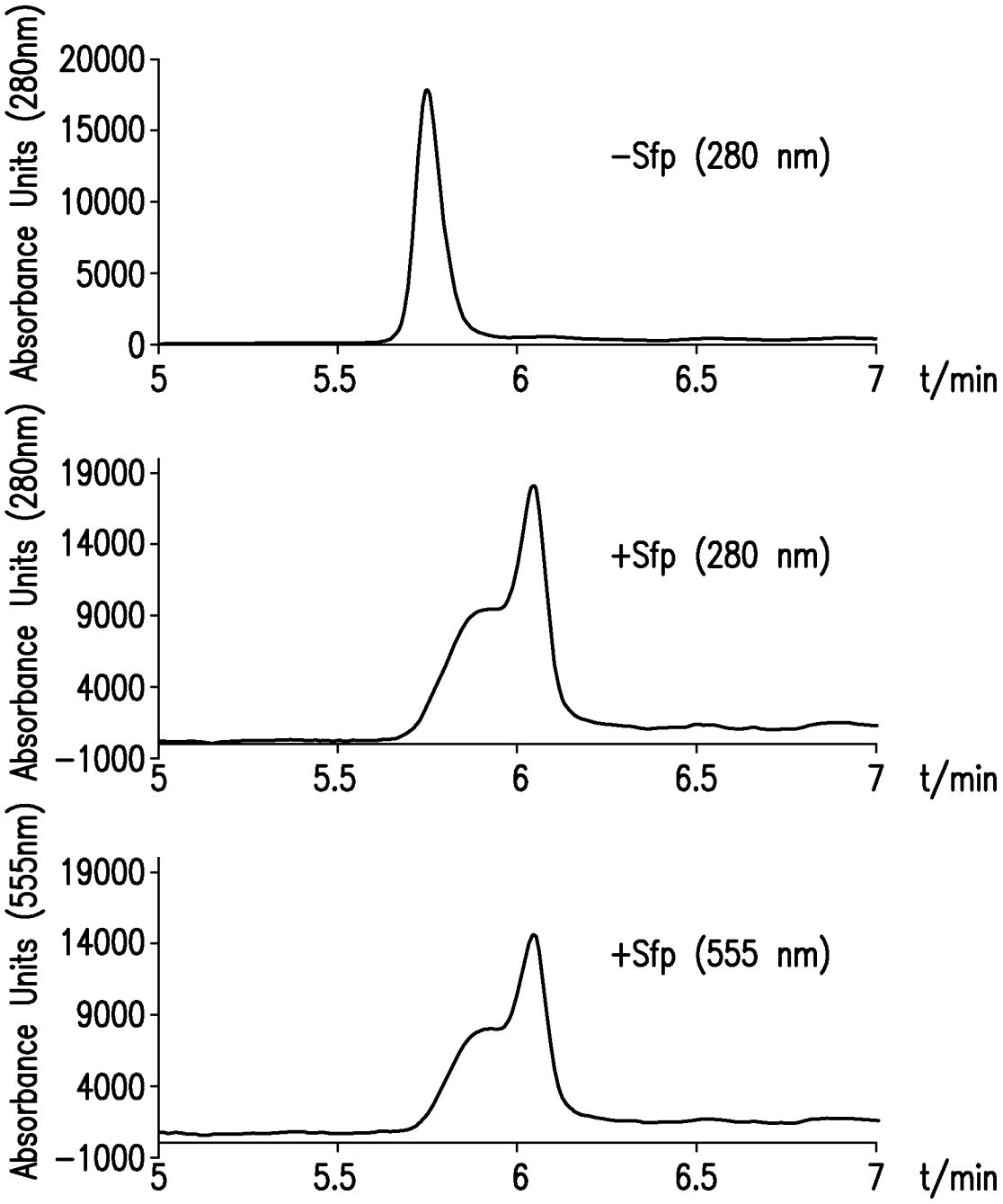

Absorbance Units (280nm)
20000
15000
10000
5000
0
−Sfp (280 nm)
5
5.5
6
6.5
7
t/min
Absorbance Units (280nm)
19000
14000
9000
4000
−1000
+Sfp (280 nm)
5
5.5
6
6.5
7
t/min
Absorbance Units (555nm)
19000
14000
9000
4000
−1000
+Sfp (555 nm)
5
5.5
6
6.5
7
t/min

FIG.9B

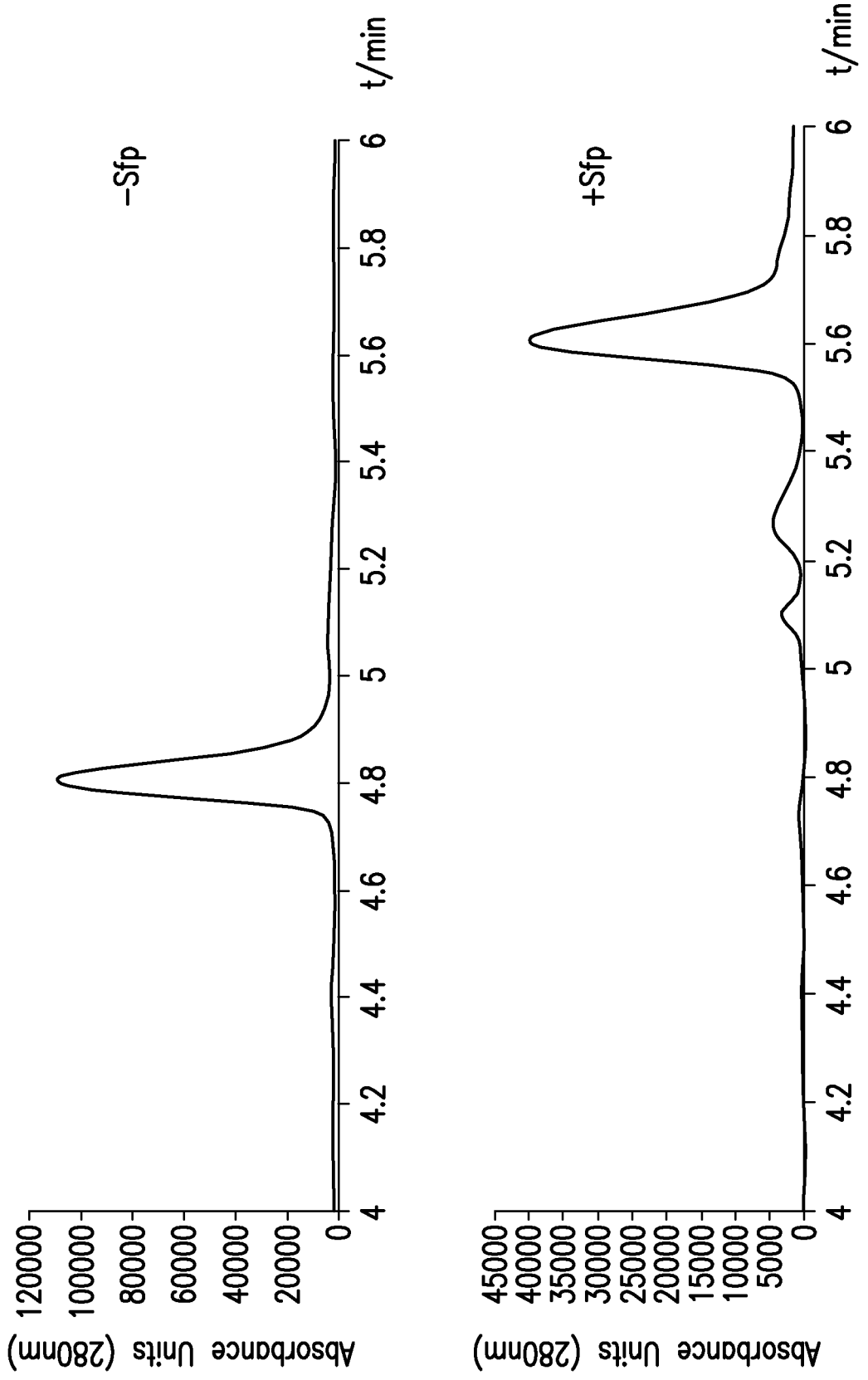

−Sfp
+Sfp
Absorbance Units (280nm)
t/min
0
20000
40000
60000
80000
100000
120000
5000
10000
15000
20000
25000
30000
35000
40000
45000
4
4.2
4.4
4.6
4.8
5
5.2
5.4
5.6
5.8
6

FIG.10A

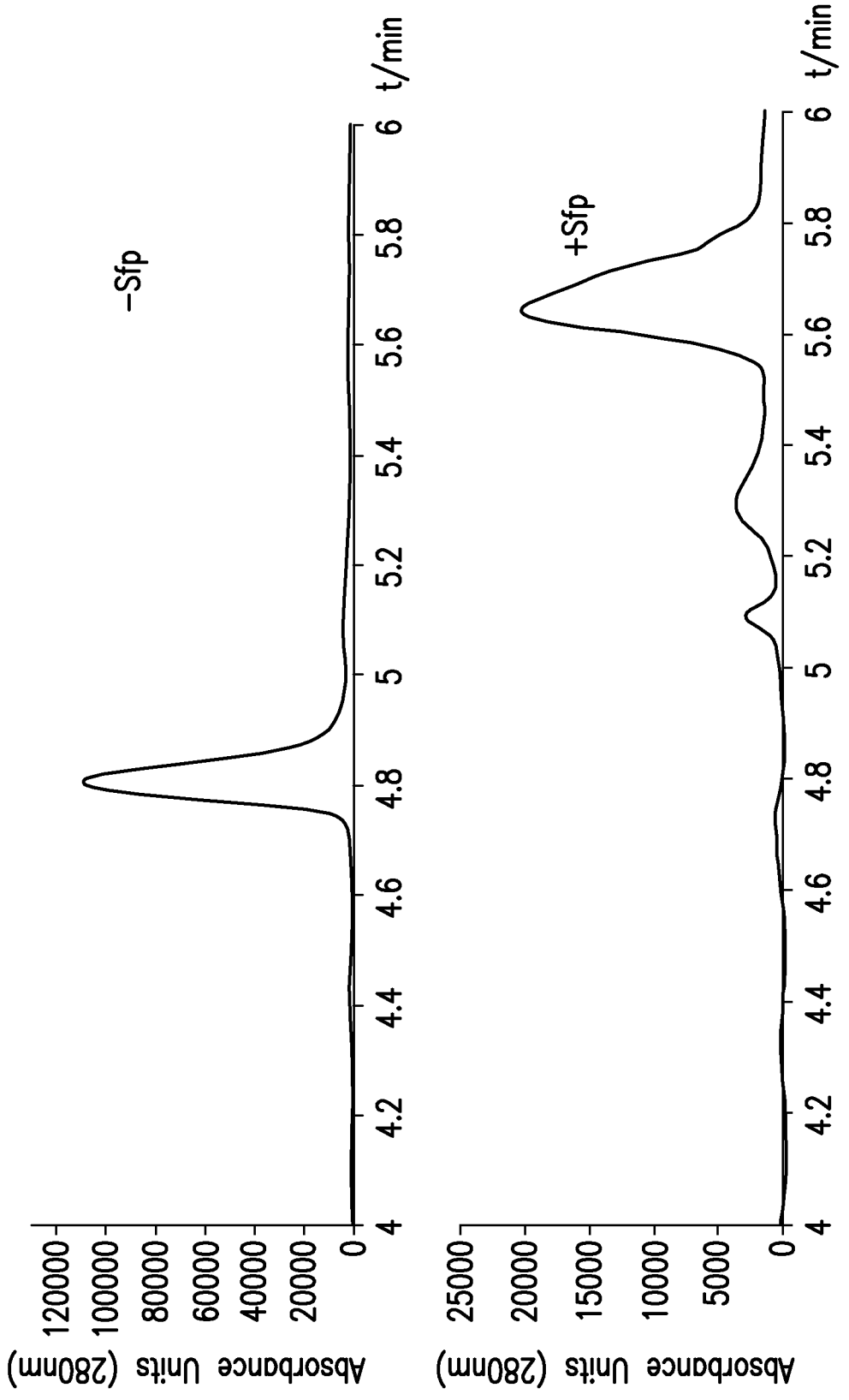

−Sfp
+Sfp
Absorbance Units (280nm)
t/min
0
20000
40000
60000
80000
100000
120000
5000
10000
15000
20000
25000
4
4.2
4.4
4.6
4.8
5
5.2
5.4
5.6
5.8
6

FIG.10B

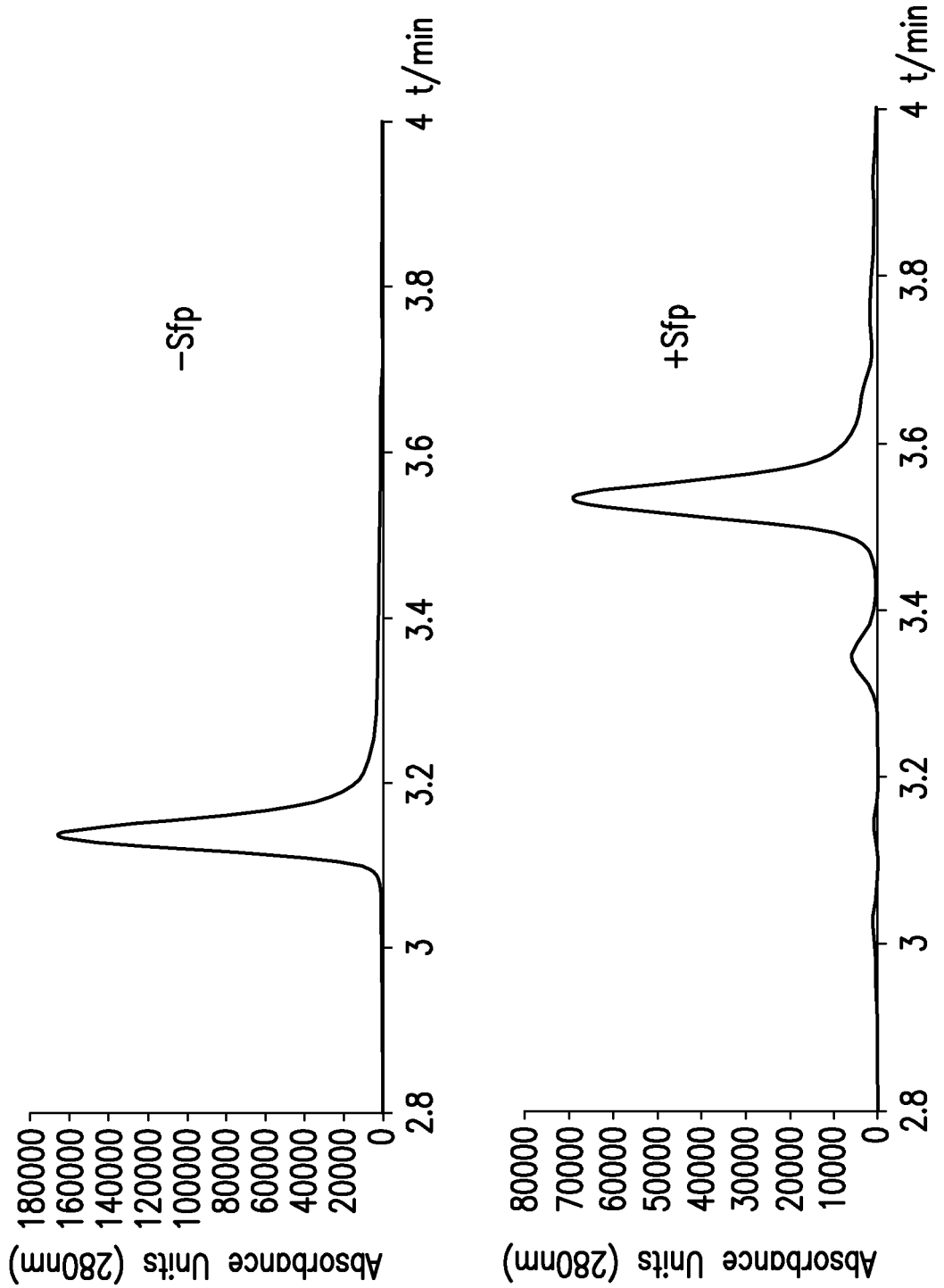

−Sfp
+Sfp
Absorbance Units (280nm)
Absorbance Units (280nm)
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
80000
70000
60000
50000
40000
30000
20000
10000
0
2.8
3
3.2
3.4
3.6
3.8
4 t/min

FIG.11A

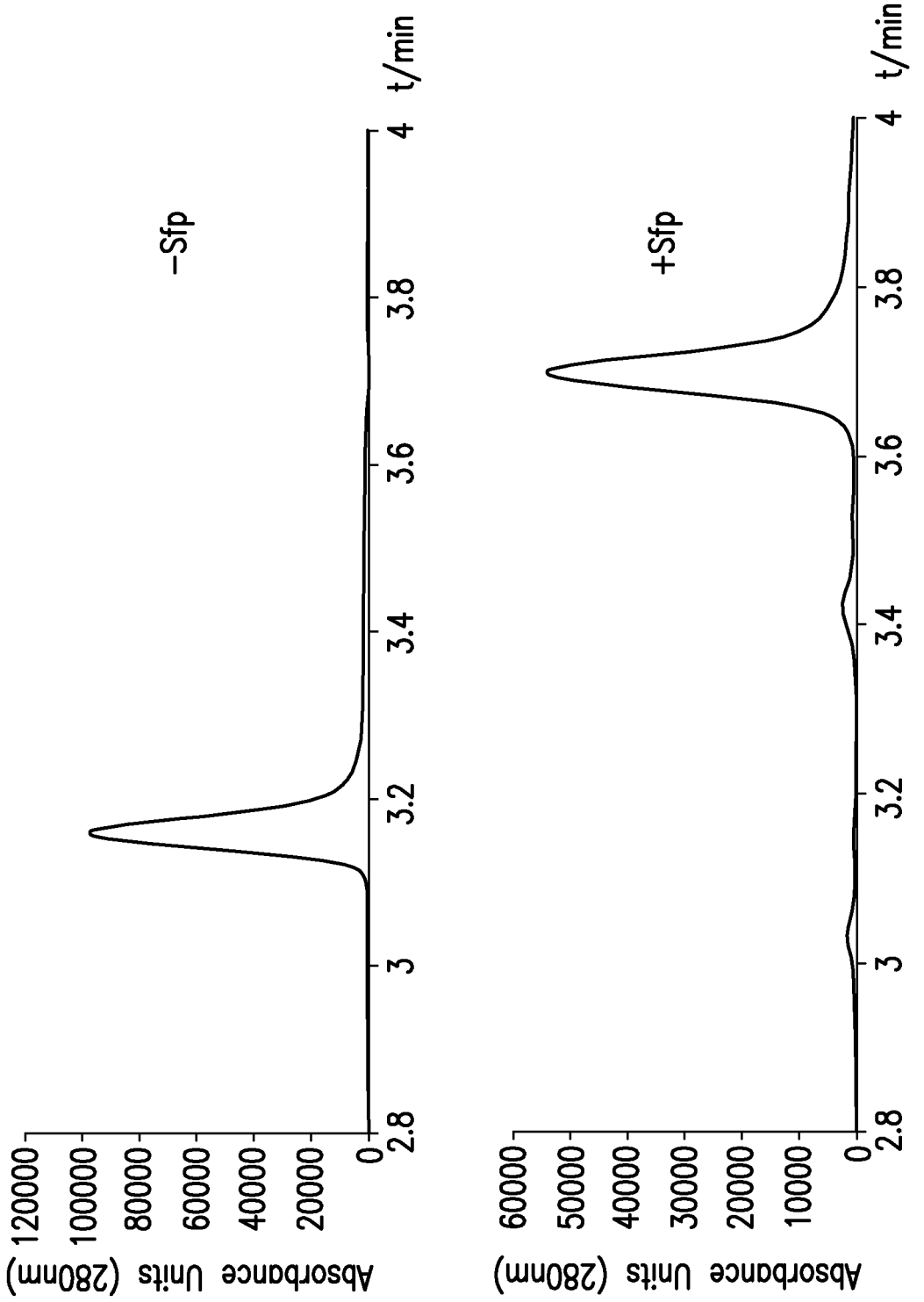

−Sfp
+Sfp
Absorbance Units (280nm)
t/min
0
20000
40000
60000
80000
100000
120000
10000
30000
50000
2.8
3
3.2
3.4
3.6
3.8
4

FIG.11B

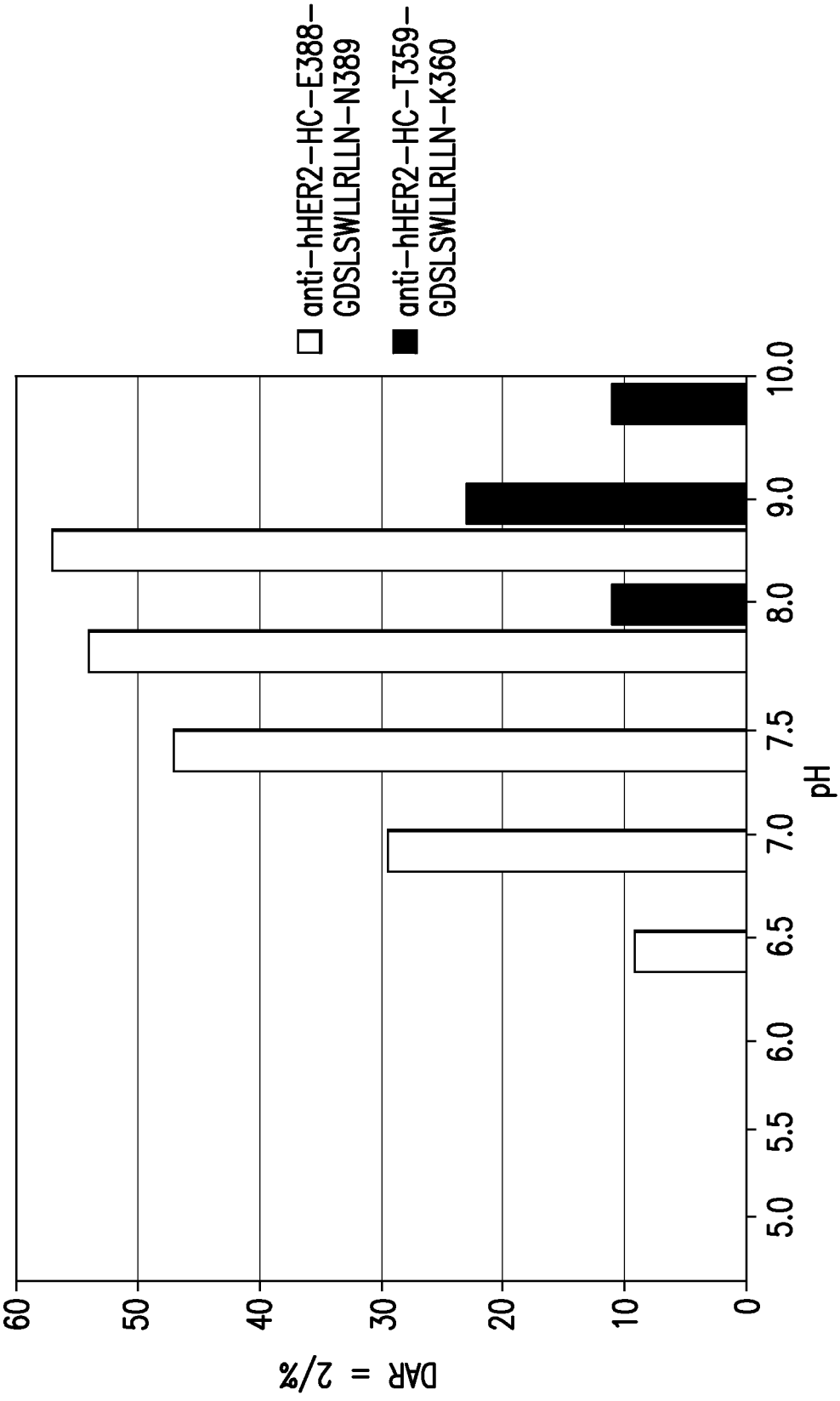

anti-hHER2-HC-E388-GDSLSWLLRLLN-N389
anti-hHER2-HC-T359-GDSLSWLLRLLN-K360
DAR = 2/%
0
10
20
30
40
50
60
5.0
5.5
6.0
6.5
7.0
7.5
8.0
9.0
10.0
pH

FIG.12

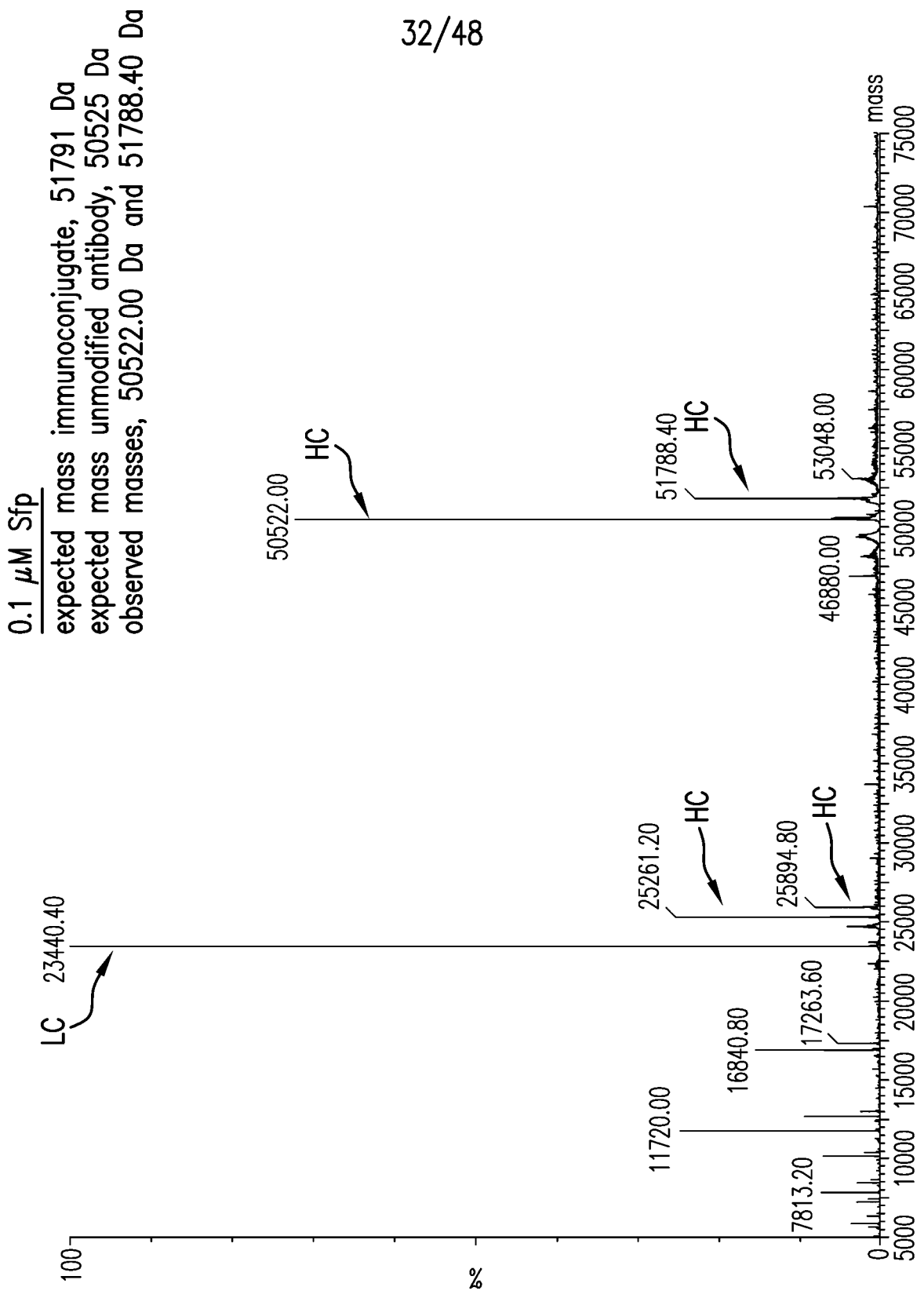

0.1 μM Sfp
expected mass immunoconjugate, 51791 Da
expected mass unmodified antibody, 50525 Da
observed masses, 50522.00 Da and 51788.40 Da
LC
23440.40
HC
50522.00
HC
51788.40
HC
25261.20
HC
25894.80
11720.00
16840.80
17263.60
7813.20
46880.00
53048.00
100
%
0
5000
10000
15000
20000
25000
30000
35000
40000
45000
50000
55000
60000
65000
70000
75000
mass

FIG. 13A

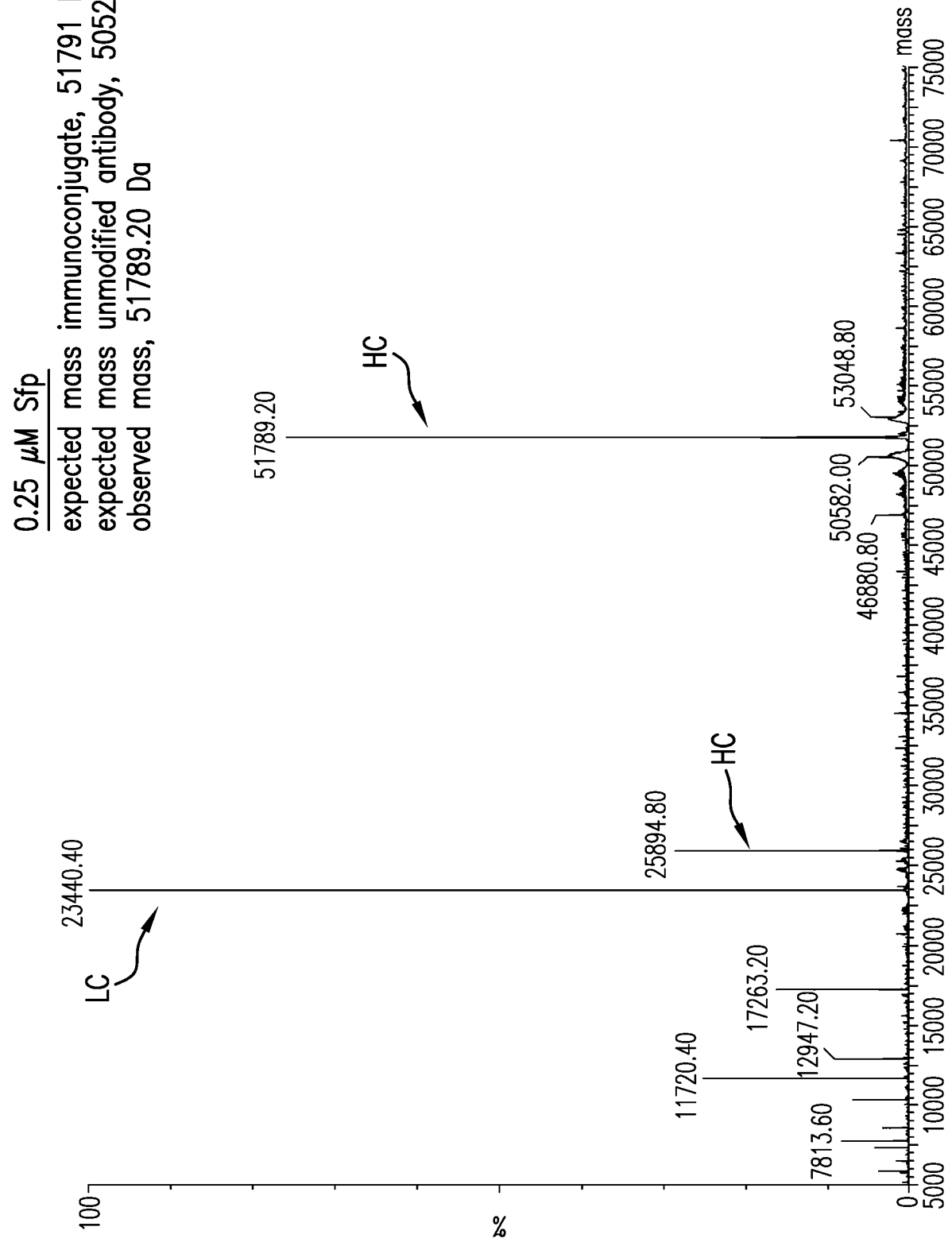

0.25 μM Sfp
expected mass immunoconjugate, 51791 Da
expected mass unmodified antibody, 50525 Da
observed mass, 51789.20 Da
LC
HC
HC
23440.40
25894.80
51789.20
53048.80
50582.00
46880.80
17263.20
12947.20
11720.40
7813.60
100
%
0
5000
10000
15000
20000
25000
30000
35000
40000
45000
50000
55000
60000
65000
70000
75000
mass

FIG.13B

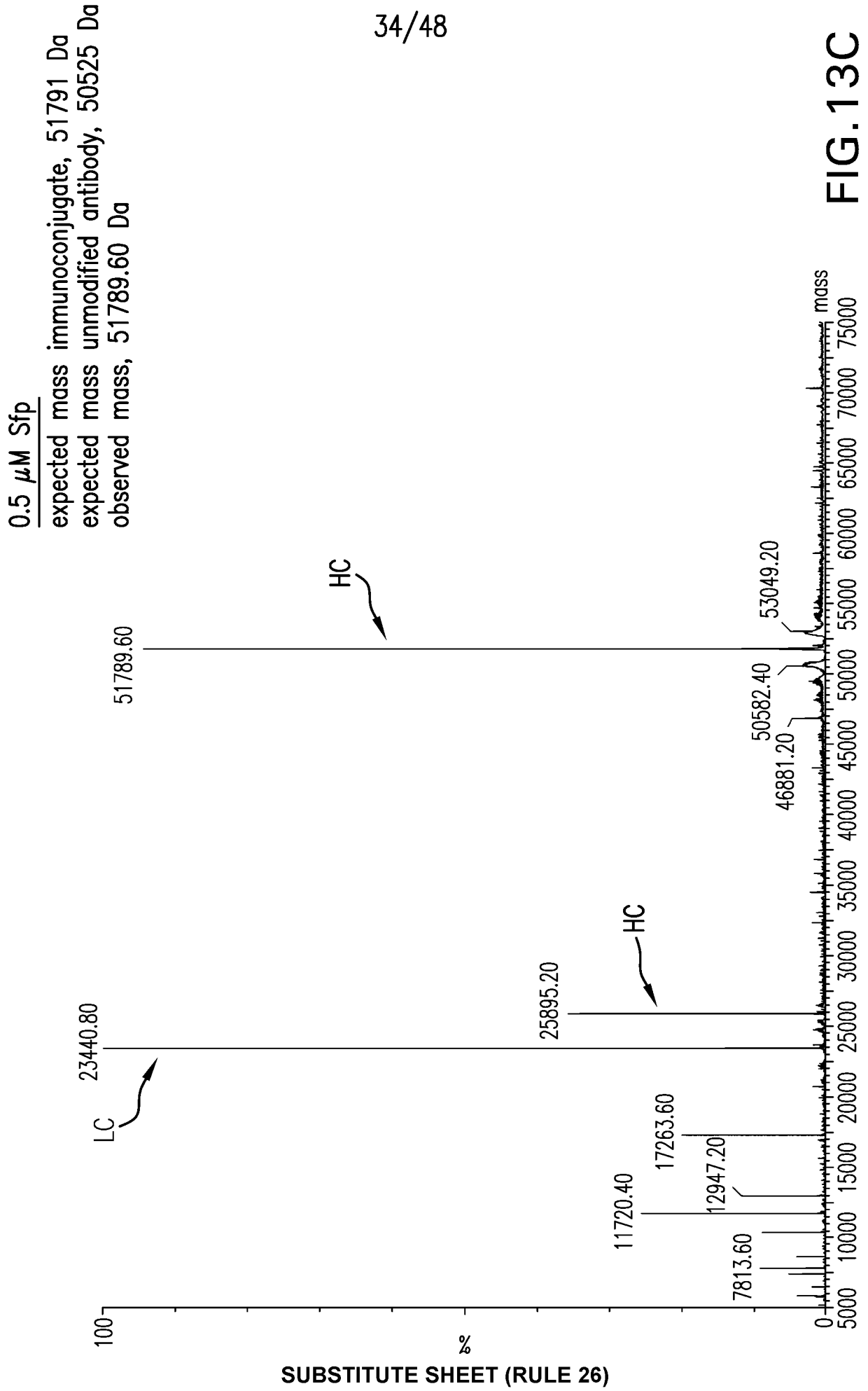

0.5 μM Sfp
expected mass immunoconjugate, 51791 Da
expected mass unmodified antibody, 50525 Da
observed mass, 51789.60 Da
HC
HC
LC
51789.60
53049.20
50582.40
46881.20
25895.20
23440.80
17263.60
12947.20
11720.40
7813.60
100
0
%
5000 10000 15000 20000 25000 30000 35000 40000 45000 50000 55000 60000 65000 70000 75000
mass

FIG.13C

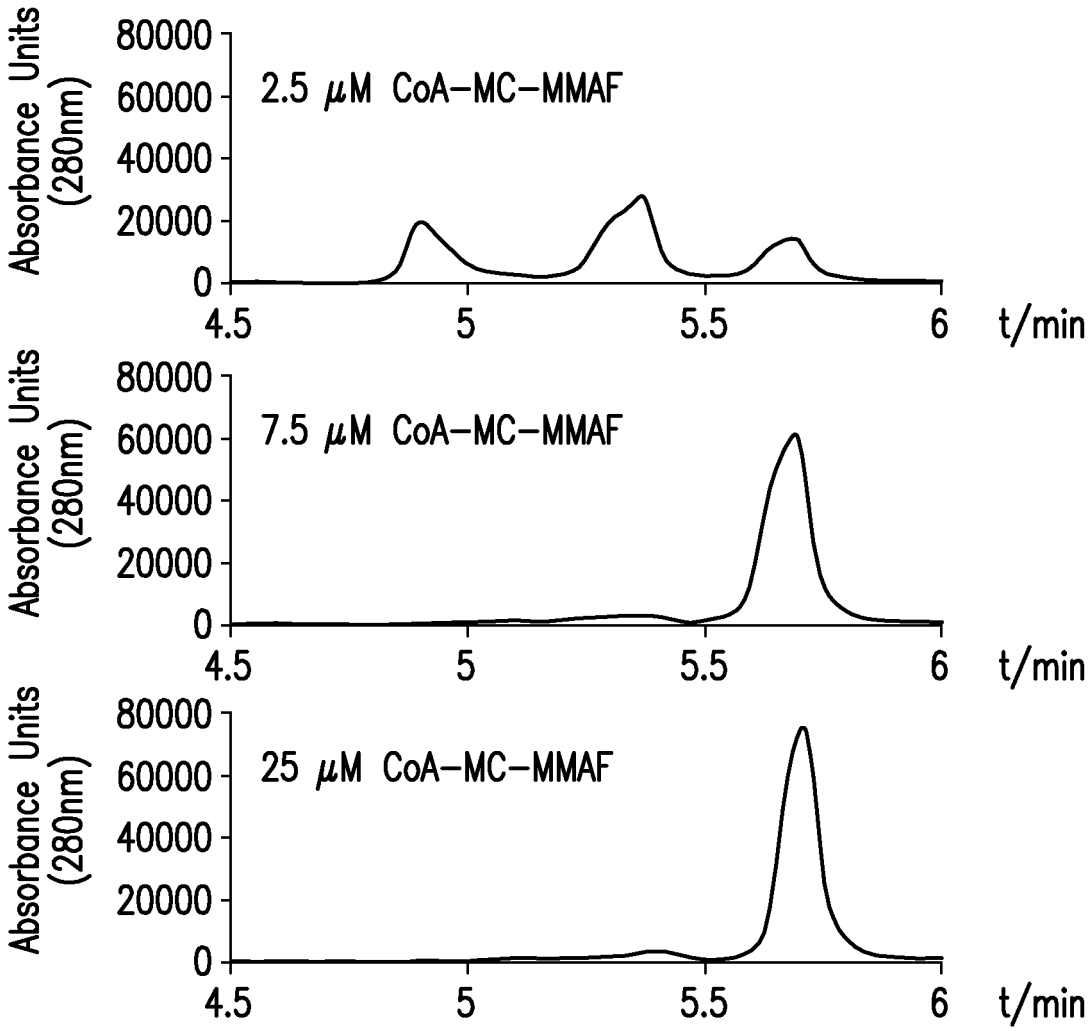

Absorbance Units (280nm)
80000
60000
40000
20000
0
2.5 μM CoA-MC-MMAF
4.5
5
5.5
6
t/min
Absorbance Units (280nm)
80000
60000
40000
20000
0
7.5 μM CoA-MC-MMAF
4.5
5
5.5
6
t/min
Absorbance Units (280nm)
80000
60000
40000
20000
0
25 μM CoA-MC-MMAF
4.5
5
5.5
6
t/min

FIG.14A

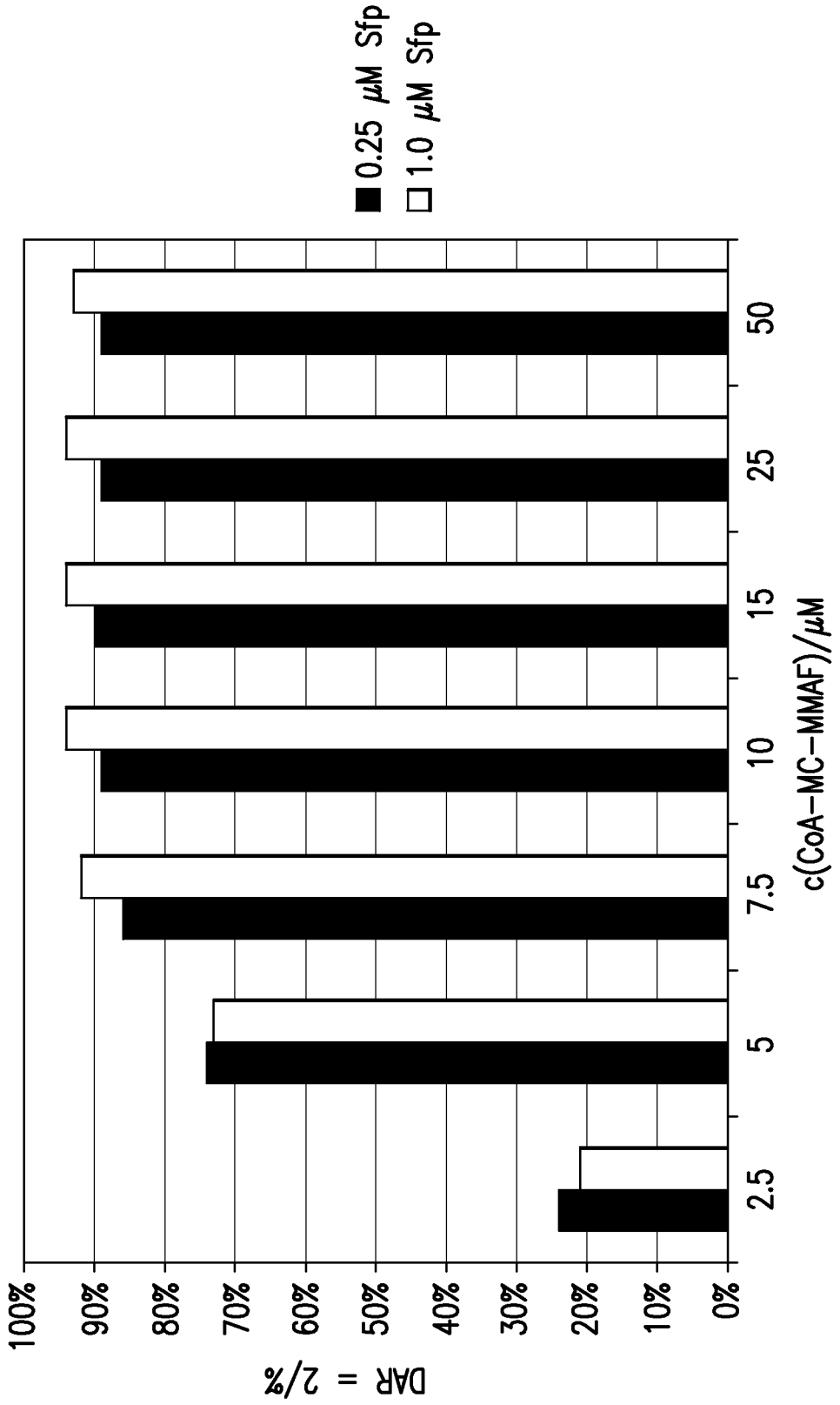

0.25 μM Sfp
1.0 μM Sfp
DAR = 2/%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2.5
5
7.5
10
15
25
50
c(CoA-MC-MMAF)/μM

FIG.14B

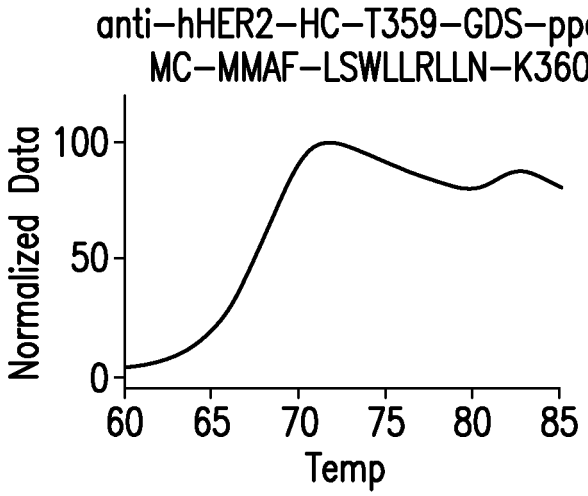


FIG.15A

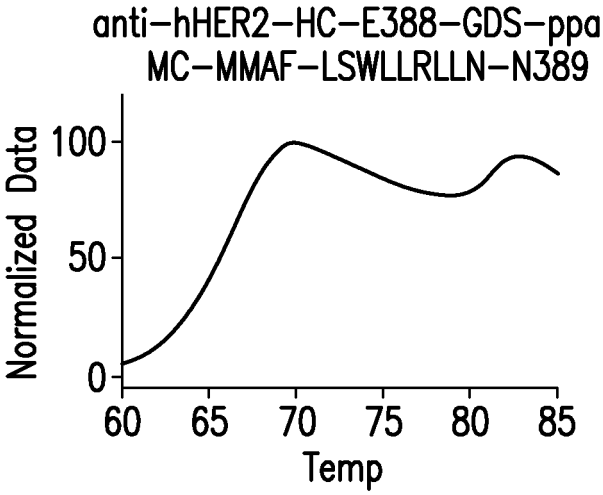


FIG.15B

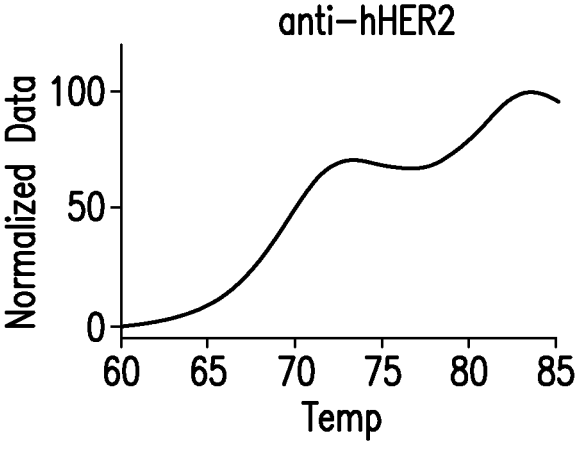


FIG.15C

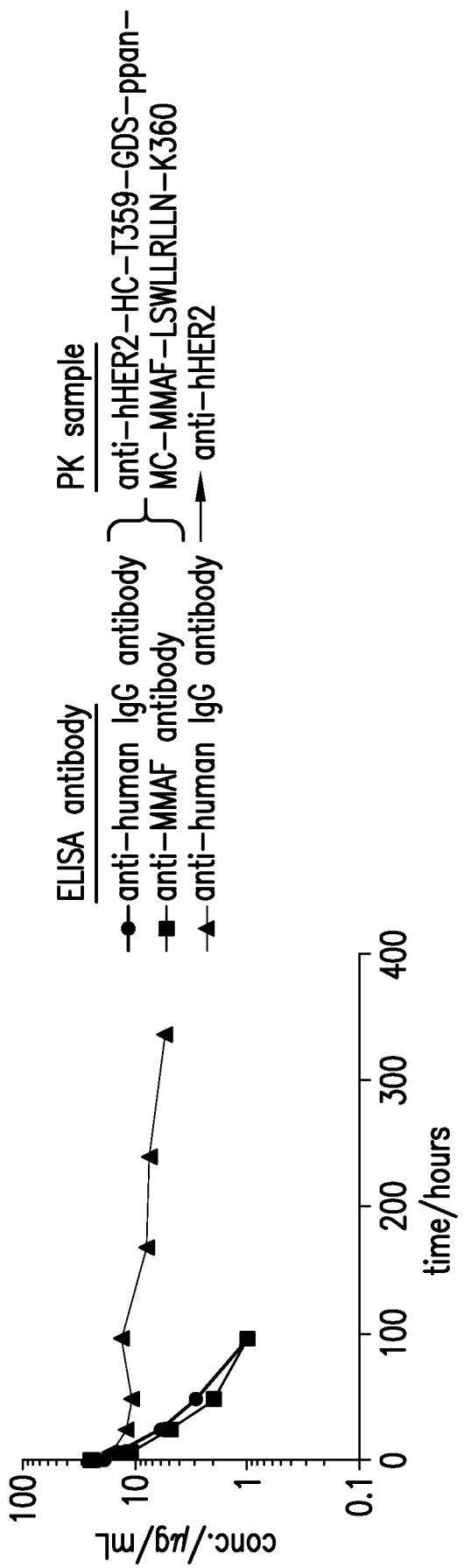


FIG.16A



FIG.16B

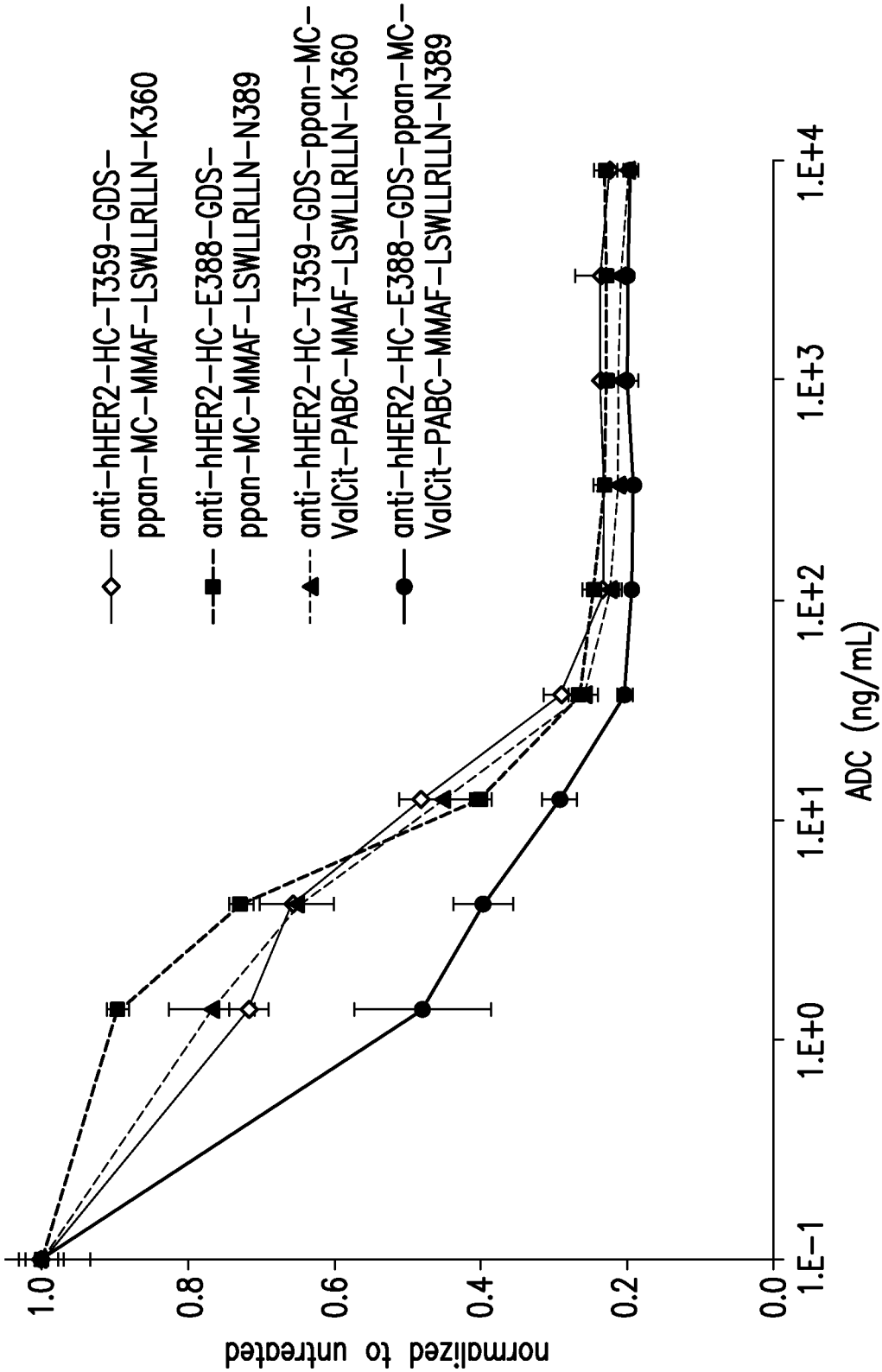

anti-hHER2-HC-T359-GDS-ppan-MC-MMAF-LSWLLRLLN-K360
anti-hHER2-HC-E388-GDS-ppan-MC-MMAF-LSWLLRLLN-N389
anti-hHER2-HC-T359-GDS-ppan-MC-ValCit-PABC-MMAF-LSWLLRLLN-K360
anti-hHER2-HC-E388-GDS-ppan-MC-ValCit-PABC-MMAF-LSWLLRLLN-N389
normalized to untreated
1.0
0.8
0.6
0.4
0.2
0.0
1.E-1
1.E+0
1.E+1
1.E+2
1.E+3
1.E+4
ADC (ng/mL)

FIG.17

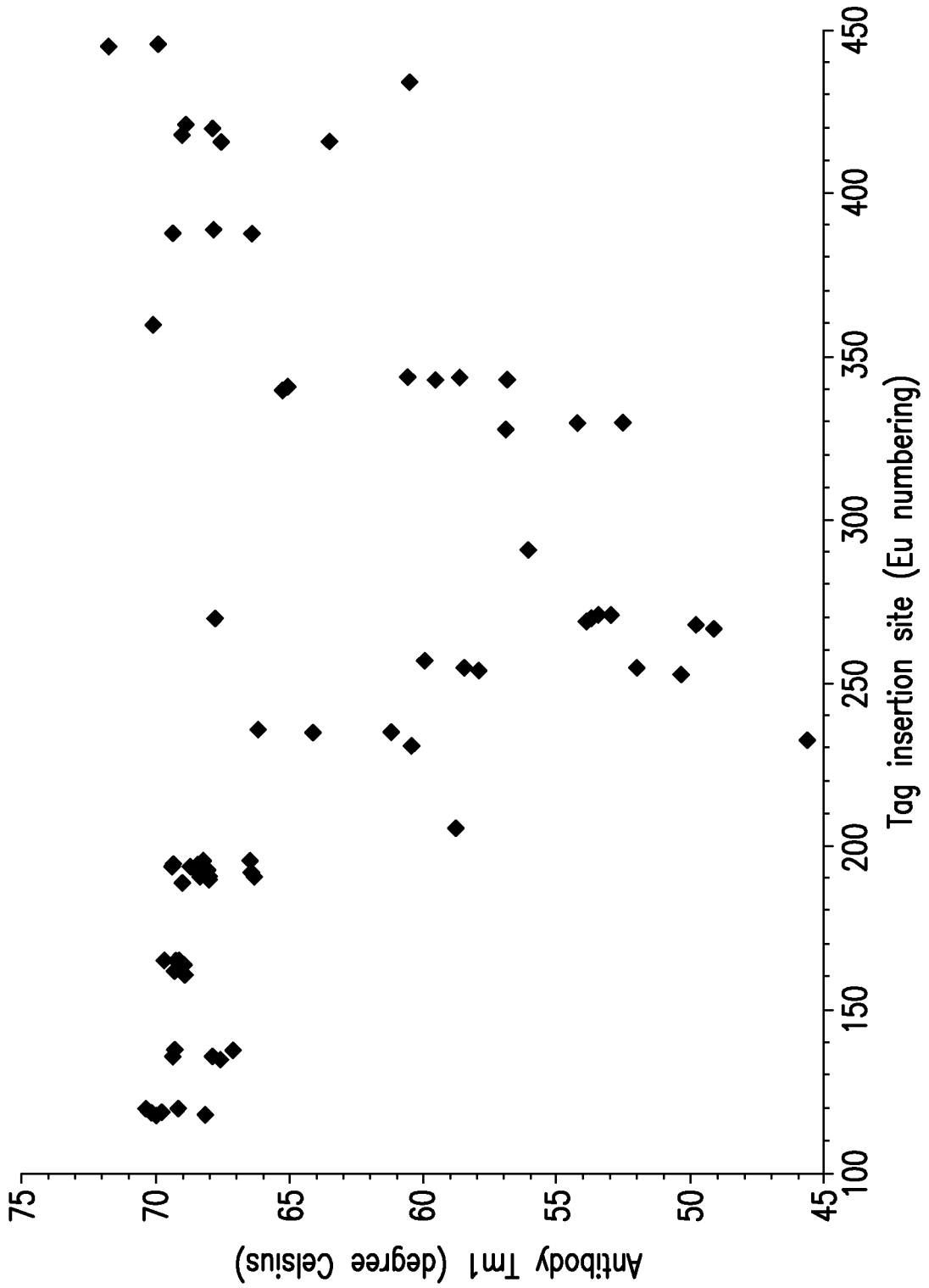

Antibody Tm1 (degree Celsius)
75
70
65
60
55
50
45
Tag insertion site (Eu numbering)
100
150
200
250
300
350
400
450

FIG.18

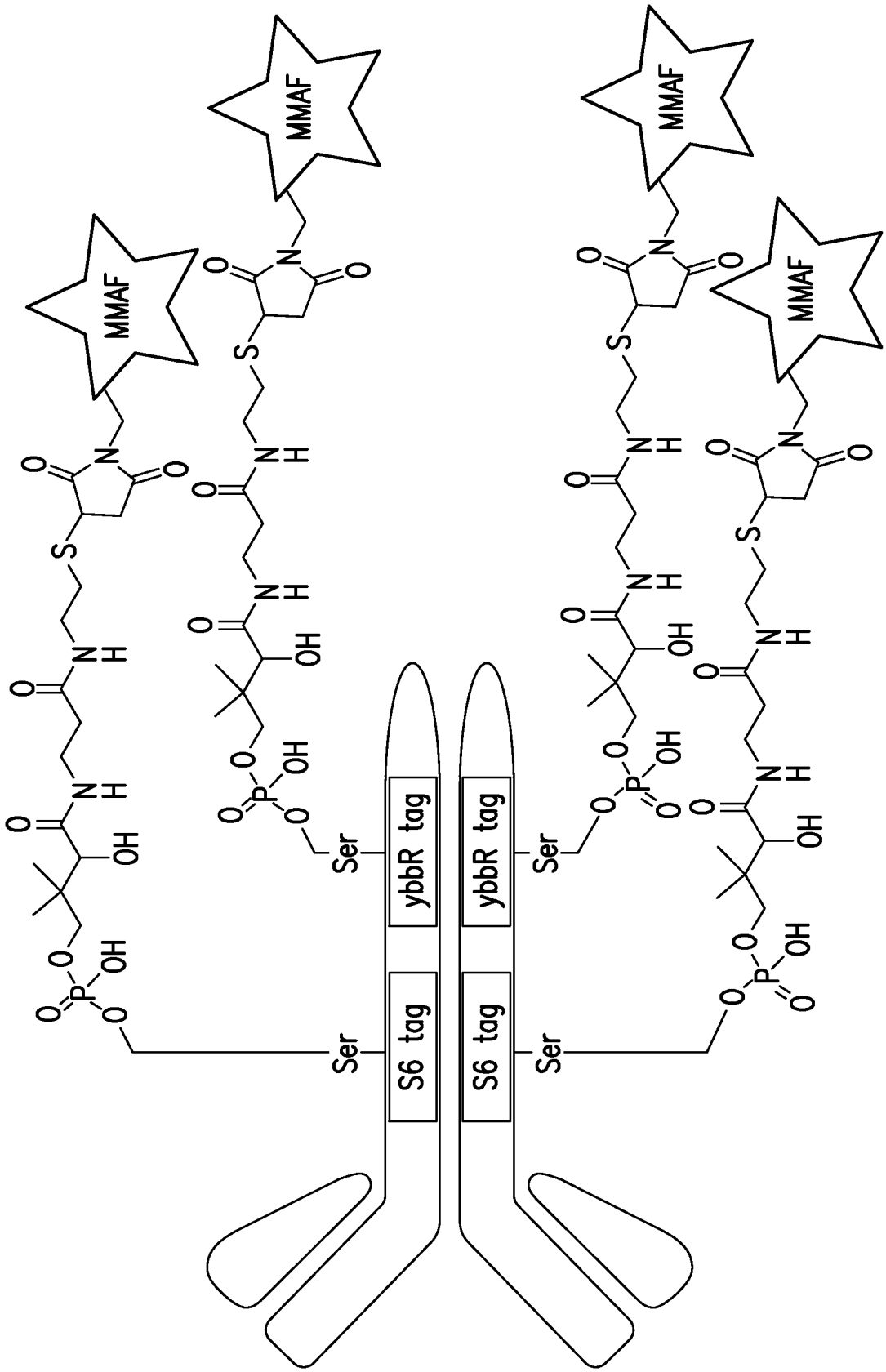

MMAF
MMAF
MMAF
MMAF
ybbR tag
ybbR tag
S6 tag
S6 tag
Ser
Ser
Ser
Ser

FIG.19A

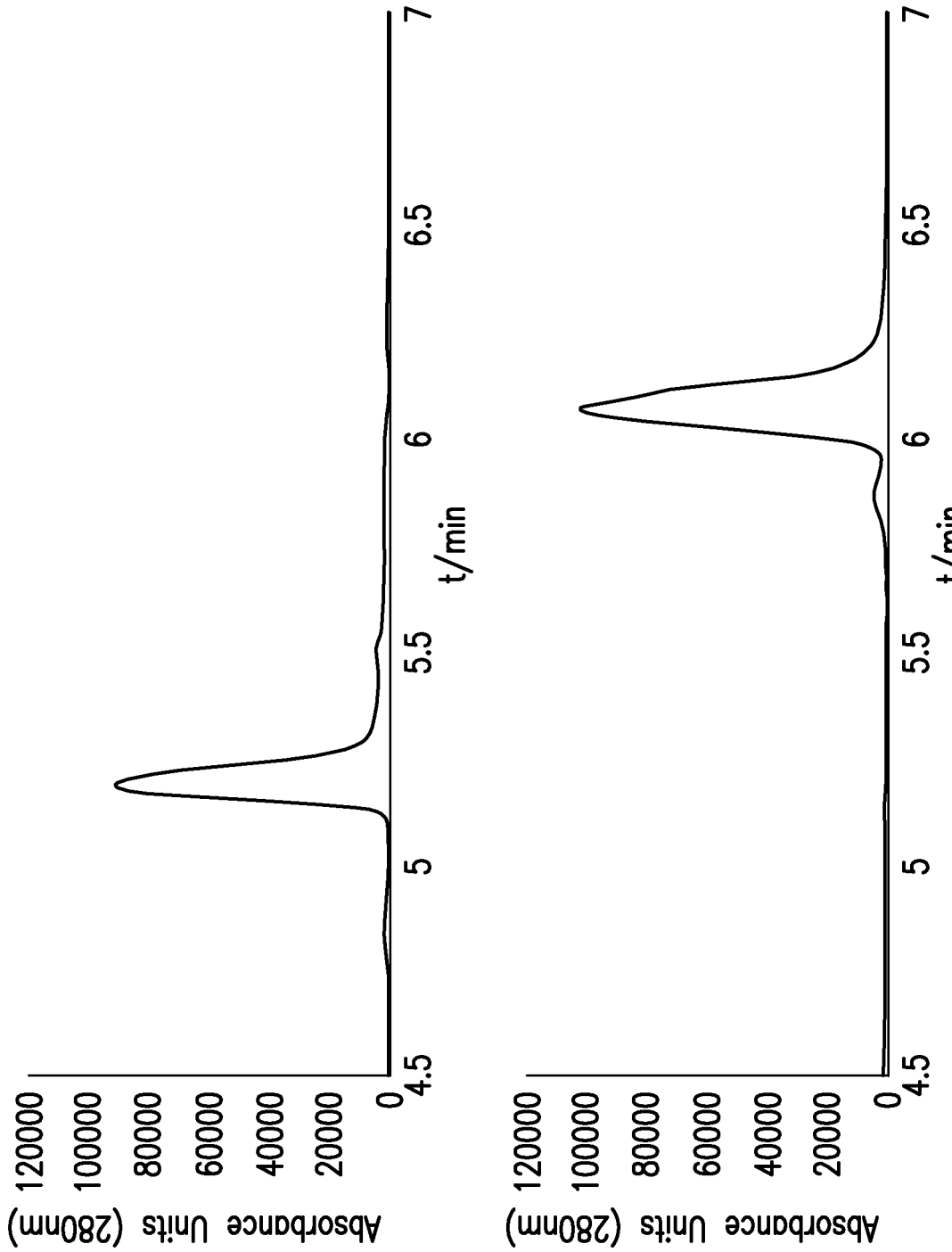

Absorbance Units (280nm)
120000
100000
80000
60000
40000
20000
0
4.5
5
5.5
6
6.5
7
t/min
Absorbance Units (280nm)
120000
100000
80000
60000
40000
20000
0
4.5
5
5.5
6
6.5
7
t/min

FIG.19B

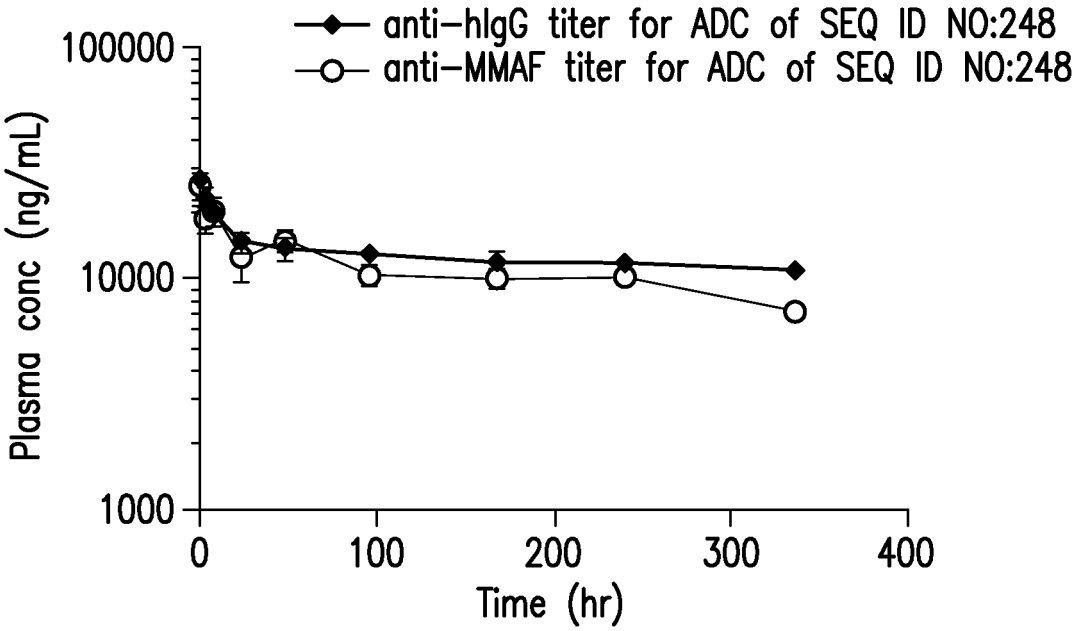


FIG.20A

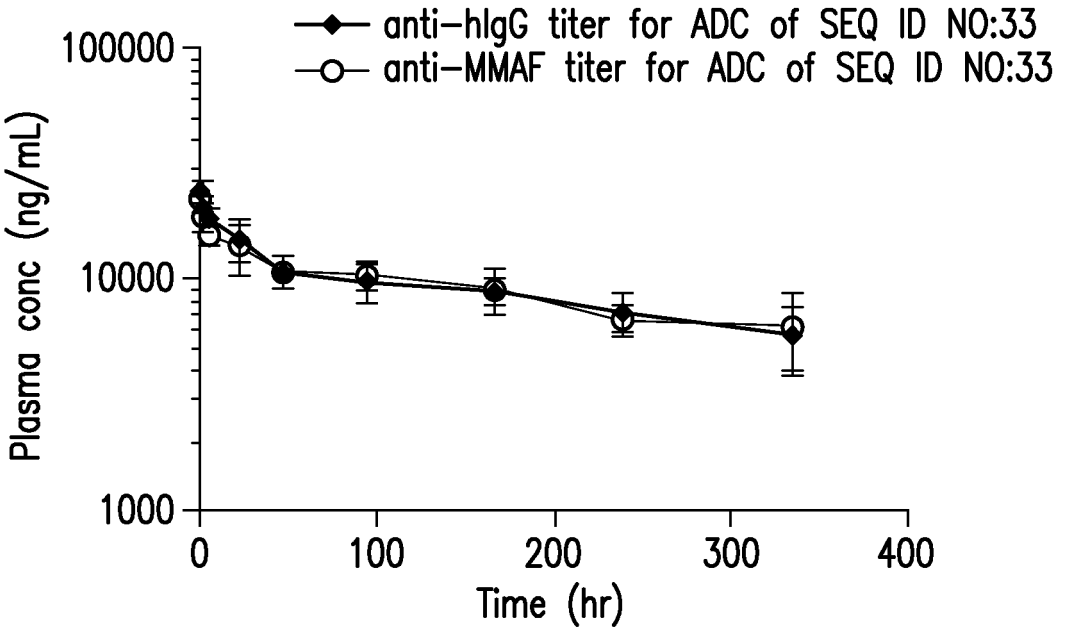


FIG.20B

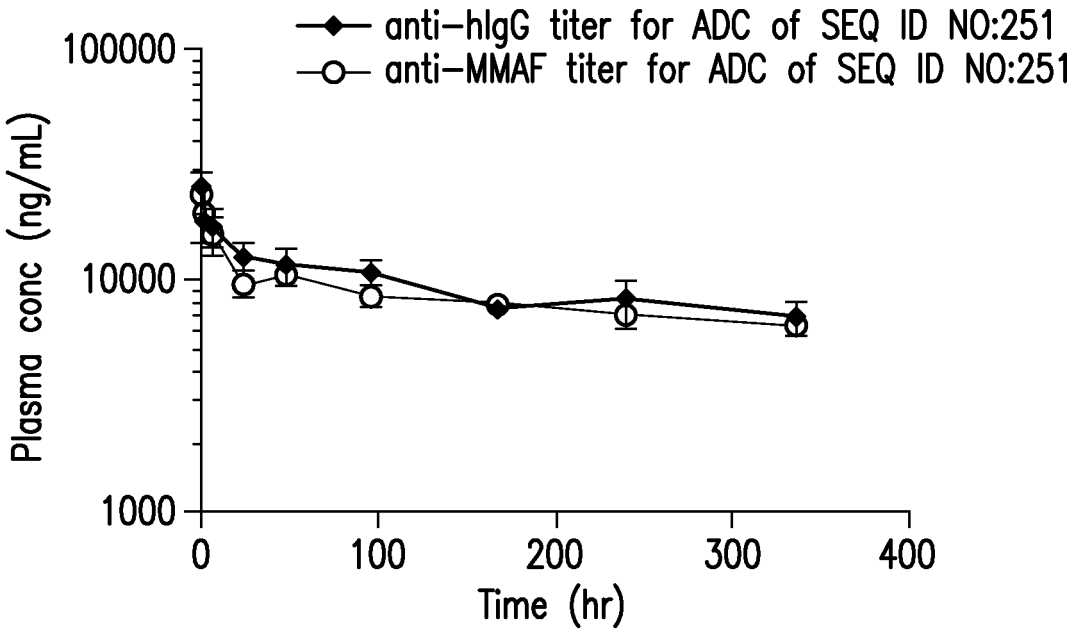


FIG.20C

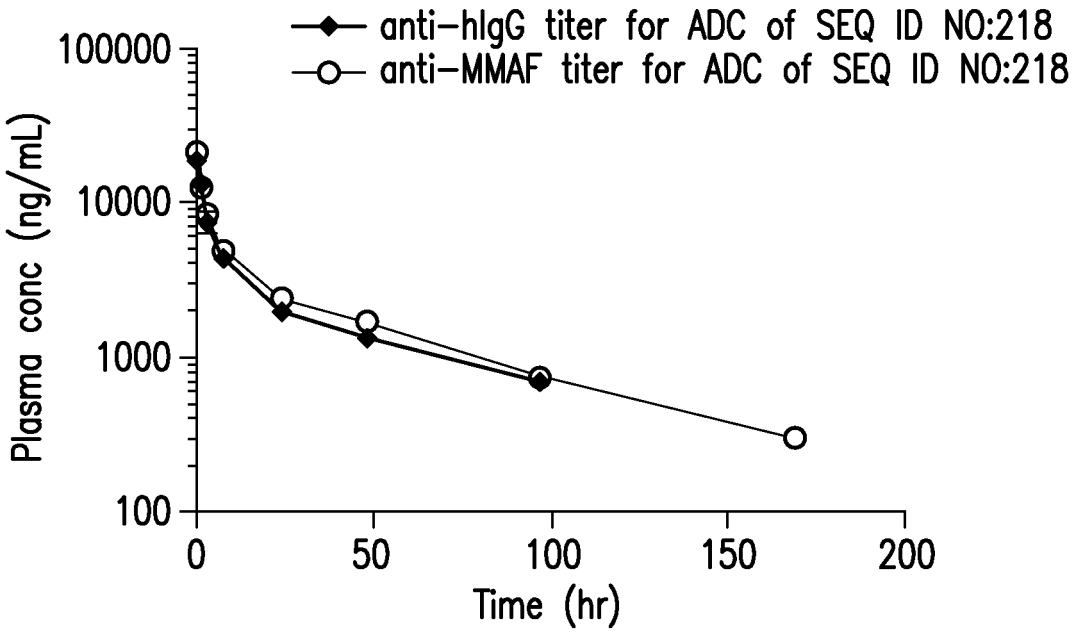


FIG.20D

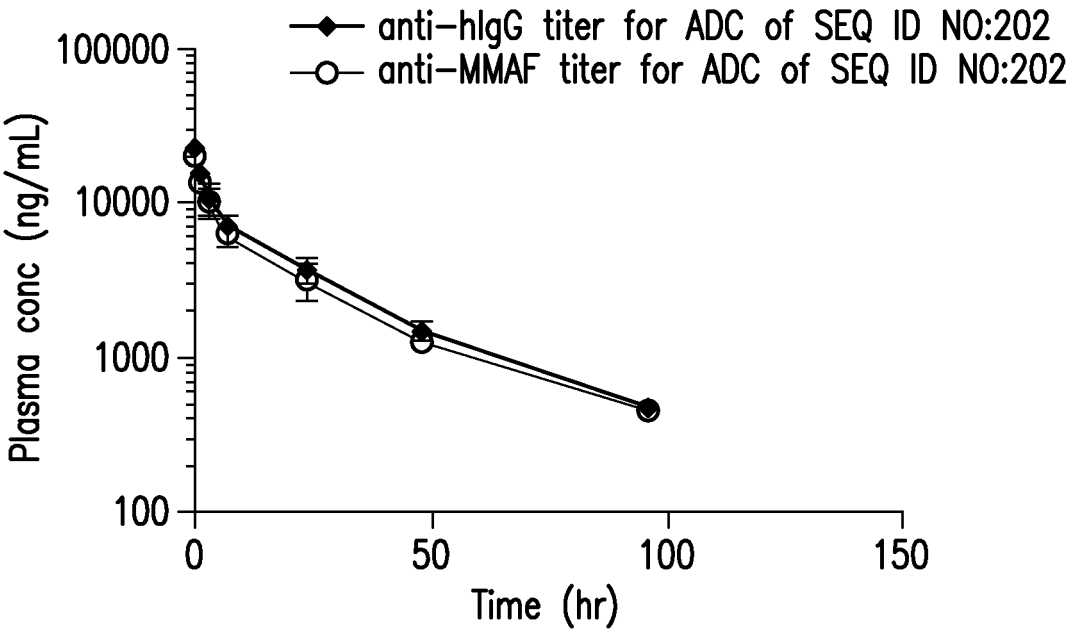


FIG.20E

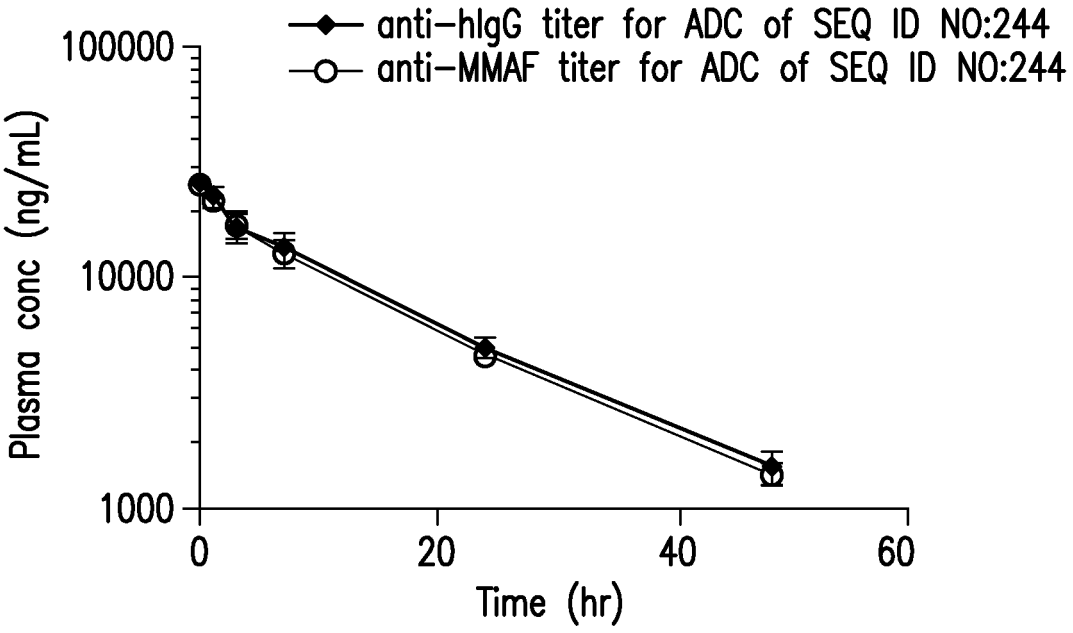


FIG.20F

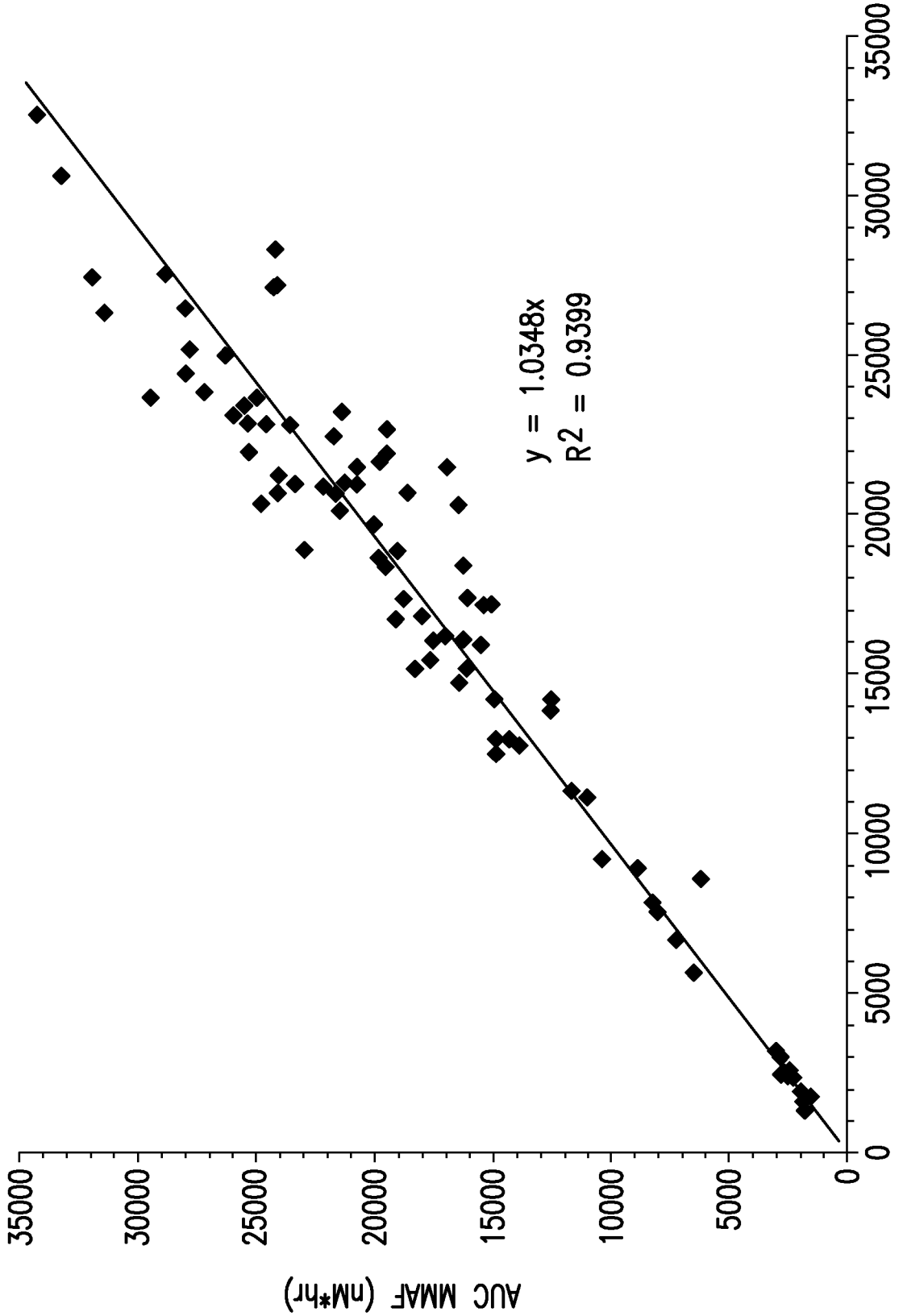

y = 1.0348x
R2 = 0.9399
AUC hIgG (nM*hr)
AUC MMAF (nM*hr)
0
5000
10000
15000
20000
25000
30000
35000

FIG.21

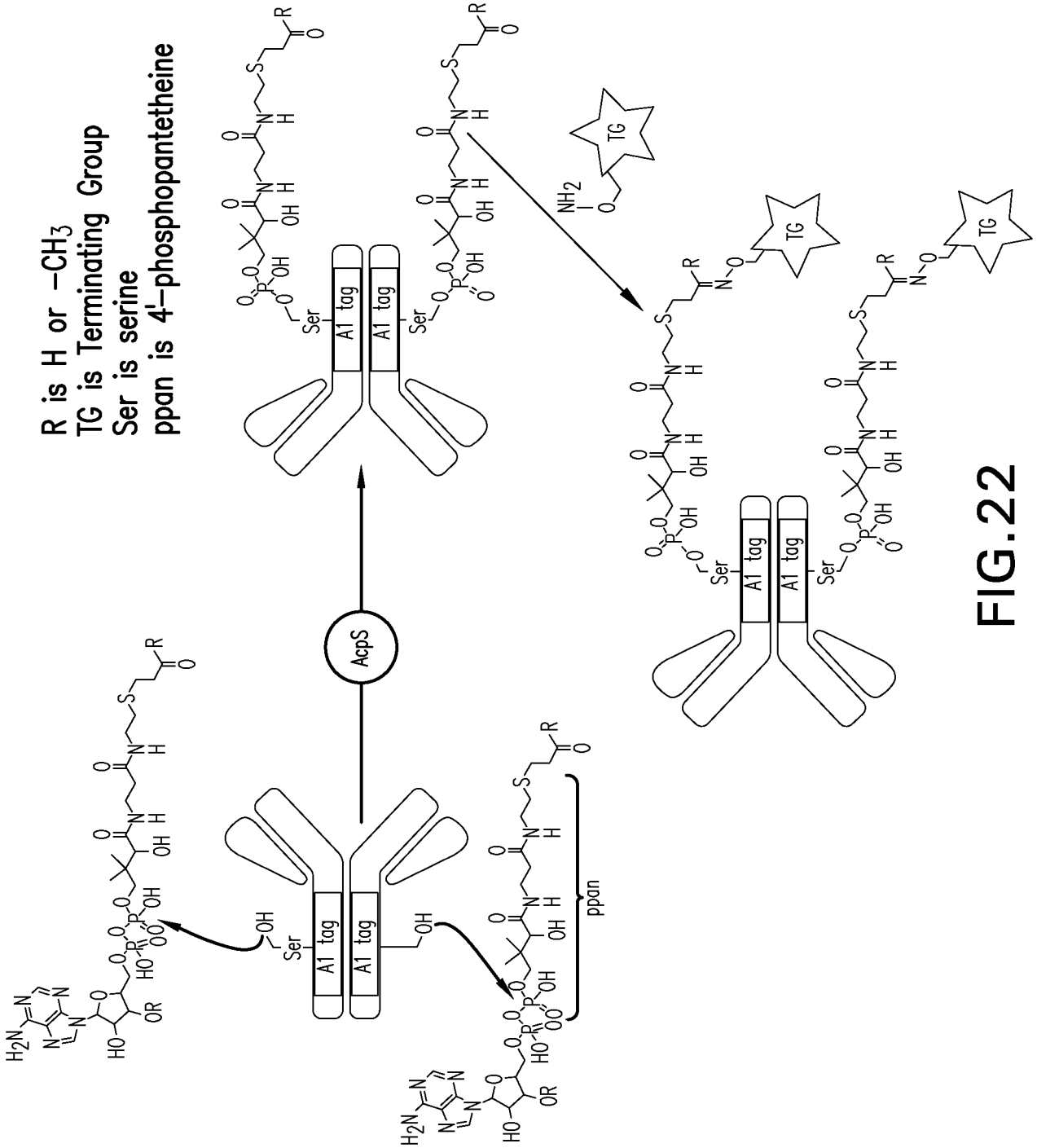

R is H or –CH3
TG is Terminating Group
Ser is serine
ppan is 4'–phosphopantetheine
AcpS
A1 tag
Ser
ppan
TG
NH2

FIG.22

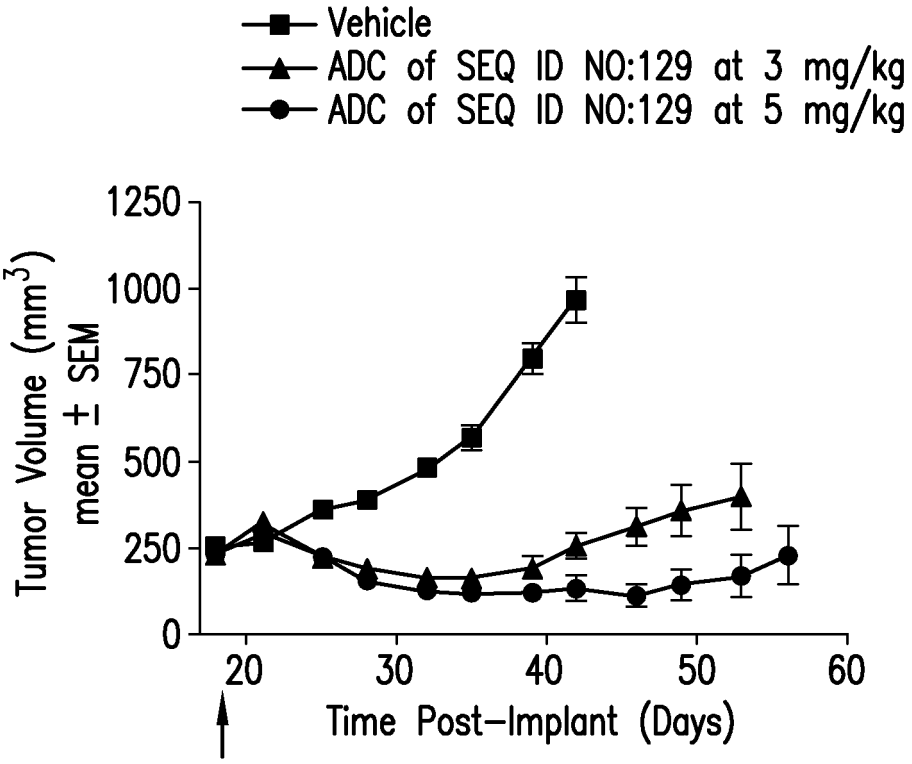

Vehicle
ADC of SEQ ID NO:129 at 3 mg/kg
ADC of SEQ ID NO:129 at 5 mg/kg
Tumor Volume (mm^3)
mean ± SEM
1250
1000
750
500
250
0
20
30
40
50
60
Time Post-Implant (Days)

FIG.23