

MCA-925 PR
Shari Spector
Novel Immunoglobulin...

Figure 1

DNA Sequences of the wt IgG binding domains of Protein A

SEQ ID NO:1- E domain DNA sequence

GCGCAACAAAACGCTTTCTATCAGGTAAGTGAACATGCCTAACCTGAACGCCGATCAGCG
TAACGGCTTCATCCAAAGCCTGAAGGACGACCCGAGCCAGTCCGCAAACGTTCTGGGTG
AAGCTCAAAAACCTGAACGACAGCCAGGCACCGAAAGCTGAC

SEQ ID NO:2- D domain DNA sequence

GCCCAACAGAACAAATTTAACAAAGACCAGCAGTCCGCGTTCTACGAGATTCTGAACAT
GCCTAACCTGAATGAAGAACAGCGCAACGGTTTTATTTCAGTCTCTGAAGGACGATCCTT
CTCAATCCACCAACGTAAGTGGCGAAGCGAAGAACTGAACGAATCTCAGGCTCCGAAG

SEQ ID NO:3- A domain DNA sequence

GCCGACAACAACTTCAACAAAGAGCAGCAAAACGCTTTCTACGAAATCCTGAATATGCC
AAATCTGAACGAAGAGCAGCGTAACGGTTTCATCCAATCTCTGAAAGACGATCCGTCCC
AGTCCGCGAATCTGCTGGCGGAGGCTAAAAGCTGAACGAATCCCAGGCTCCGAAA

SEQ ID NO:4- B domain DNA sequence

GCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCCTGCATCTGCC
GAACCTGAACGAAGAACAACGCAACGGTTTCATTTCAGAGCCTGAAAGACGACCCATCTC
AGTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAA

SEQ ID NO:5- C domain DNA sequence

GCGGATAACAAATTCACAAGGAGCAACAGAAACGCATTCTATGAAATCTGCACCTGCC
GAATCTGACGGAGGAGCAACGTAACGGCTTTATCCAGTCCCTGAAGGATGATCCGTCTG
TGCTTAAAGAGATCCTGGCGGAGGCAAAAAAAGCTGAATGATGCACAAGCTCCGAAA

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Figure 2

SEQ ID NO:6- Z domain DNA sequence

GTAGACAACAAATTC AATAAAGAACAGCAGAACGCTTTC TATGAAATCCTGCACCTGCC
GAACCTGAACGAAGAACAGCGTAACGCGTTTATCCAGTCCCTGAAAGACGACCCGAGCC
AGAGCGCAAATCTGCTGGCGGAAGCGAAAAAGCTGAACGATGCCCAGGCCGCGAAA

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Figure 3

main sequence alignment

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-----AQQNAFYQVILNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDSQAPK 51 (SEQ ID NO:7)  
NKFNKDQQSAFYVILLNMPNLNEEQRNGFIQSLKDDPSQSTNVLGEAKKLNESQAPK 61 (SEQ ID NO:8)  
IN - FNKEQQNAFYVILLNMPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNESQAPK 58 (SEQ ID NO:9)  
NKFNKDQQNAFYVILLNMPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDAQAPK 58 (SEQ ID NO:10)  
NKFNKDQQNAFYVILLNMPNLNEEQRNGFIQSLKDDPSVSKEILLAEAKKLNDAQAPK 58 (SEQ ID NO:11)  
** ***** :***** * :***** :***** :*****
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Figure 4

SEQ ID NO:12- Z domain amino acid sequence

VDNKFNKEQQ NAFYEILHLP NLNEEQRNAF IQSLKDDPSQ SANLLAEAKKL
NDAQAPK

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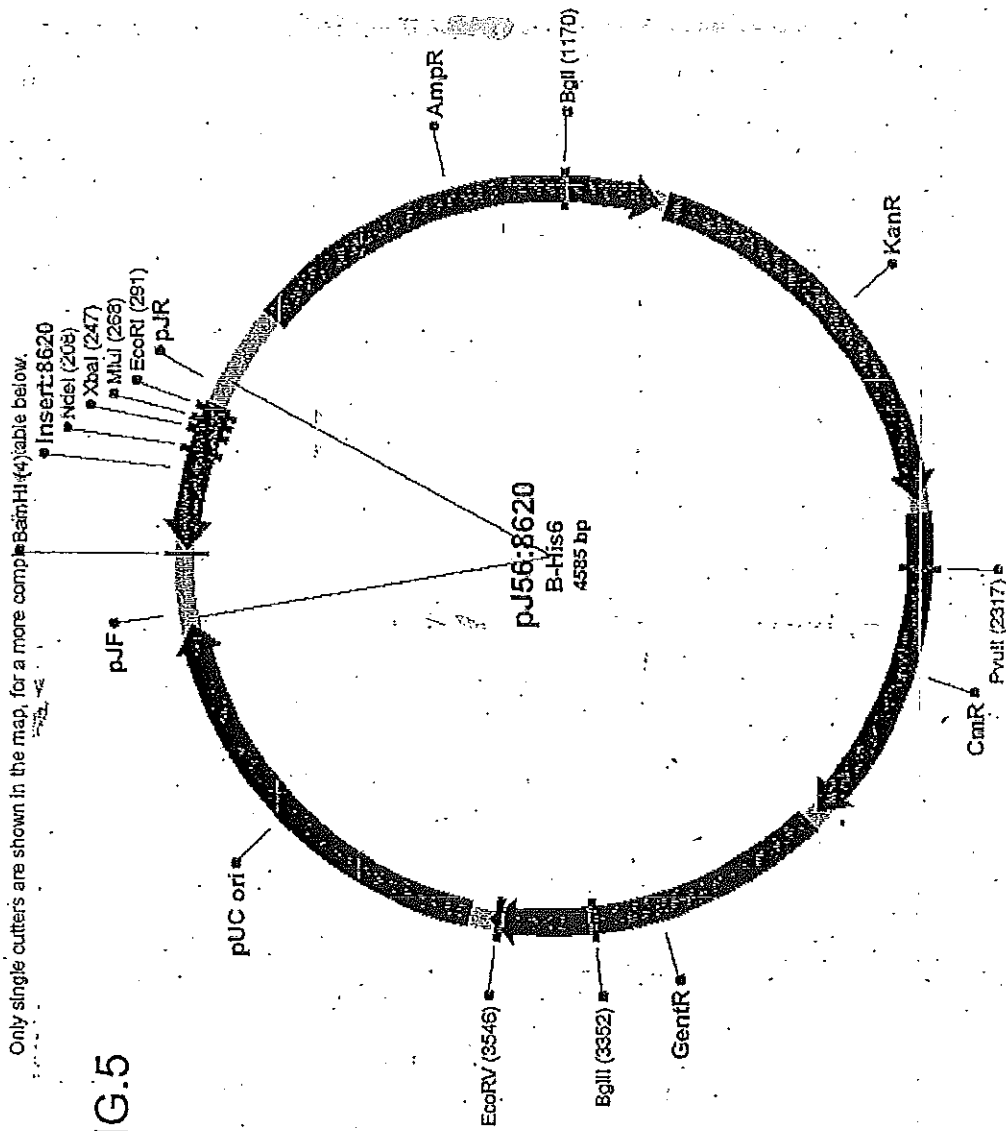


FIG.5

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Figure 6

B domain variant DNA sequences

SEQ ID NO: 14- G29K

GCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCCTGCATCTGCC
GAACCTGAACGAAGAACAACGCAACAAATTCATTTCAGAGCCTGAAAGACGACCCATCTC
AGTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAA

SEQ ID NO: 13- G29L

GCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCCTGCATCTGCC
GAACCTGAACGAAGAACAACGCAACCGCTTCATTTCAGAGCCTGAAAGACGACCCATCTC
AGTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAA

SEQ ID NO:15- G29R

GCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCCTGCATCTGCC
GAACCTGAACGAAGAACAACGCAACCTGTTTCATTTCAGAGCCTGAAAGACGACCCATCTC
AGTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAA

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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

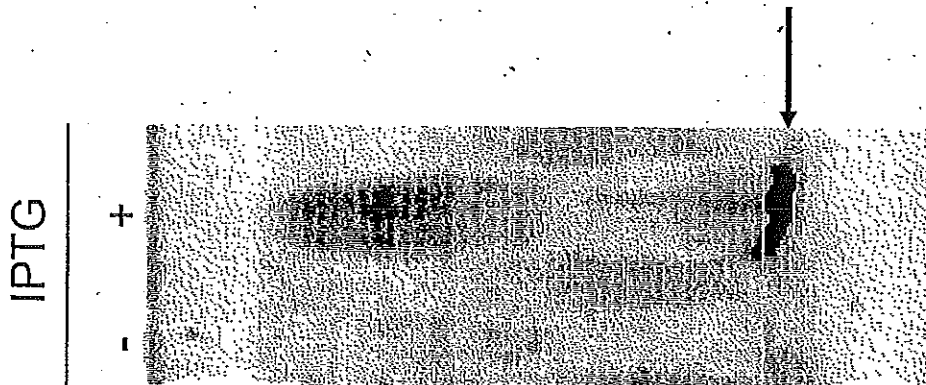
SEQ ID NO:18- G29R

ADNKFNKEQQ NAFYEILHLP NLNEEQRNRF IQSLKDDPSQ SANLLAEAKKL
NDAQAPK

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Figure 8

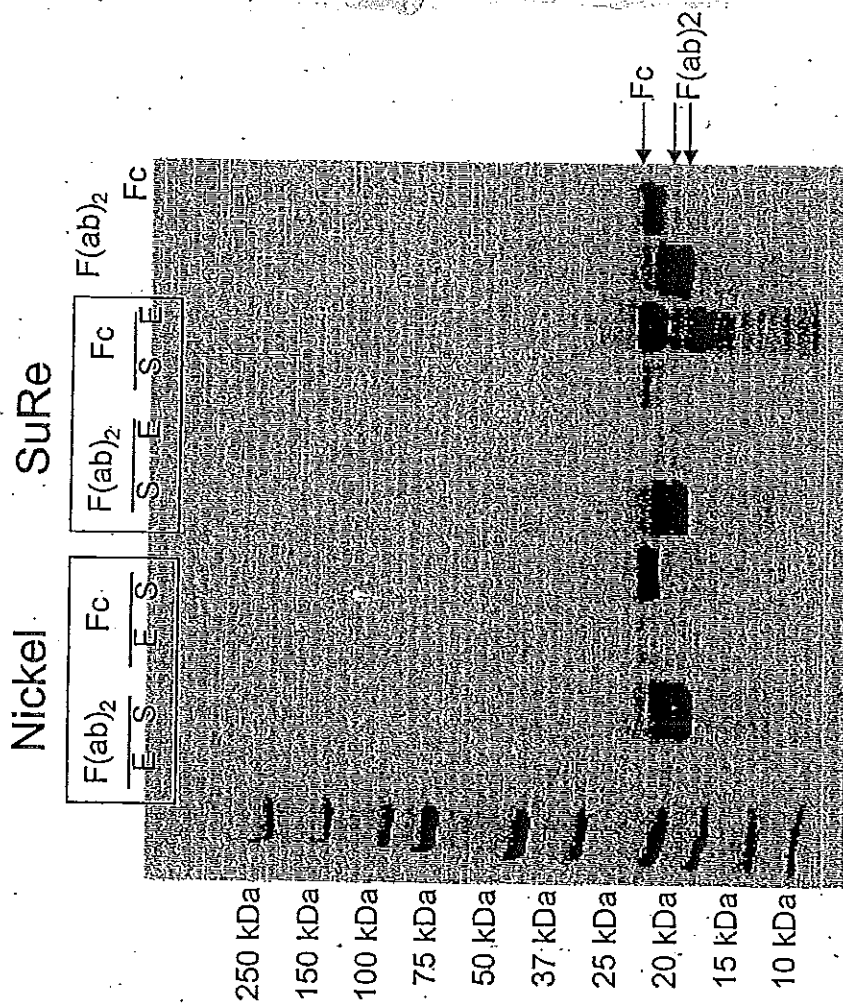
Protein A expression
from pET11a:8620/E.
coli BL21(DE3) was
detected by Western
blotting with Chicken IgY
anti-protein A antibody



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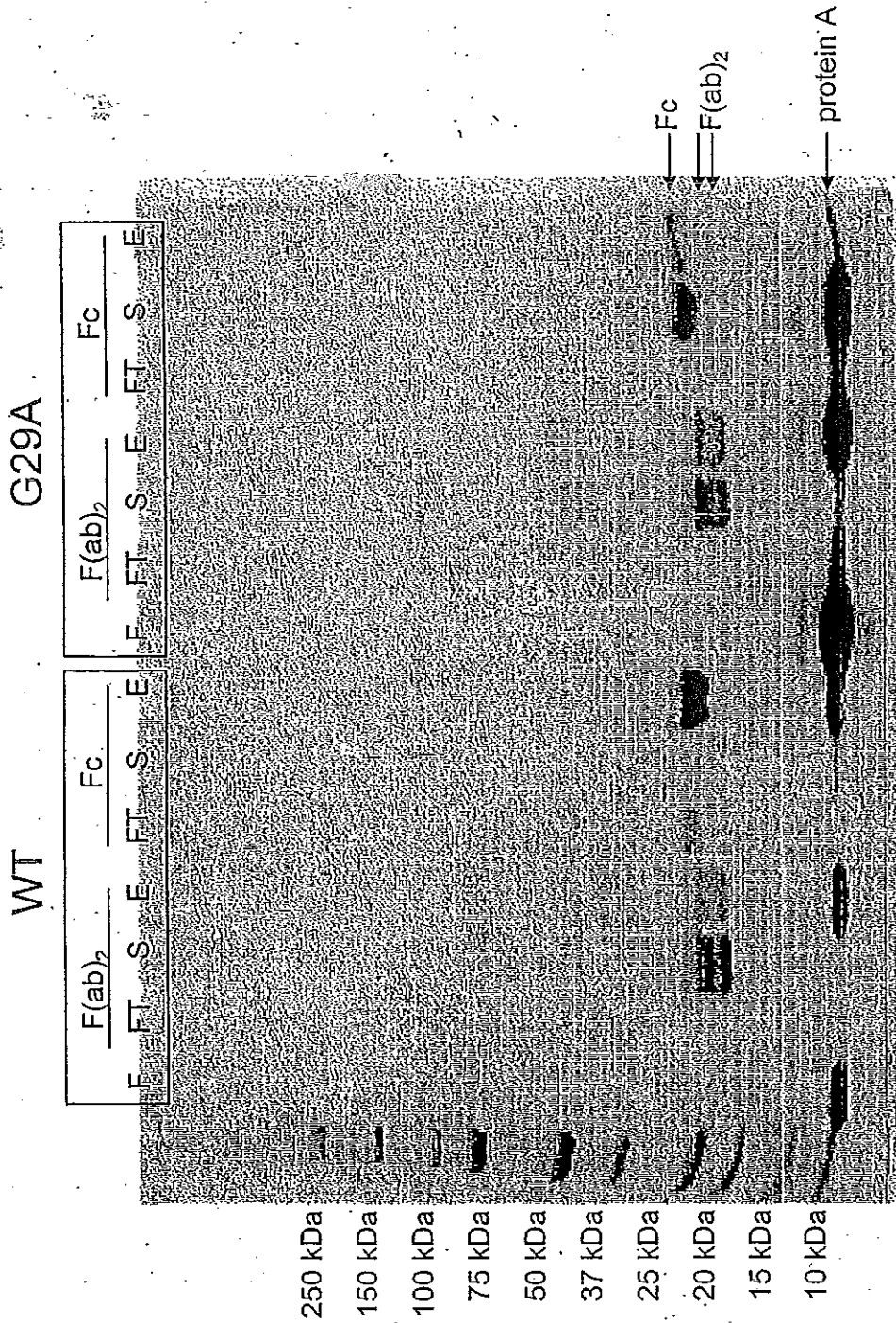
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Figure 9



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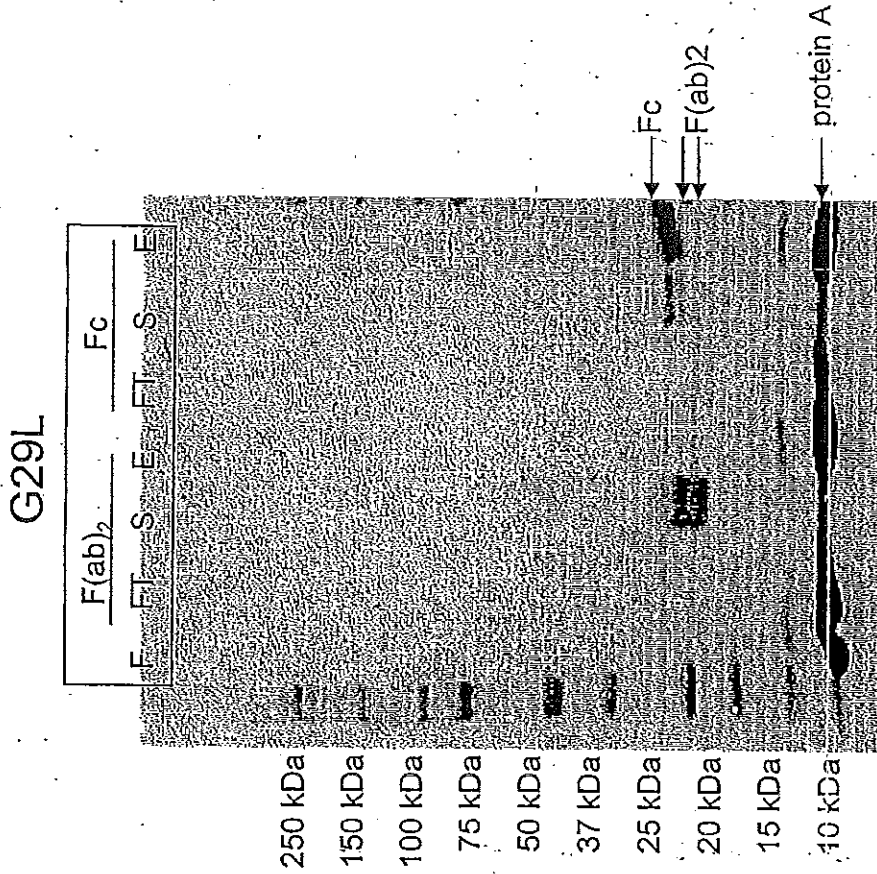
Figure 10



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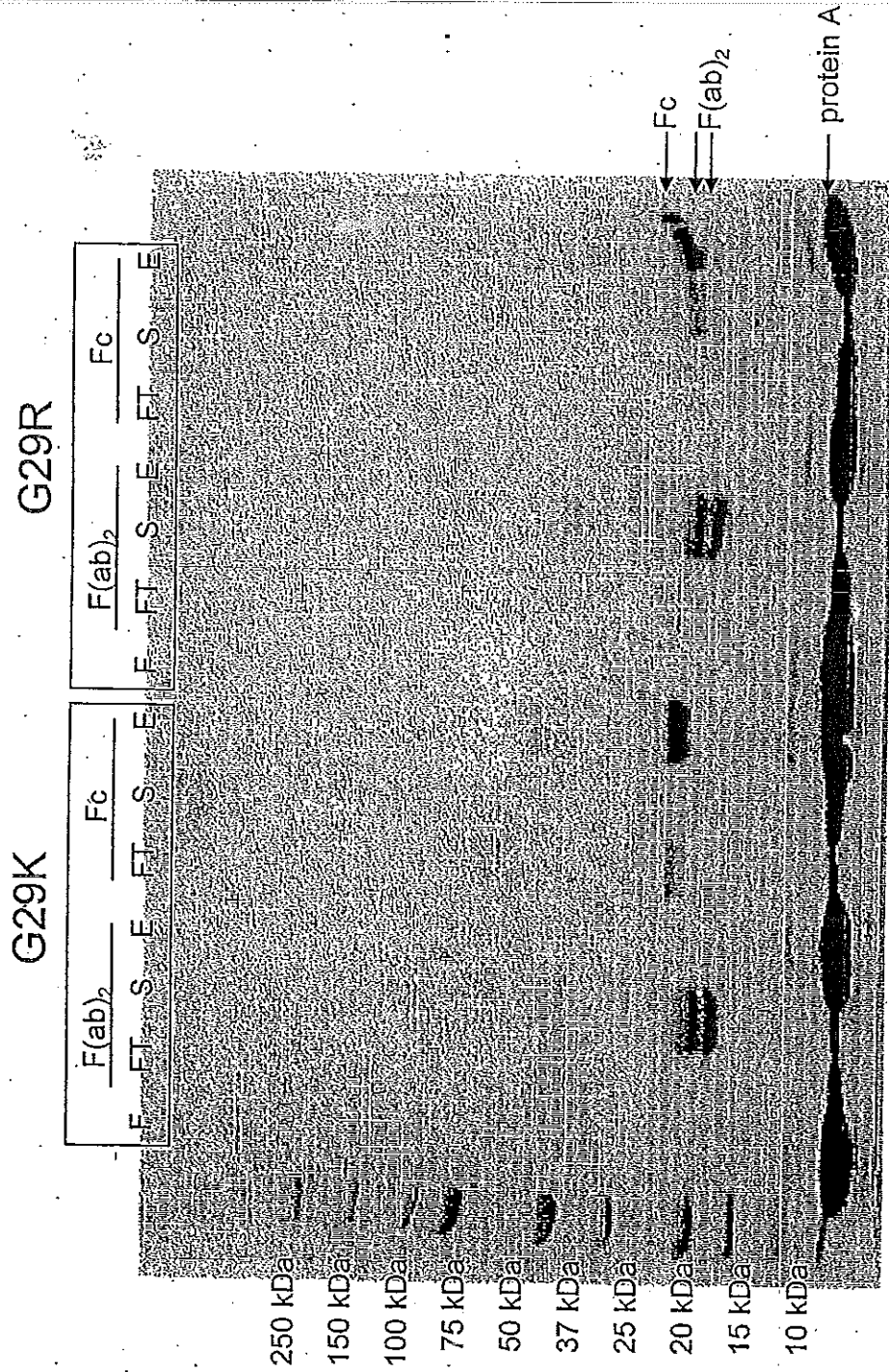
Figure 11



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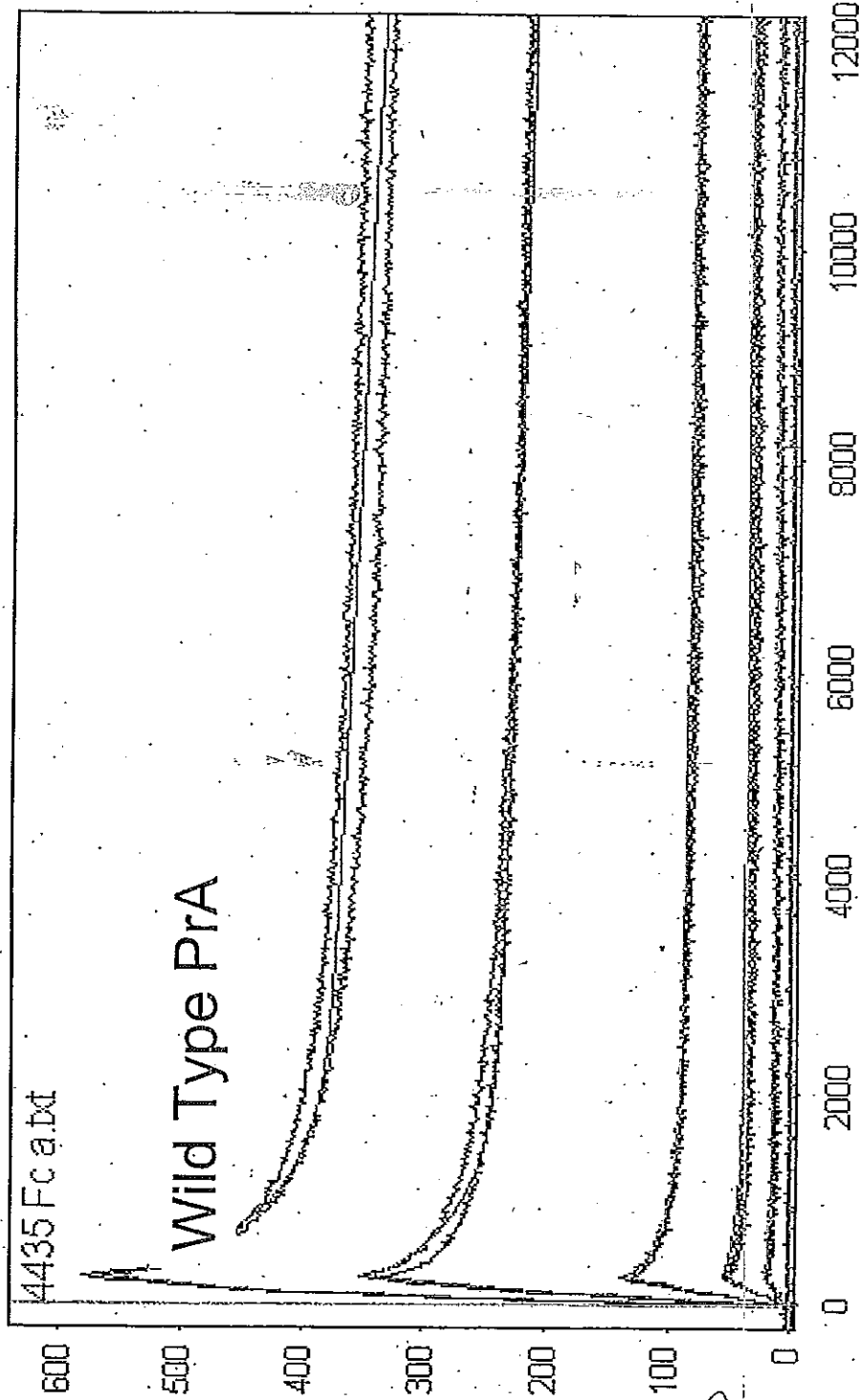
Figure 12



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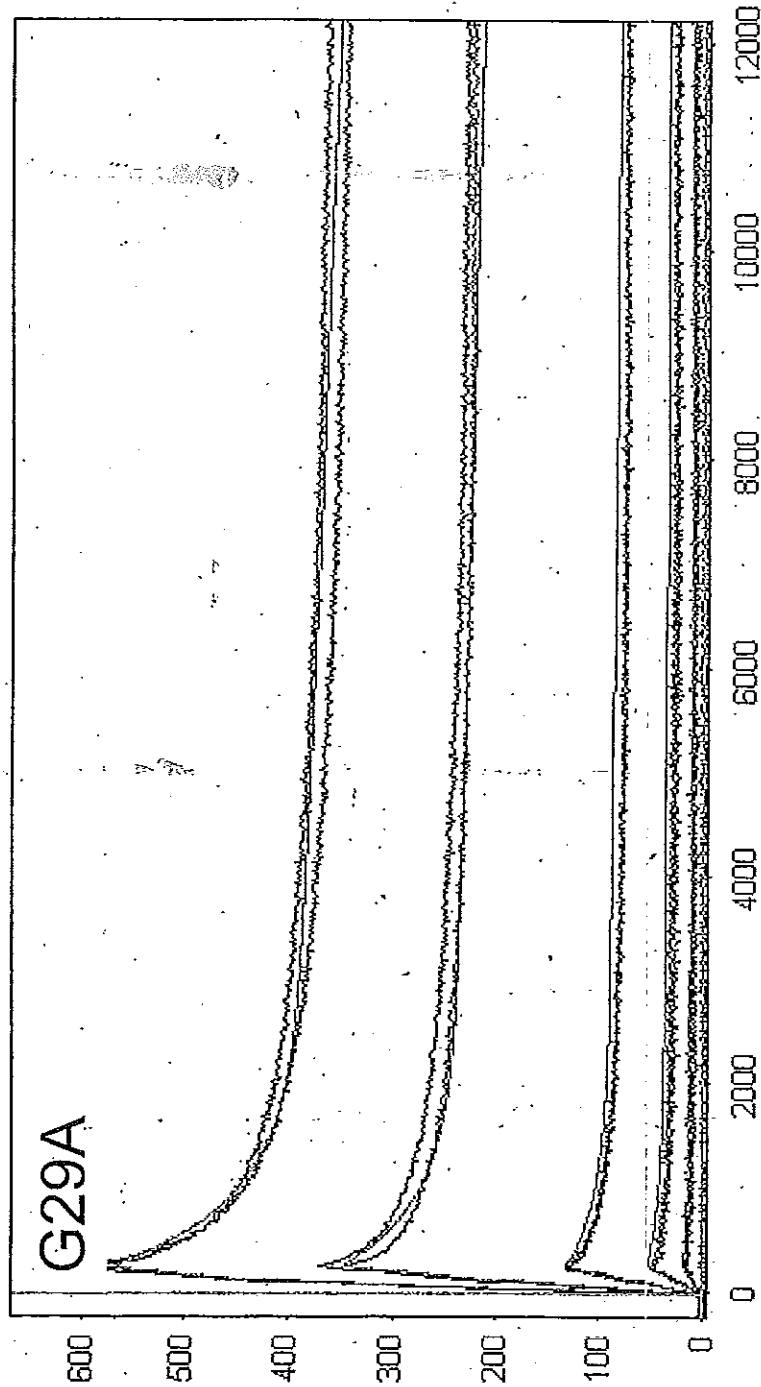
Figure 13A



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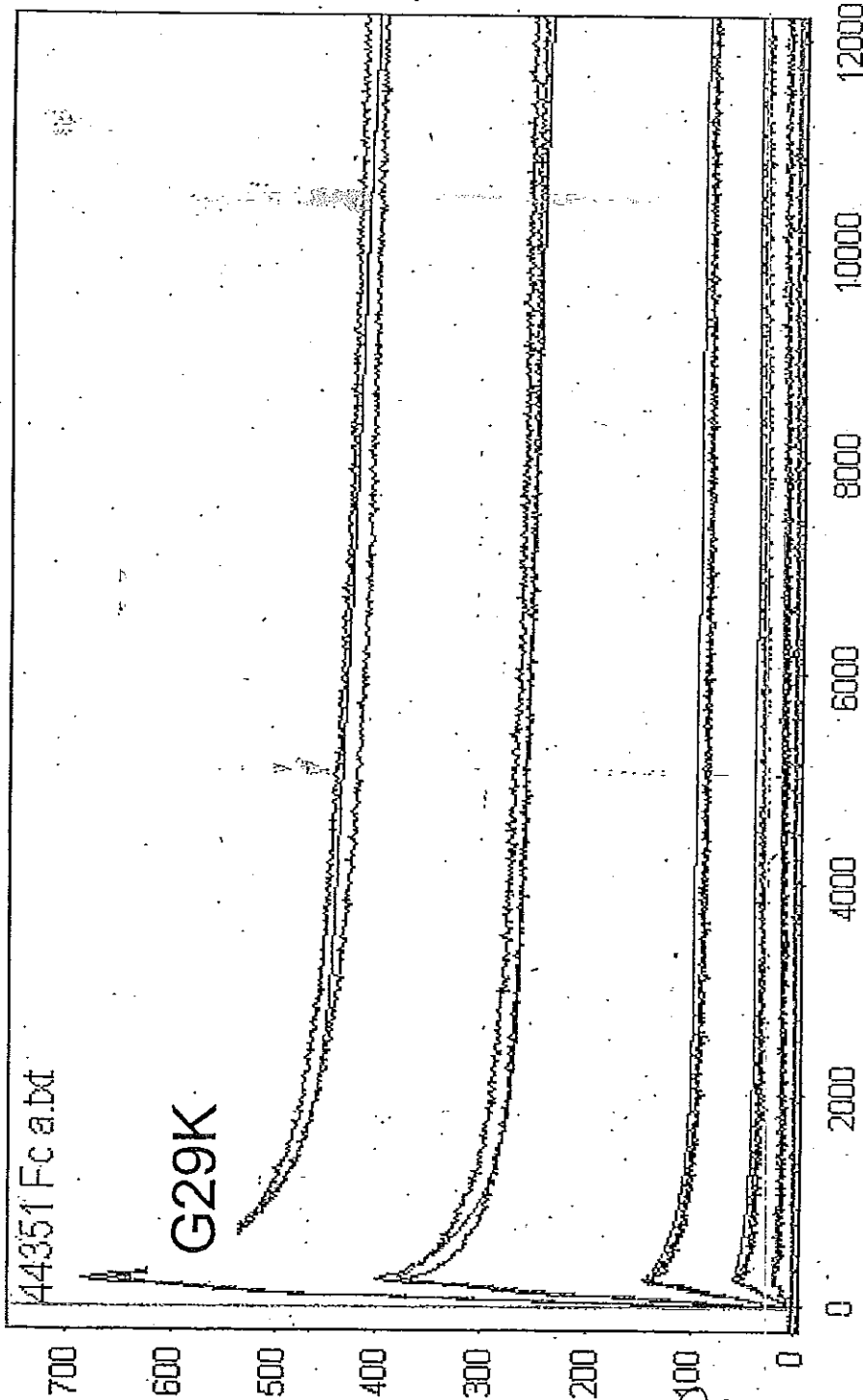
Figure 13B



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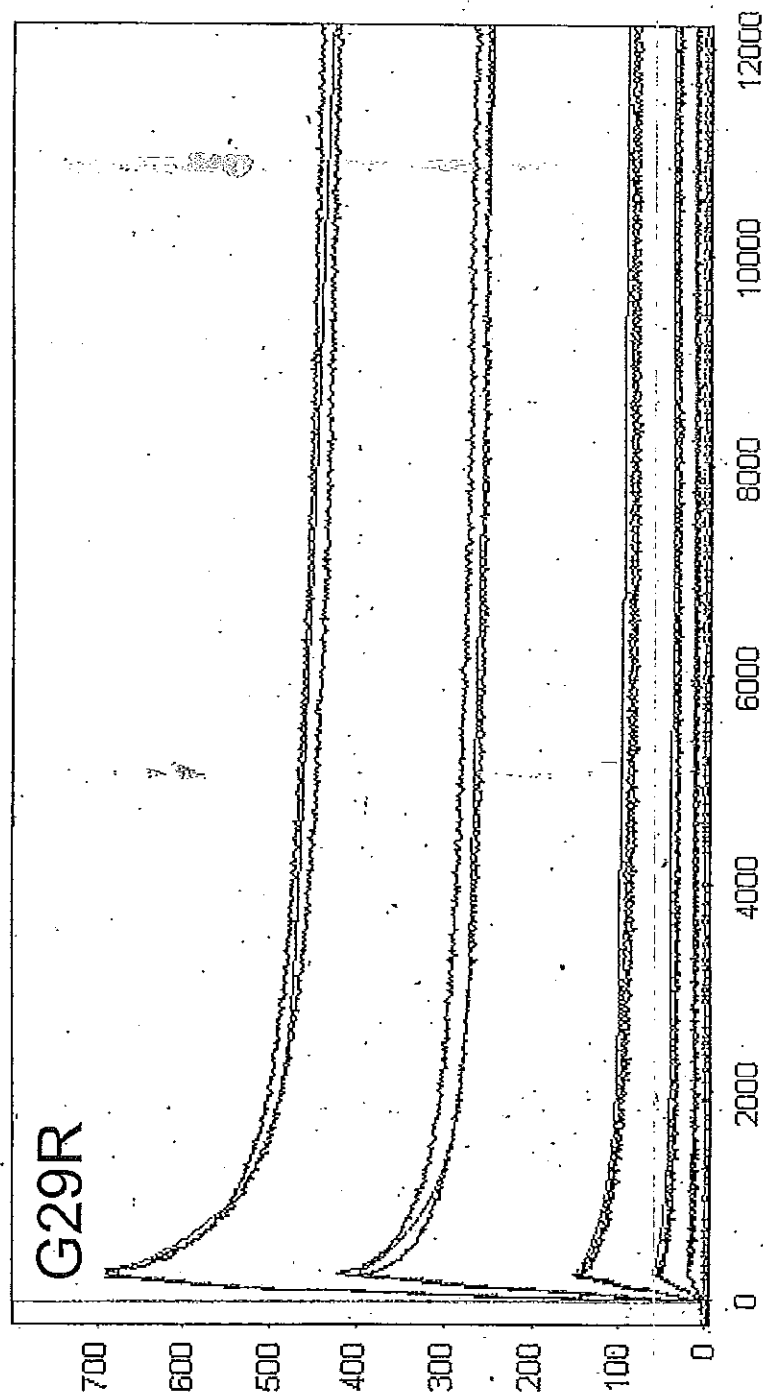
Figure 13C



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Figure 13D

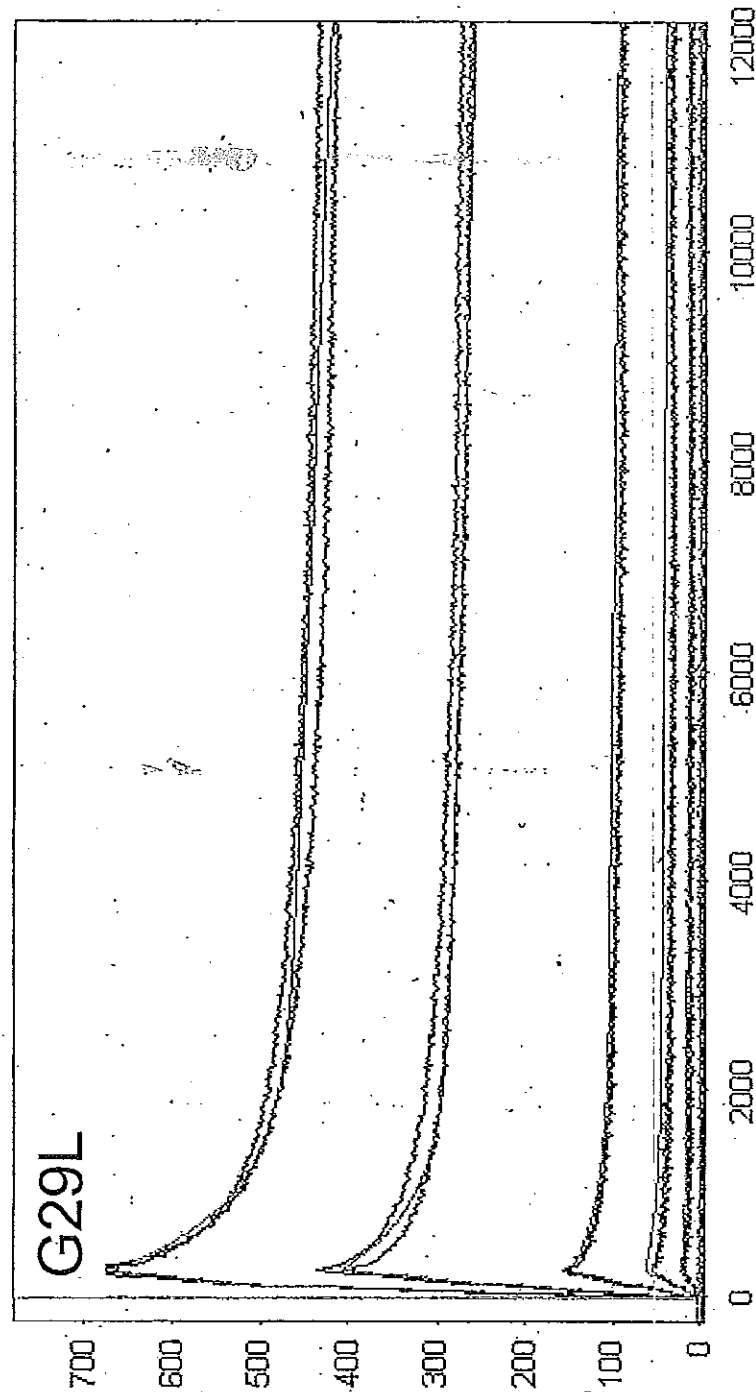


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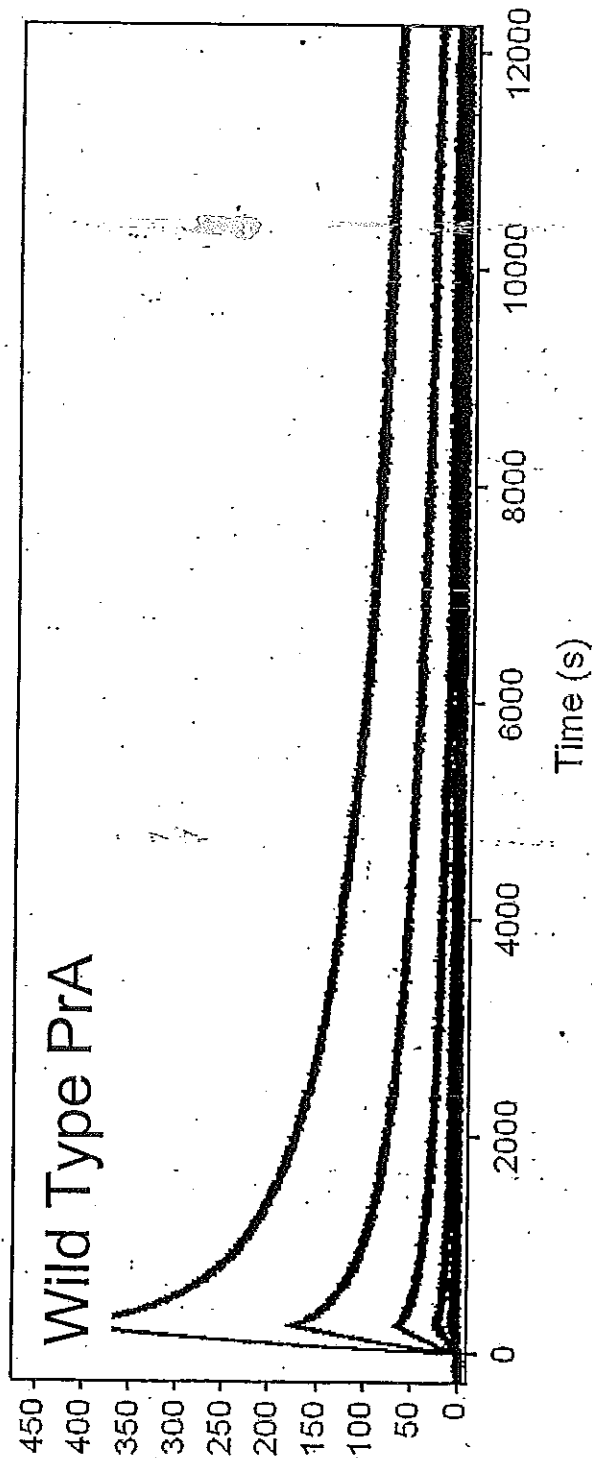
Figure 13E



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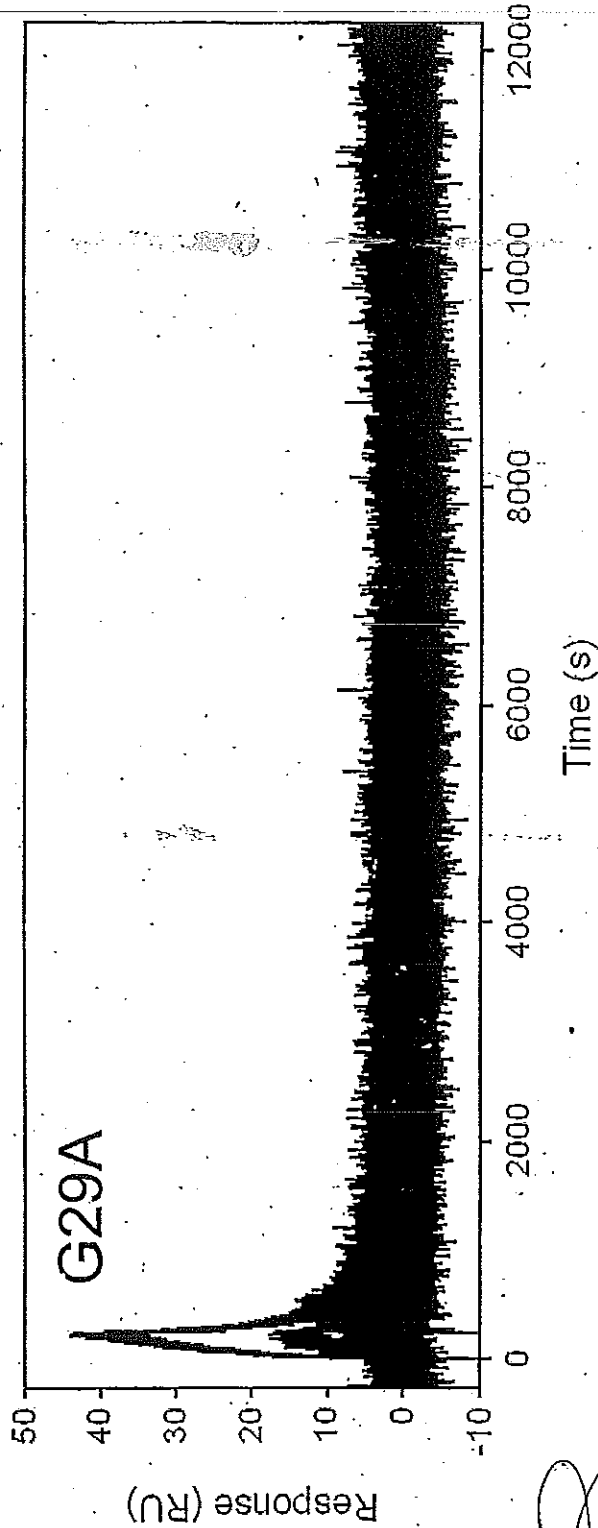
Figure 14A



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Figure 14B

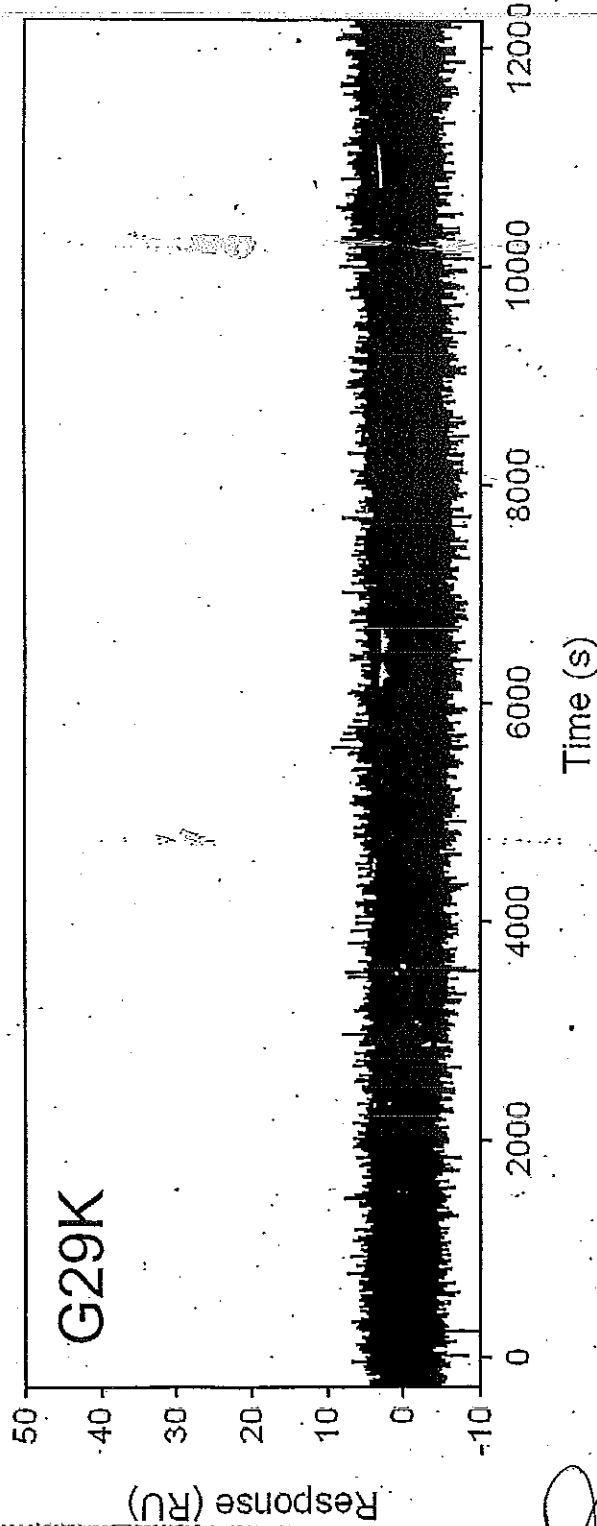


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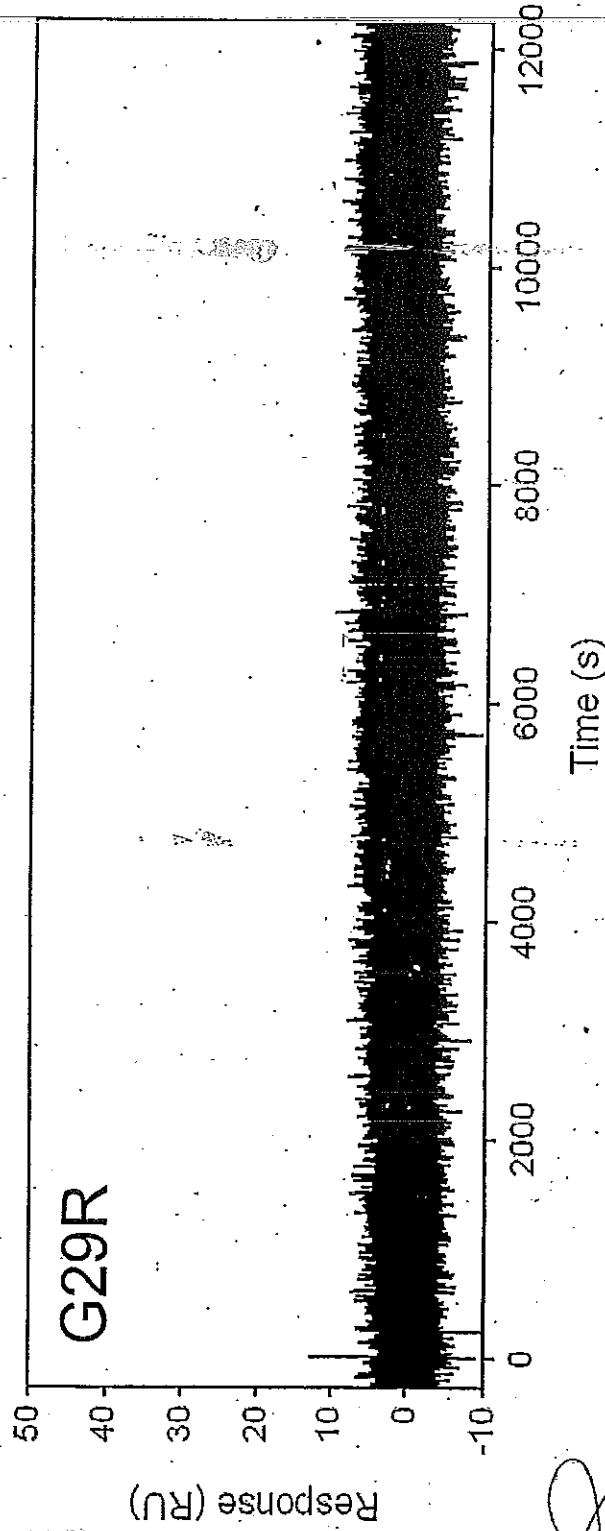
Figure 14C



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Figure 14D

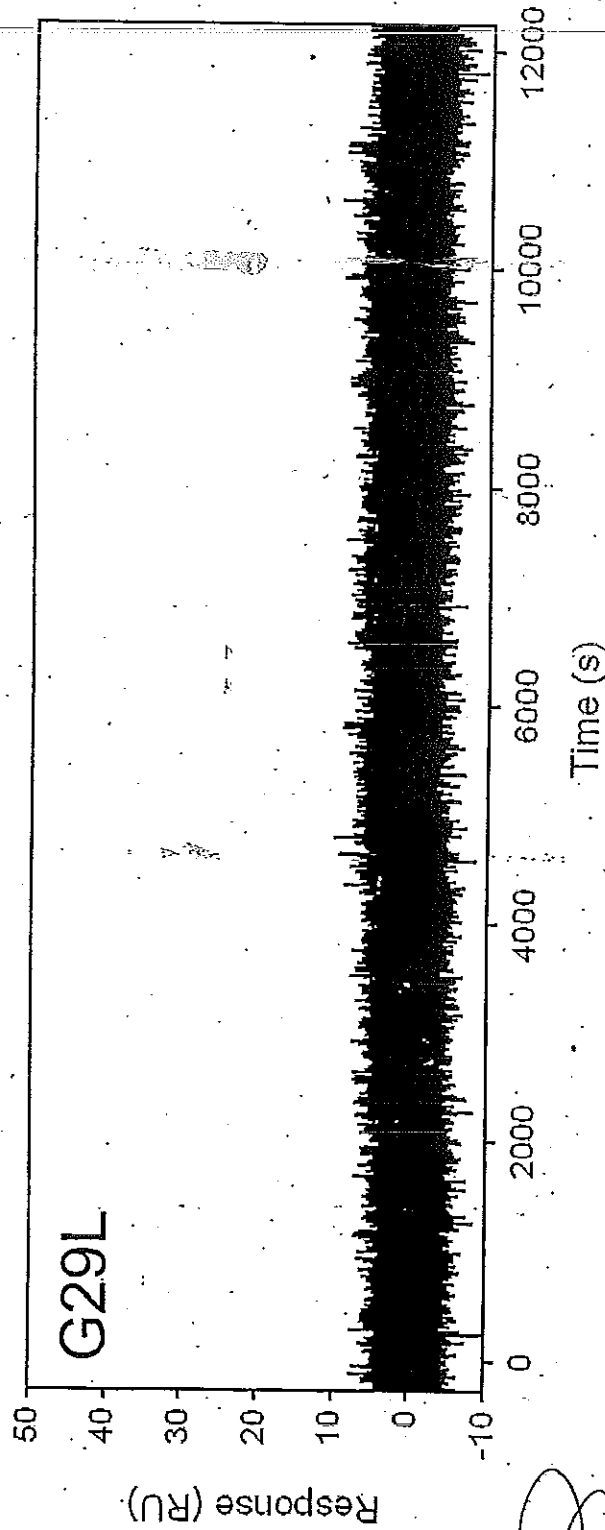


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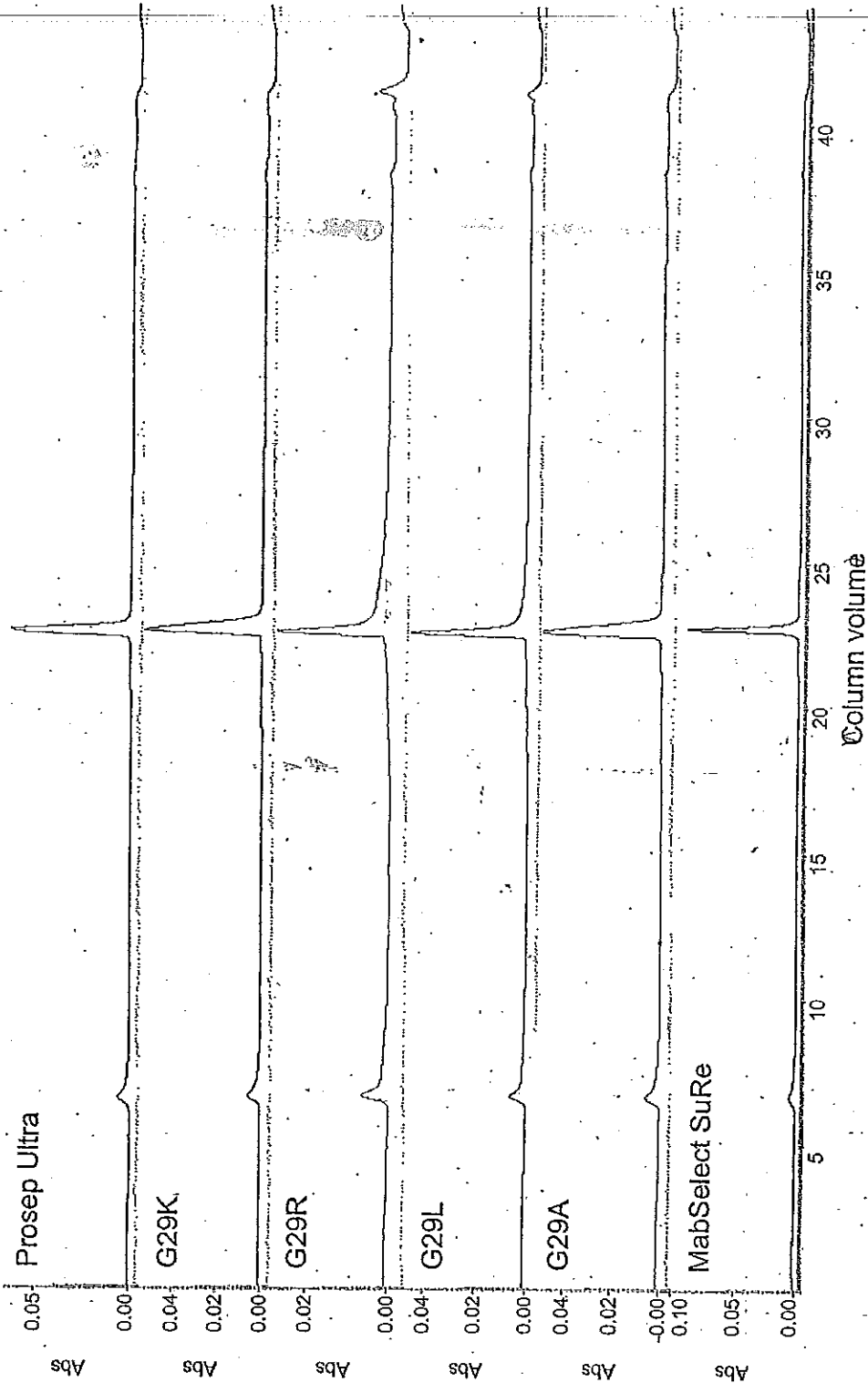
Figure 14E



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Figure 15A: Fc pulse results



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Figure 15B: F(ab)₂ pulse results

