Figure 1

DNA Sequences of the wt IgG binding domains of Protein A

SEQ ID NO:1- E domain DNA sequence
GCGCAACAAAACGCTTTCTATCAGGTACTGAACATGCCTAACCTGAACGCCGATCAGCG
TAACGGCTTCATCCAAAGCCTGAAGGACGACCCGAGCCAGTCCGCAAACGTTCTGGGTG
AAGCTCAAAAACTGAACGACAGCCAGGCACCGAAAGCTGAC

SEQ ID NO:2- D domain DNA sequence
GCCCAACAGAACAAATTTAACAAAGACCAGCAGTCCGCGTTCTACGAGATTCTGAACAT
GCCTAACCTGAATGAAGAACAGCGCAACGGTTTTATTCAGTCTCTGAAGGACGATCCTT
CTCAATCCACCAACGTACTGGGCGAAGCGAAGAAACTGAACGAATCTCAGGCTCCGAAG

SEQ ID NO:3- A domain DNA sequence
GCCGACAACAACTTCAACAAAGAGCAGCAAAACGCTTTCTACGAAATCCTGAATATGCC
AAATCTGAACGAAGAGCAGCGTAACGGTTTCATCCAATCTCTGAAAGACGATCCGTCCC
AGTCCGCGAATCTGCTGGCGGAGGCTAAAAAGCTGAACGAATCCCAGGCTCCGAAA

SEQ ID NO:4- B domain DNA sequence
GCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCCTGCATCTGCC
GAACCTGAACGAAGAACAACGCAACGGTTTCATTCAGAGCCTGAAAGACGACCCATCTC
AGTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAA

SEQ ID NO:5- C domain DNA sequence
GCGGATAACAAATTCAACAAGGAGCAACAGAACGCATTCTATGAAATTCTGCACCTGCC
GAATCTGACGGAGGAGCAACGTAACGGCTTTATCCAGTCCCTGAAGGATGATCCGTCTG
TGTCTAAAGAGATCCTGGCGGAGGCAAAAAAACTGAATGATGCACAAGCTCCGAAA

Figure 2

SEQ ID NO:6- Z domain DNA sequence

GTAGACAACAAATTCAATAAAGAACAGCAGAACGCTTTCTATGAAATCCTGCACCTGCC
GAACCTGAACGAAGAACAGCGTAACGCGTTTATCCAGTCCCTGAAAGACGACCCGAGCC
AGAGCGCAAATCTGCTGGCGGAAGCGAAAAAGCTGAACGATGCCCAGGCGCCGAAA

Figure 3

IgG binding domain sequence alignment

```
E       ----------AQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDSQAPK 51 (SEQ ID NO:7)
D       ADAQQNKFNKDQQSAFYEILNMPNLNEEQRNGFIQSLKDDPSQSTNVLGEAKKLNESQAPK 61 (SEQ ID NO:8)
A       --ADNN-FNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNESQAPK 58 (SEQ ID NO:9)
B       ---ADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDAQAPK 58 (SEQ ID NO:10)
C       ---ADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDAQAPK 58 (SEQ ID NO:11)
            :      ** ***:::*::***. :************** * ::*.**:***::****
```

Figure 4

SEQ ID NO:12- Z domain amino acid sequence
VDNKFNKEQQ NAFYEILHLP NLNEEQRNAF IQSLKDDPSQ SANLLAEAKKL NDAQAPK

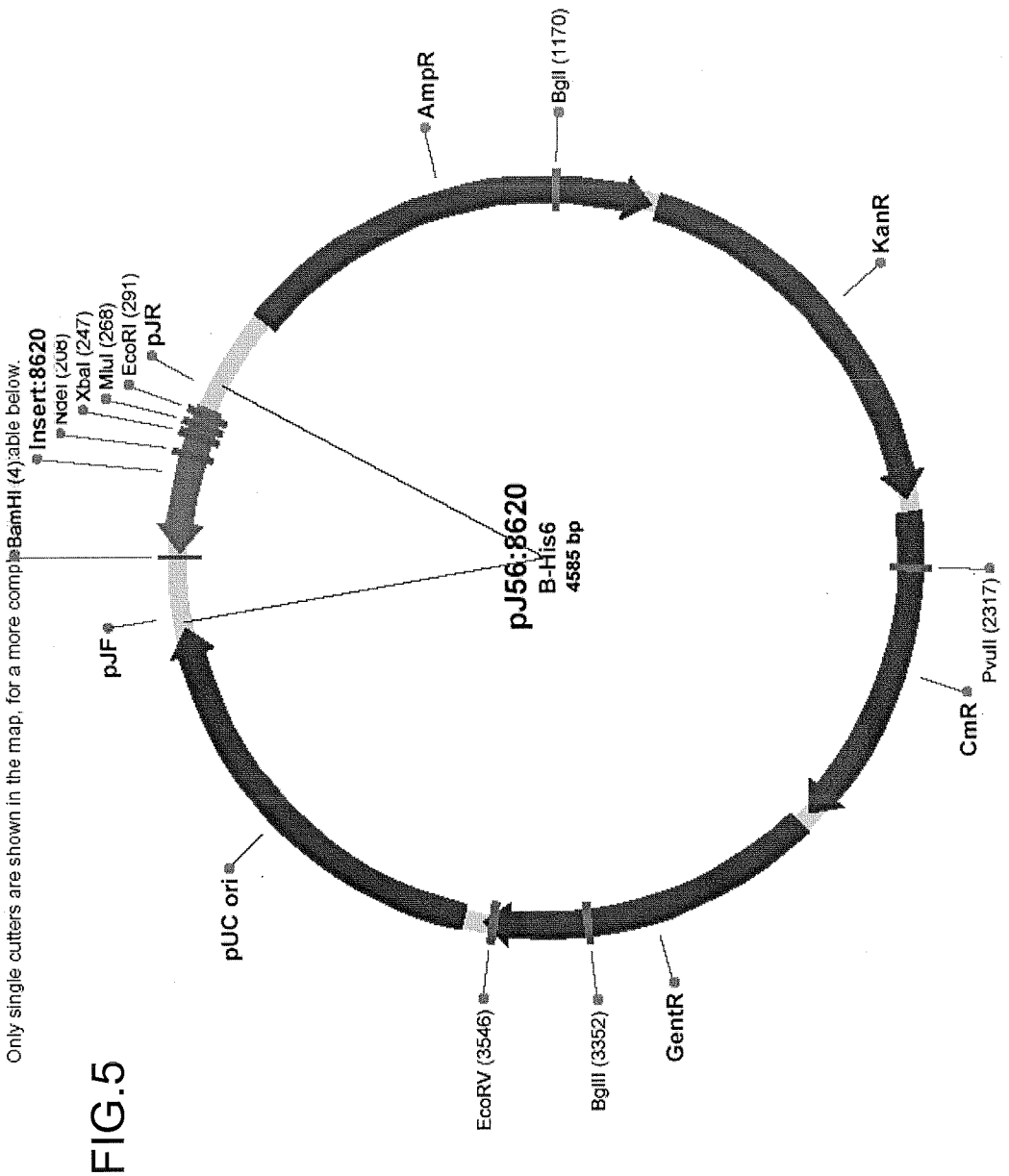

Only single cutters are shown in the map, for a more comp
BamHI (4)able below.
Insert:8620
NdeI (208)
XbaI (247)
MluI (268)
EcoRI (291)
pJR
pJF
pUC ori
EcoRV (3546)
BglII (3352)
GentR
CmR
PvuII (2317)
KanR
BglI (1170)
AmpR
pJ56:8620
B-His6
4585 bp

FIG.5

Figure 6

B domain variant DNA sequences

SEQ ID NO: 14- G29K
GCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCCTGCATCTGCC
GAACCTGAACGAAGAACAACGCAACAAATTCATTCAGAGCCTGAAAGACGACCCATCTC
AGTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAA

SEQ ID NO: 13- G29L
GCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCCTGCATCTGCC
GAACCTGAACGAAGAACAACGCAACCGCTTCATTCAGAGCCTGAAAGACGACCCATCTC
AGTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAA

SEQ ID NO:15- G29R
GCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCCTGCATCTGCC
GAACCTGAACGAAGAACAACGCAACCTGTTCATTCAGAGCCTGAAAGACGACCCATCTC
AGTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAA

Figure 7

B domain variant amino acid sequences

SEQ ID NO:17- G29K
ADNKFNKEQQ NAFYEILHLP NLNEEQRNKF IQSLKDDPSQ SANLLAEAKKL NDAQAPK

SEQ ID NO:16- G29L
ADNKFNKEQQ NAFYEILHLP NLNEEQRNLF IQSLKDDPSQ SANLLAEAKKL NDAQAPK

SEQ ID NO:18- G29R
ADNKFNKEQQ NAFYEILHLP NLNEEQRNRF IQSLKDDPSQ SANLLAEAKKL NDAQAPK

Figure 8

Protein A expression from pET11a:8620/*E. coli* BL21(DE3) was detected by Western blotting with Chicken IgY anti-protein A antibody

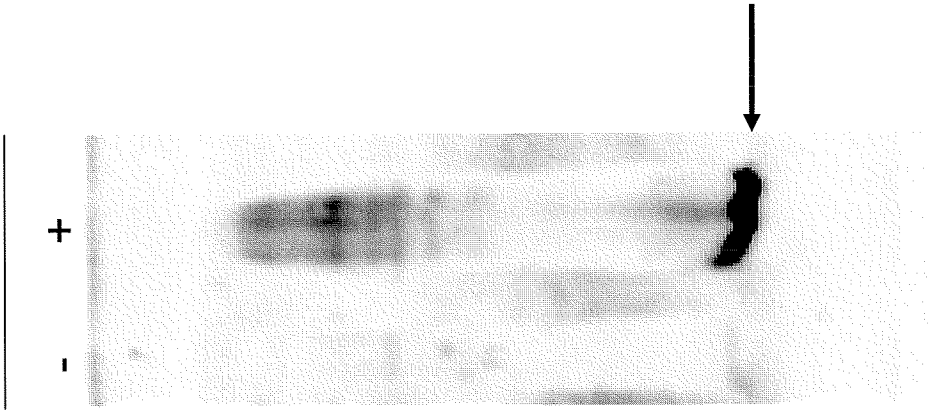


Figure 9

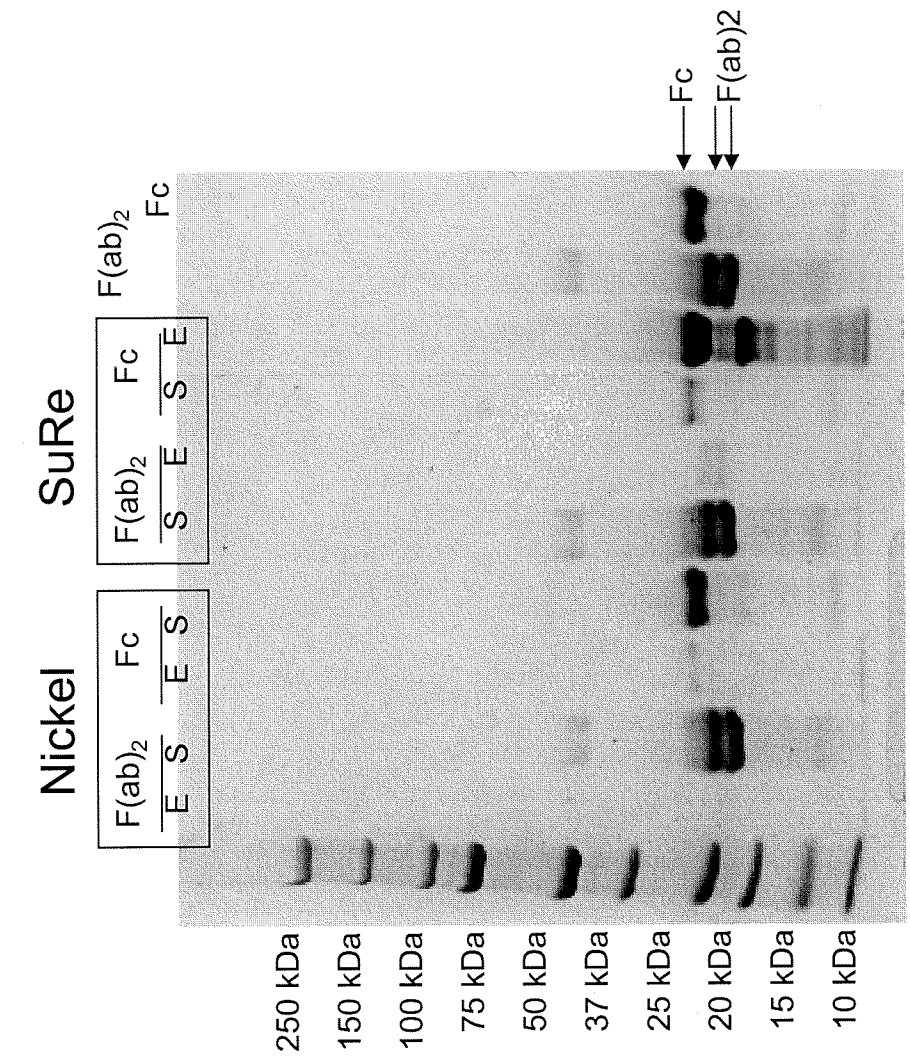

Nickel
SuRe
F(ab)2
Fc
E S
S E
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
Fc
F(ab)2

Figure 10

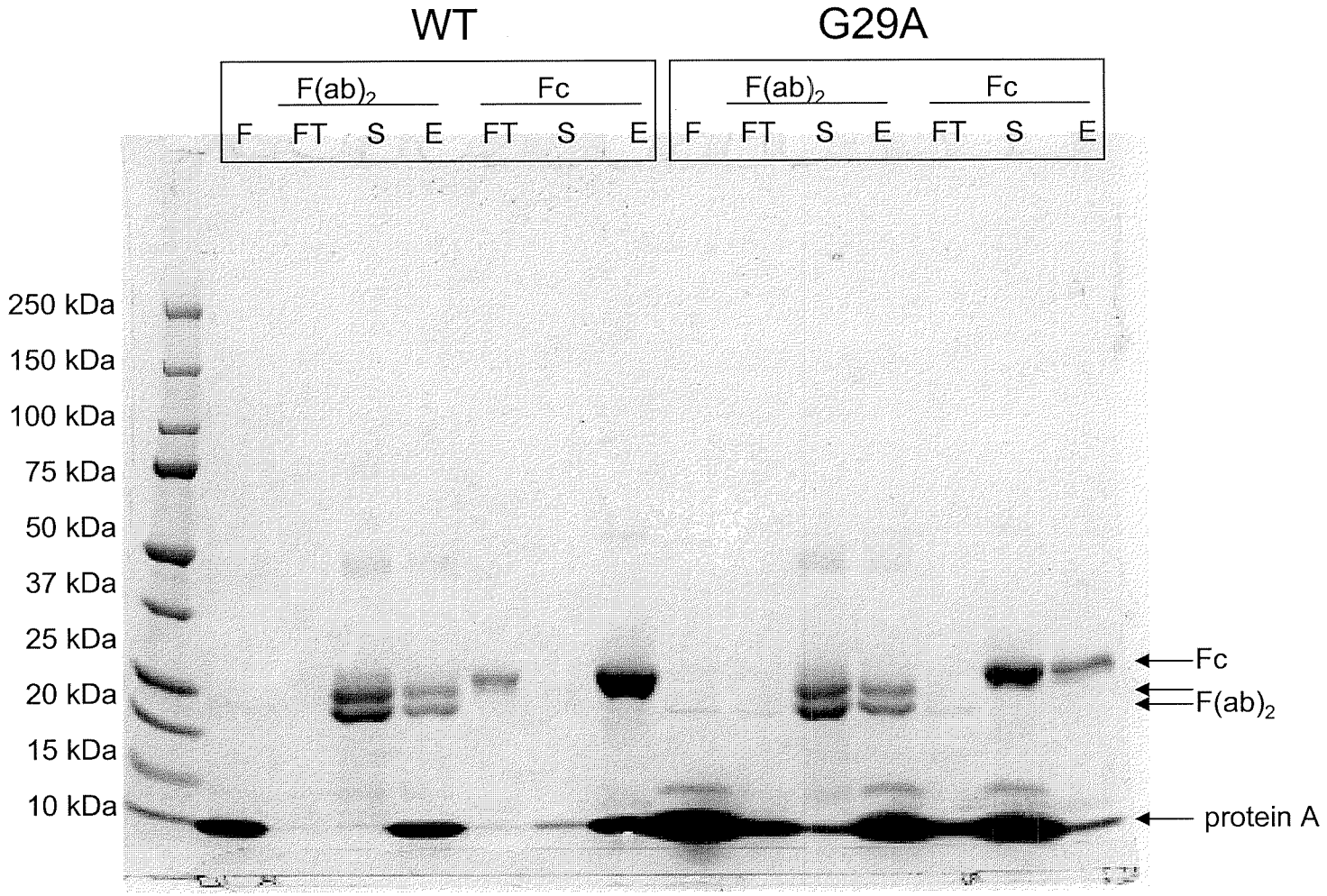

WT
G29A
F(ab)2
Fc
F FT S E FT S E
F FT S E FT S E
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
Fc
F(ab)2
protein A

Figure 11

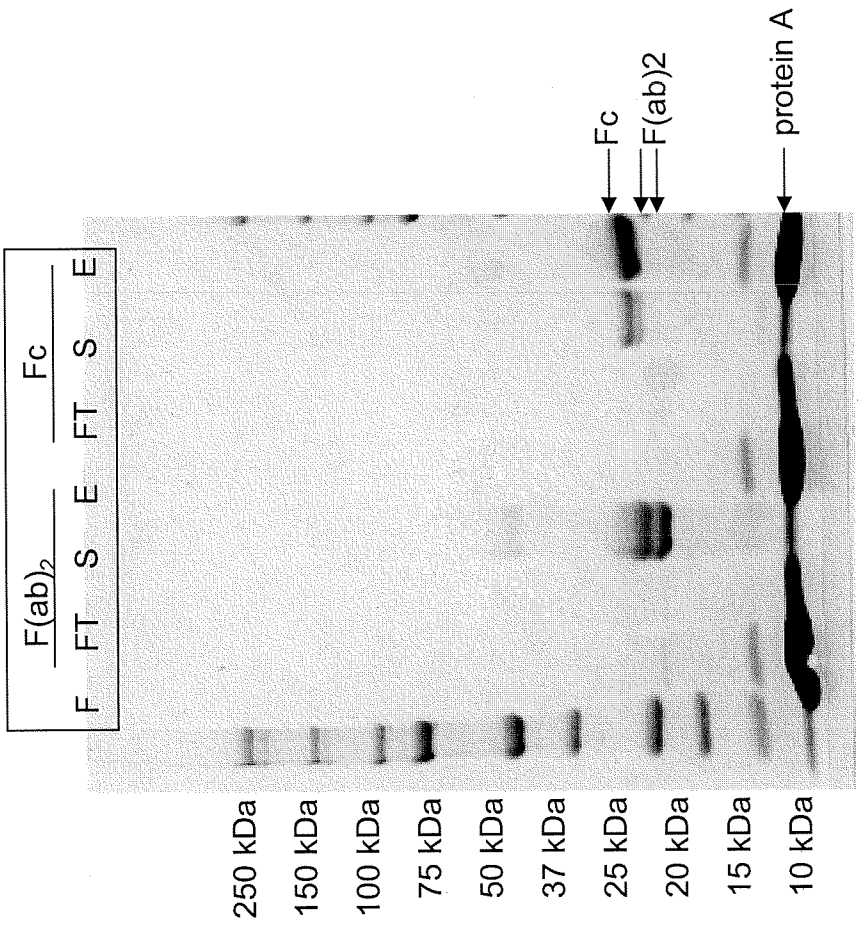

G29L
F
F(ab)2
FT
S
E
Fc
FT
S
E
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
Fc
F(ab)2
protein A

Figure 12

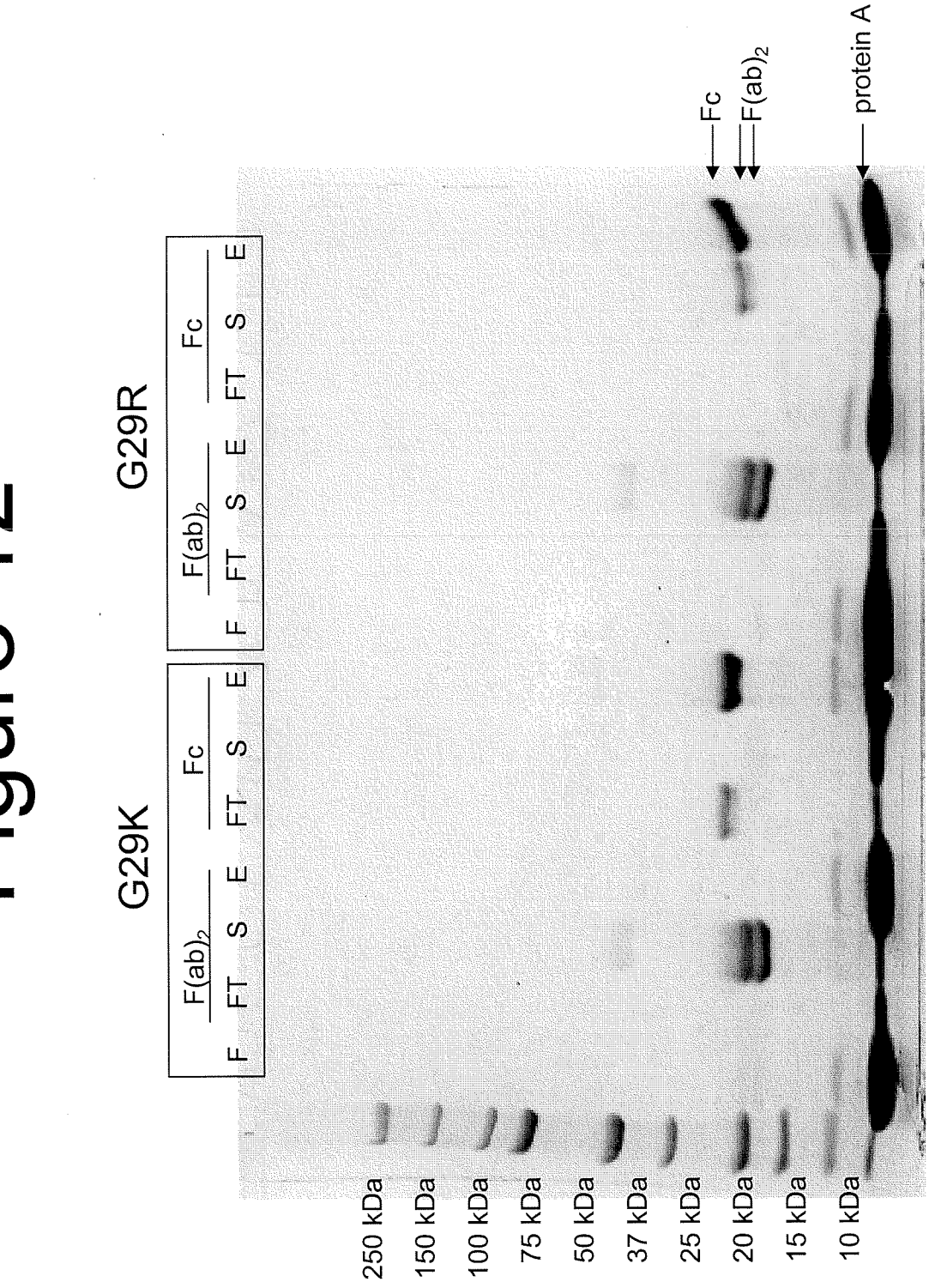

G29K
G29R
F(ab)2
Fc
F
FT
S
E
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
Fc
F(ab)2
protein A

Figure 13A

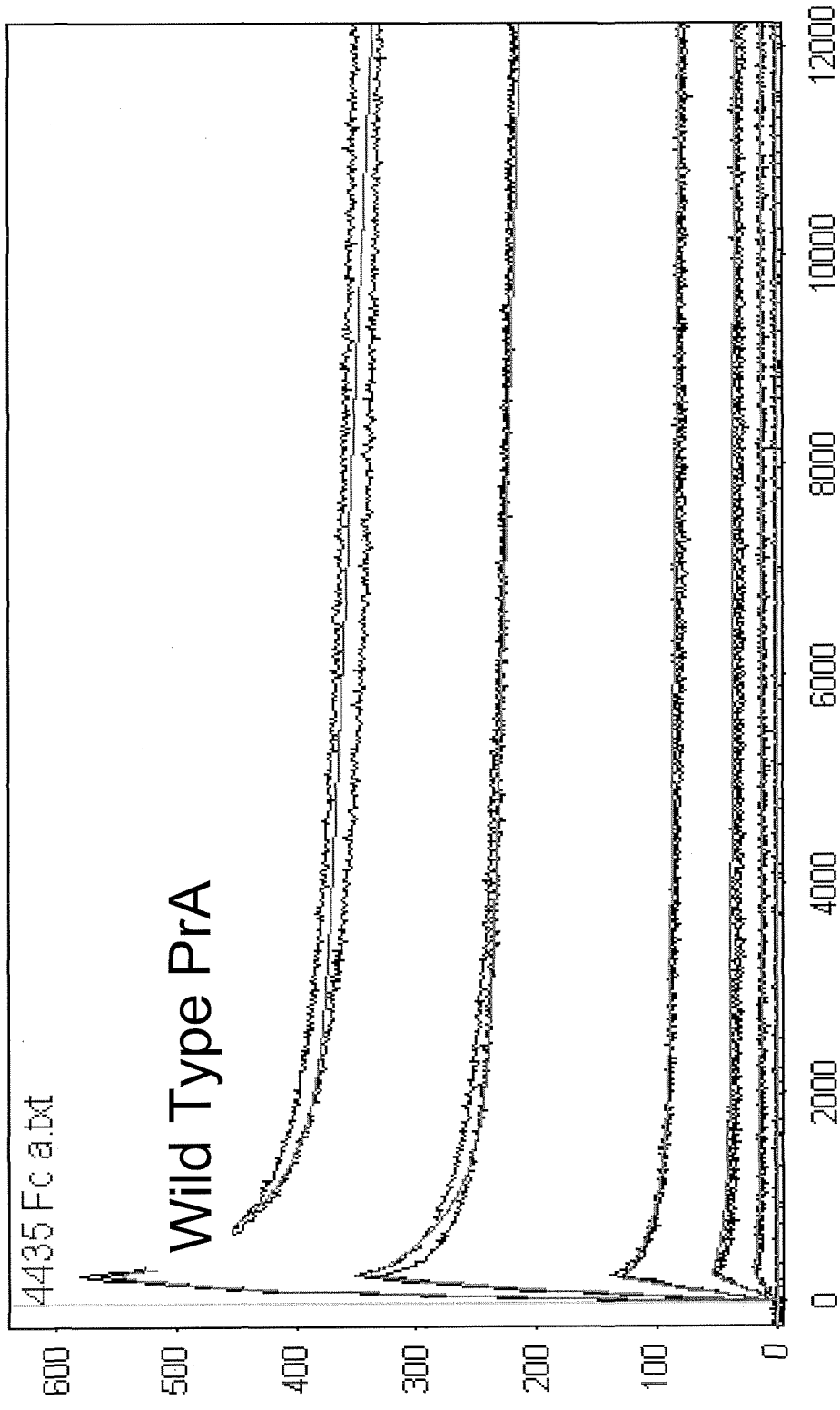

4435Fc a.txt
Wild Type PrA
600
500
400
300
200
100
0
0
2000
4000
6000
8000
10000
12000

Figure 13B

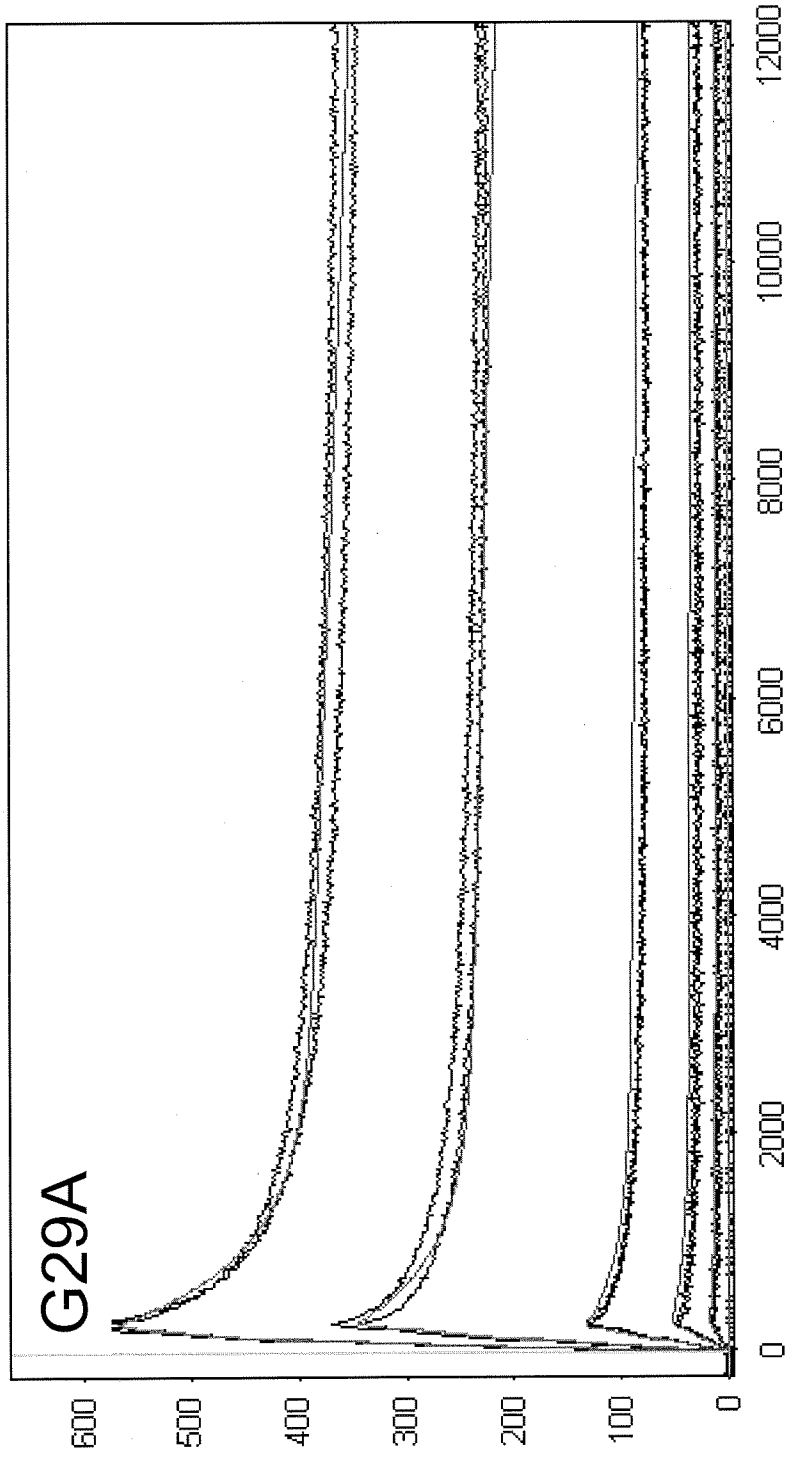

G29A
600
500
400
300
200
100
0
0
2000
4000
6000
8000
10000
12000

Figure 13C

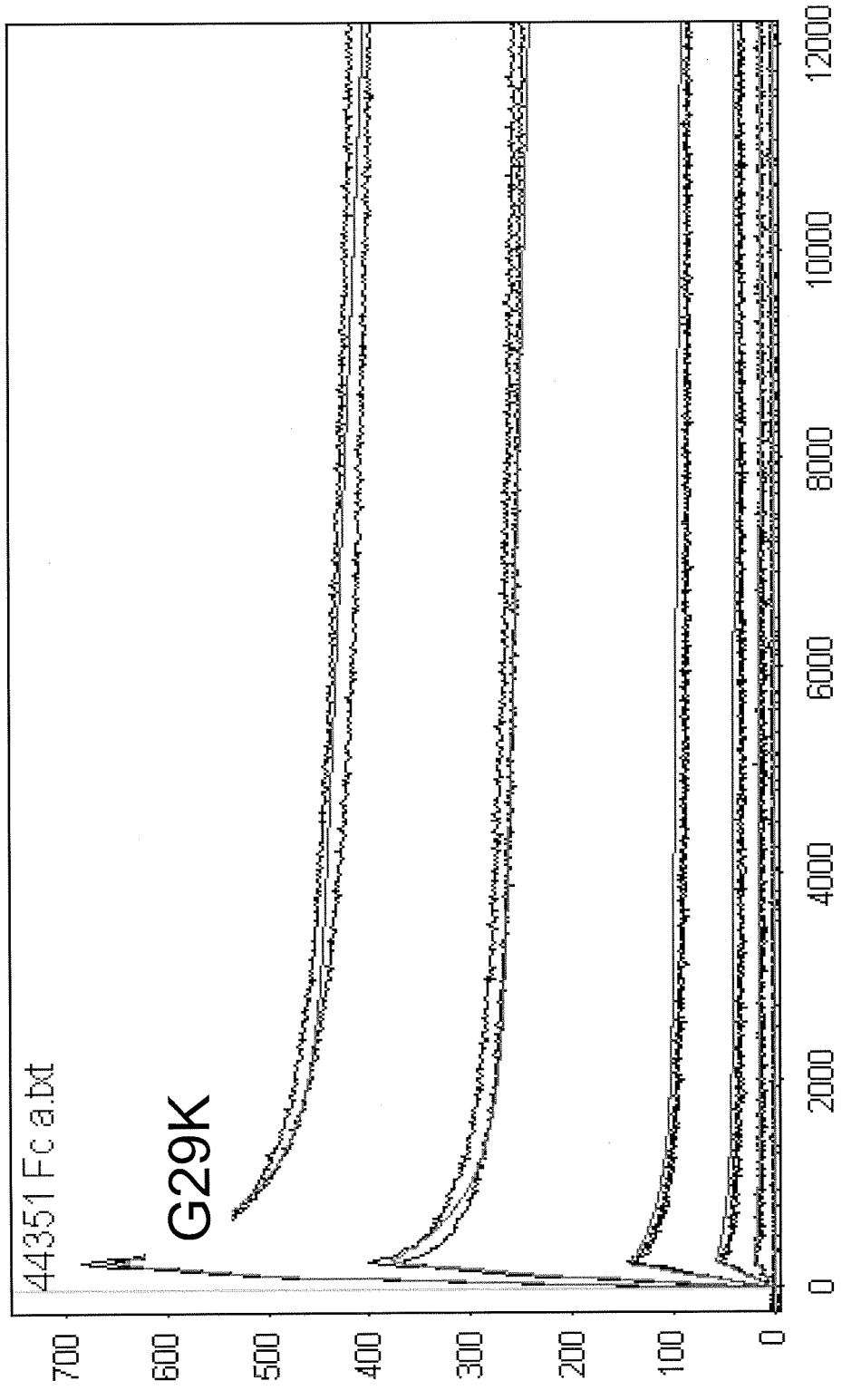

44351 Fc a.txt
G29K
700
600
500
400
300
200
100
0
0
2000
4000
6000
8000
10000
12000

Figure 13D

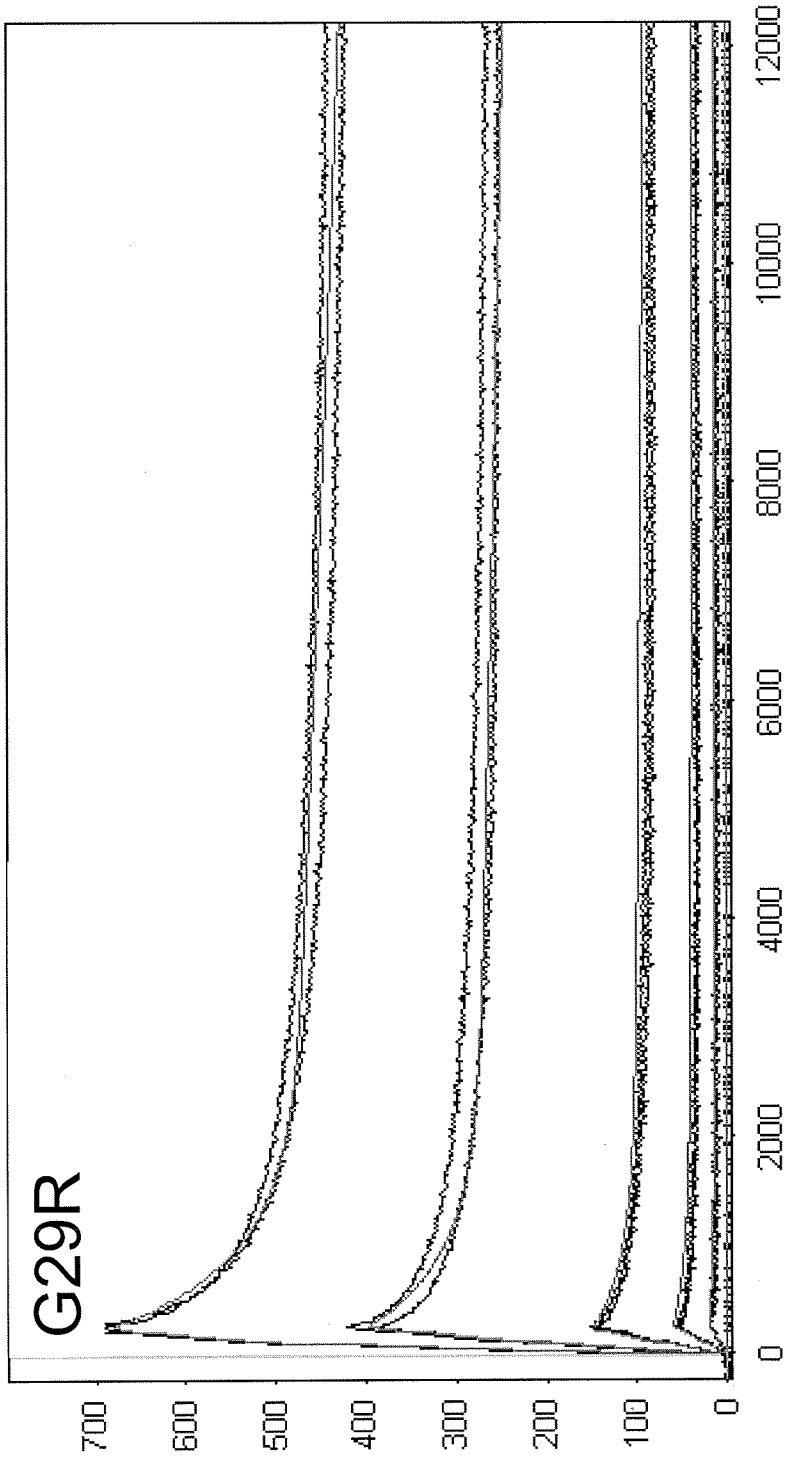

G29R
700
600
500
400
300
200
100
0
0
2000
4000
6000
8000
10000
12000

Figure 13E

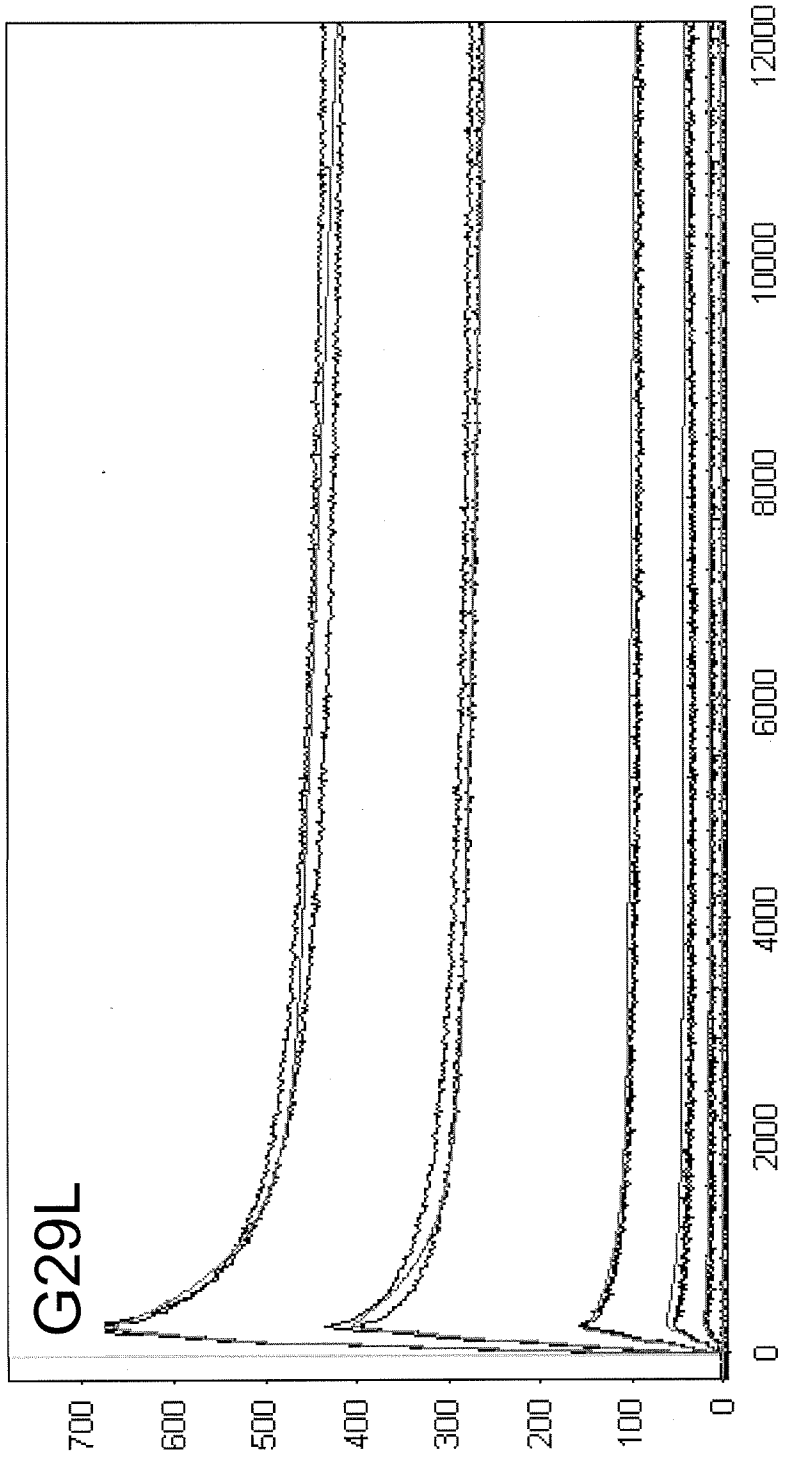

G29L
700
600
500
400
300
200
100
0
0
2000
4000
6000
8000
10000
12000

Figure 14A

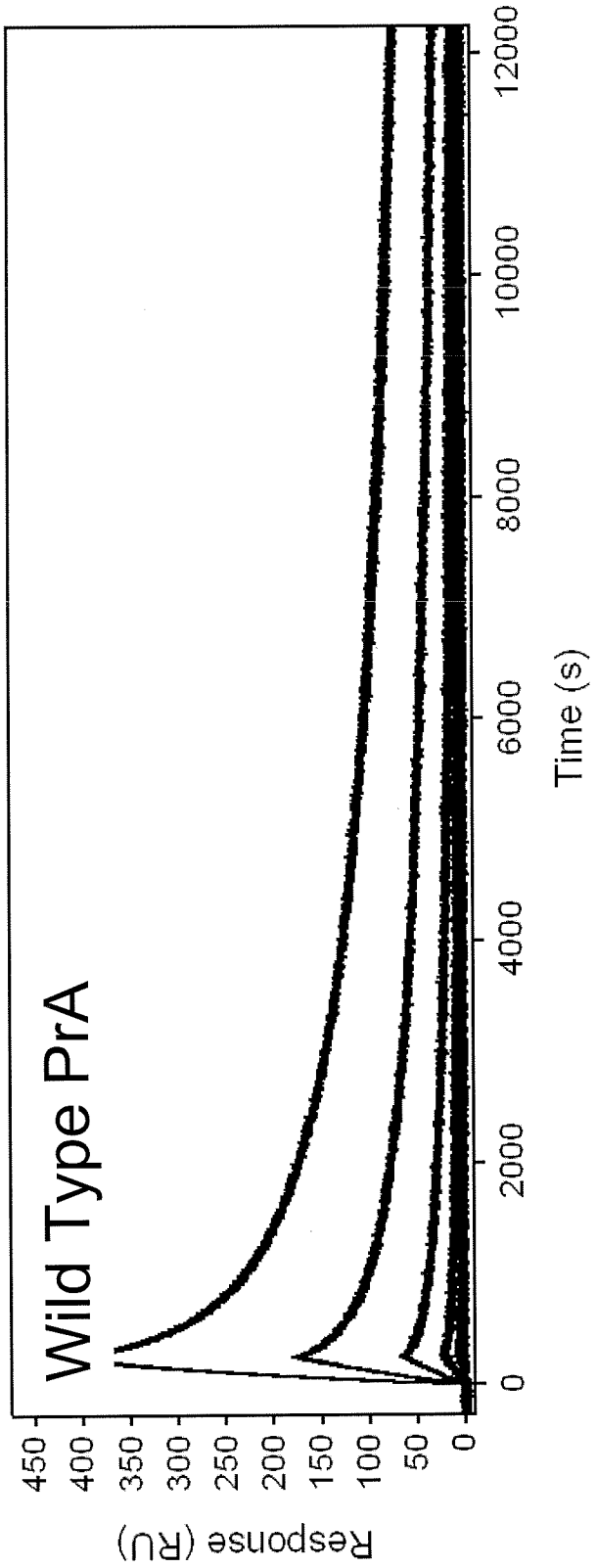

Wild Type PrA
Response (RU)
0
50
100
150
200
250
300
350
400
450
0
2000
4000
6000
8000
10000
12000
Time (s)

Figure 14B

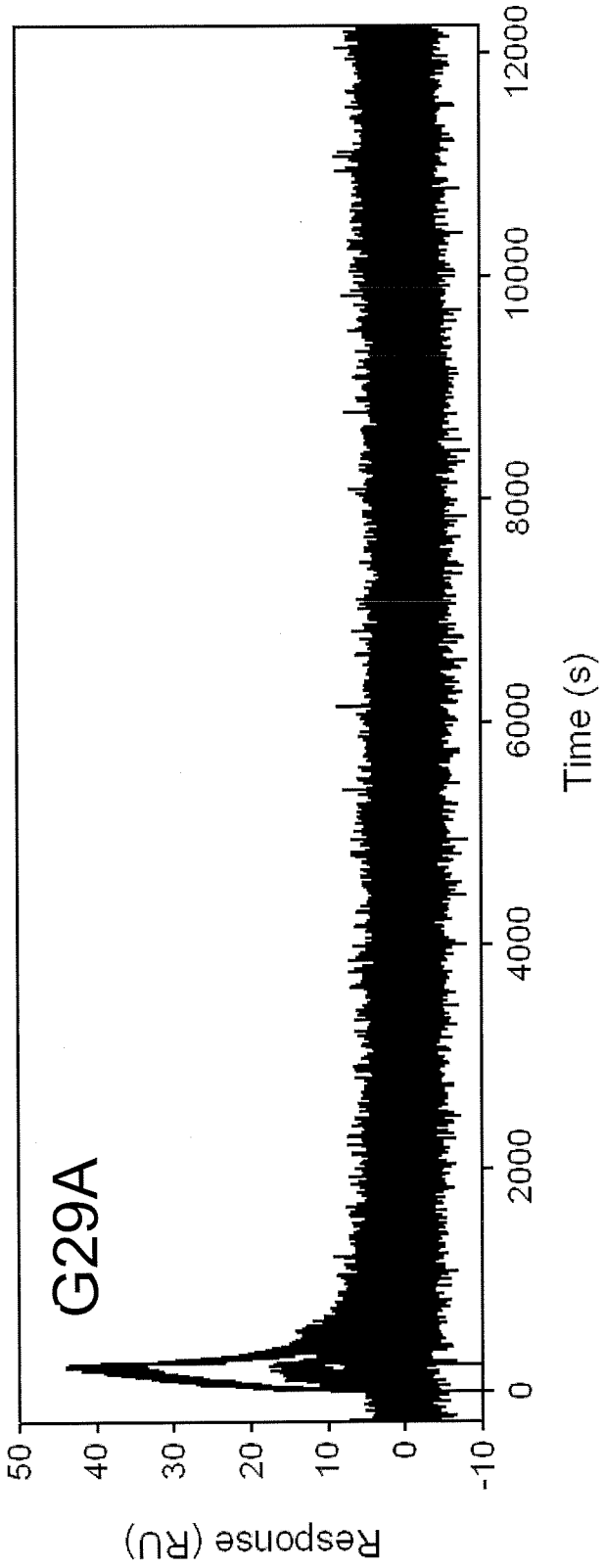

G29A
Response (RU)
50
40
30
20
10
0
-10
0
2000
4000
6000
8000
10000
12000
Time (s)

Figure 14C

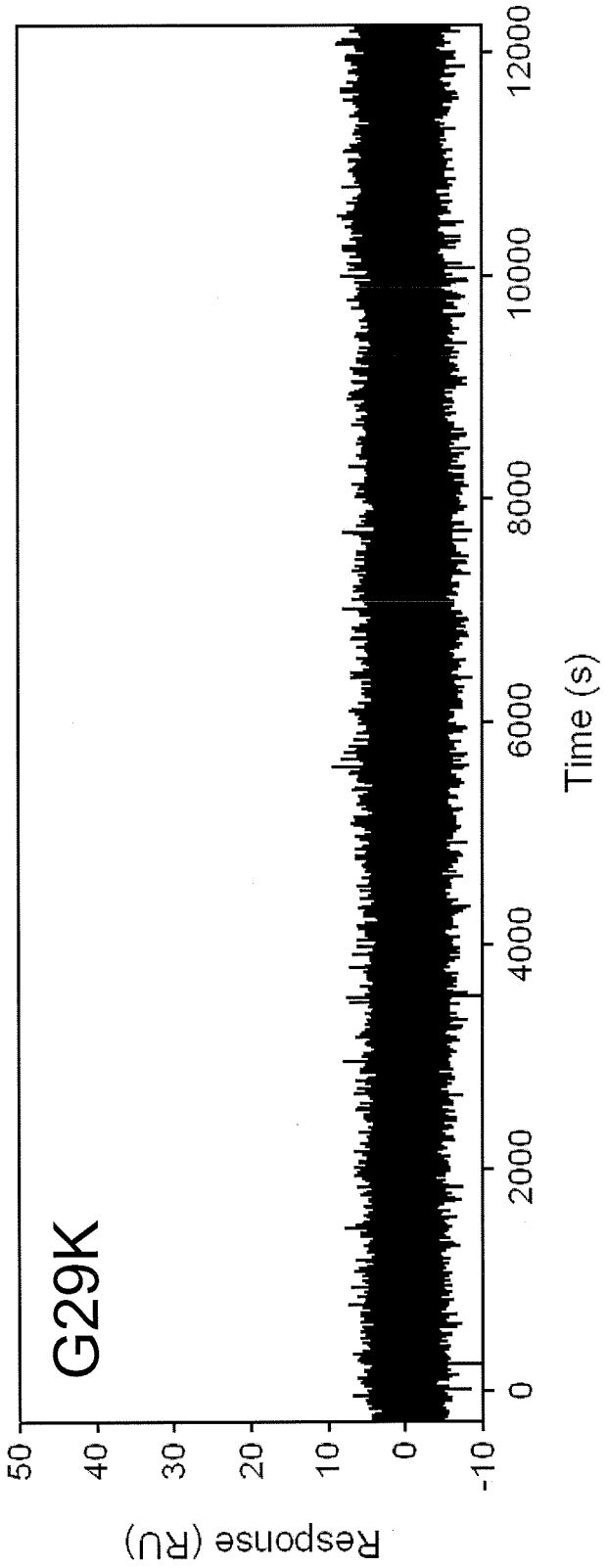

G29K
Response (RU)
50
40
30
20
10
0
-10
0
2000
4000
6000
8000
10000
12000
Time (s)

Figure 14D

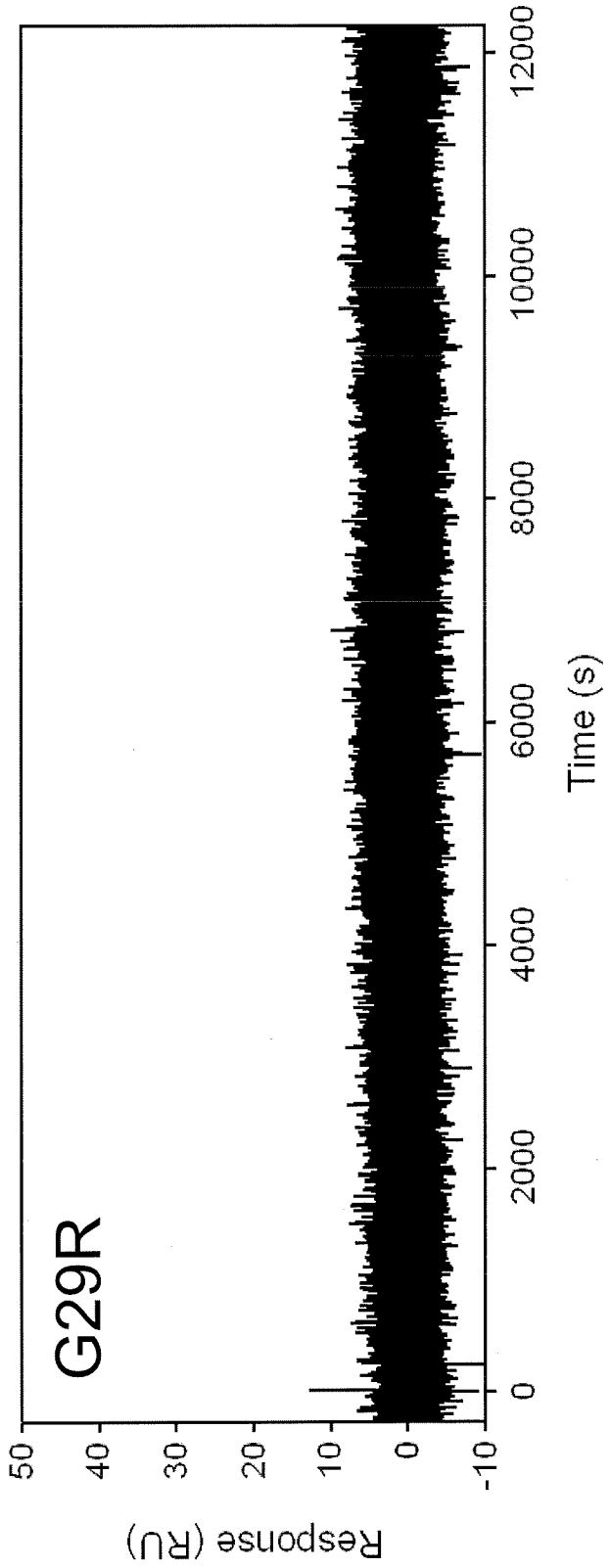

G29R
Response (RU)
50
40
30
20
10
0
-10
0
2000
4000
6000
8000
10000
12000
Time (s)

Figure 14E

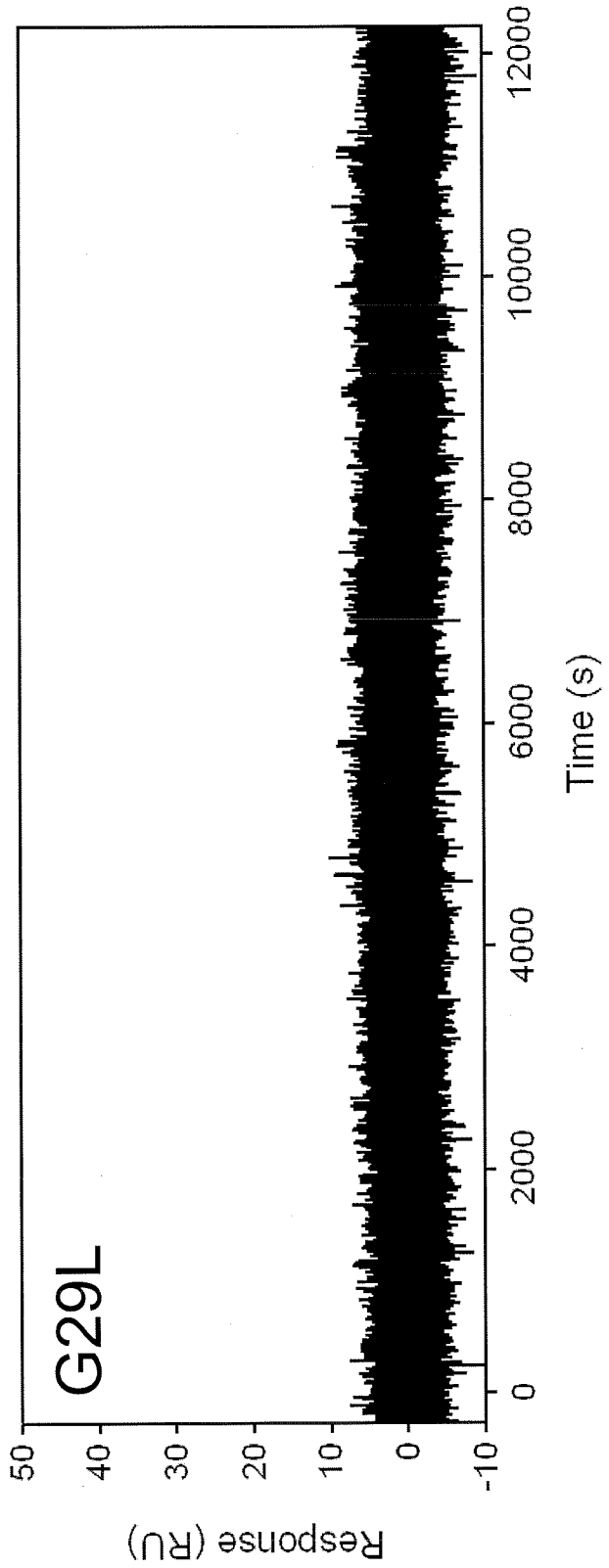

G29L
Response (RU)
50
40
30
20
10
0
-10
0
2000
4000
6000
8000
10000
12000
Time (s)

Figure 15A: Fc pulse results

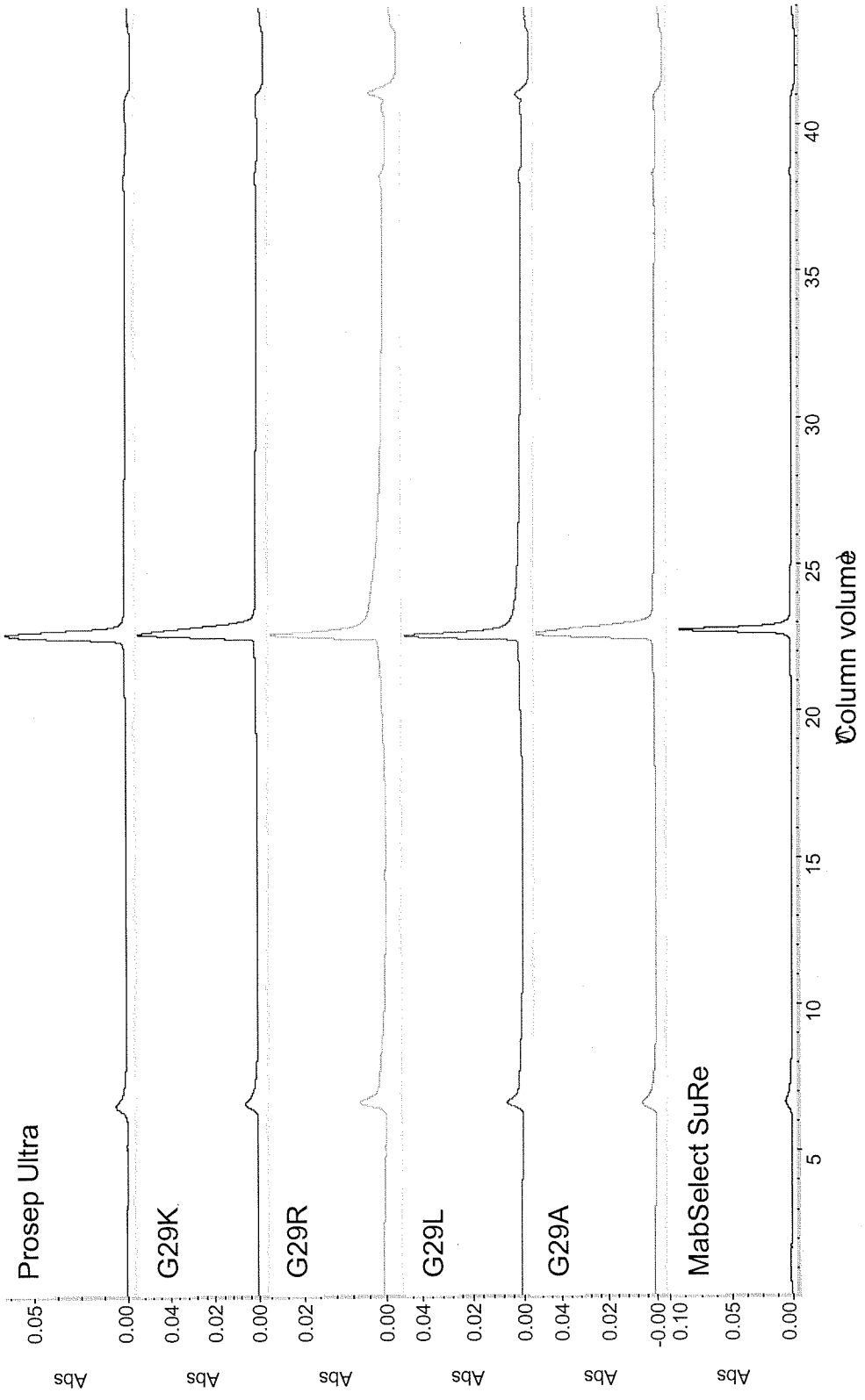


Figure 15B: F(ab)2 pulse results

