

**Figure 1**

DNA Sequences of the wt IgG binding domains of Protein A

SEQ ID NO:1- E domain DNA sequence

GCGCAACAAAACGCTTTCTATCAGGTACTGAACATGCCTAACCTGAACGCCGATCAG  
CGTAACGGCTTCATCCAAAGCCTGAAGGACGACCCGAGCCAGTCCGCAAACGTTCTG  
GGTGAAGCTCAAAAACCTGAACGACAGCCAGGCACCGAAAGCTGAC

SEQ ID NO:2- D domain DNA sequence

GCCCAACAGAACAAATTTAACAAAGACCAGCAGTCCGCGTTCTACGAGATTCTGAAC  
ATGCCTAACCTGAATGAAGAACAGCGCAACGGTTTTATTTCAGTCTCTGAAGGACGAT  
CCTTCTCAATCCACCAACGTACTGGGCGAAGCGAAGAACTGAACGAATCTCAGGCT  
CCGAAG

SEQ ID NO:3- A domain DNA sequence

GCCGACAACAACCTTCAACAAAGAGCAGCAAAACGCTTTCTACGAAATCCTGAATATG  
CCAAATCTGAACGAAGAGCAGCGTAACGGTTTCATCCAATCTCTGAAAGACGATCCG  
TCCCAGTCCGCGAATCTGCTGGCGGAGGCTAAAAAGCTGAACGAATCCCAGGCTCCG  
AAA

SEQ ID NO:4- B domain DNA sequence

GCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCCTGCATCTG  
CCGAACCTGAACGAAGAACAACGCAACGGTTTCATTTCAGAGCCTGAAAGACGACCCA  
TCTCAGTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCG  
AAA

SEQ ID NO:5- C domain DNA sequence

GCGGATAACAAATTCAACAAGGAGCAACAGAACGCATTCTATGAAATTCTGCACCTG  
CCGAATCTGACGGAGGAGCAACGTAACGGCTTTATCCAGTCCCTGAAGGATGATCCG  
TCTGTGTCTAAAGAGATCCTGGCGGAGGCAAAAAAAGCTGAATGATGCACAAGCTCCG  
AAA

**Figure 2**

SEQ ID NO:6- Z domain DNA sequence

GTAGACAACAAATTCAATAAAGAACAGCAGAACGCTTTCTATGAAATCCTGCACCTG  
CCGAACCTGAACGAAGAACAGCGTAACGCGTTTATCCAGTCCCTGAAAGACGACCCG  
AGCCAGAGCGCAAATCTGCTGGCGGAAGCGAAAAAGCTGAACGATGCCCAGGCGCCG  
AAA

Figure 3

IgG binding domain sequence alignment

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E      -----AQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDSQAPK 51 (SEQ ID NO:7)
D      ADAQQNKFNKDQQSAFYEILNMPNLNEEQRNGFIQSLKDDPSQSTNVLGEAKKLNESQAPK 61 (SEQ ID NO:8)
A      --ADNN-FNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNESQAPK 58 (SEQ ID NO:9)
B      ---ADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDAPK 58 (SEQ ID NO:10)
C      ---ADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDAPK 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

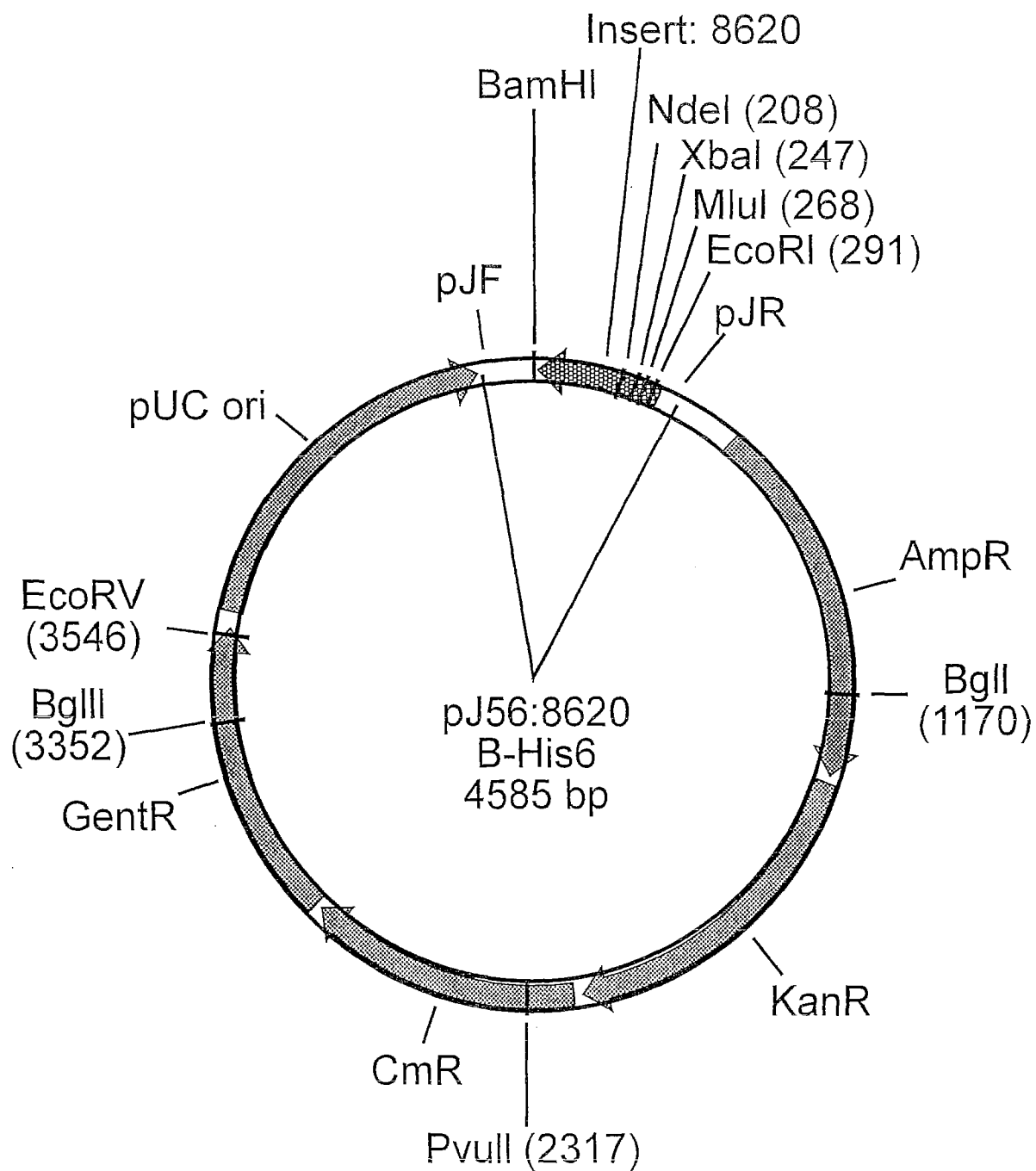


Figure 5

**Figure 6a**Wildtype B domain 6Histidine (SEQ ID NO: 13)

MADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKL  
NDAQAPKHHHHHHH

E24M (SEQ ID NO: 14)

MADNKFNKEQQNAFYEILHLPNLNMEQRNGFIQSLKDDPSQSANLLAEAKKL  
NDAQAPKHHHHHHH

E24I (SEQ ID NO: 15)

MADNKFNKEQQNAFYEILHLPNLNIEQRNGFIQSLKDDPSQSANLLAEAKKL  
NDAQAPKHHHHHHH

E24F (SEQ ID NO: 16)

MADNKFNKEQQNAFYEILHLPNLNFEQRNGFIQSLKDDPSQSANLLAEAKKL  
NDAQAPKHHHHHHH

E24T (SEQ ID NO: 17)

MADNKFNKEQQNAFYEILHLPNLNTEQRNGFIQSLKDDPSQSANLLAEAKKL  
NDAQAPKHHHHHHH

E24P (SEQ ID NO: 18)

MADNKFNKEQQNAFYEILHLPNLNPEQRNGFIQSLKDDPSQSANLLAEAKKL  
NDAQAPKHHHHHHH

E24W (SEQ ID NO: 19)

MADNKFNKEQQNAFYEILHLPNLNWEQRNGFIQSLKDDPSQSANLLAEAKKL  
NDAQAPKHHHHHHH

E24R (SEQ ID NO: 20)

MADNKFNKEQQNAFYEILHLPNLNREQRNGFIQSLKDDPSQSANLLAEAKKL  
NDAQAPKHHHHHHH

E24V (SEQ ID NO: 21)

MADNKFNKEQQNAFYEILHLPNLNVEQRNGFIQSLKDDPSQSANLLAEAKKL  
NDAQAPKHHHHHHH

E24L (SEQ ID NO: 22)

MADNKFNKEQQNAFYEILHLPNLNLEQRNGFIQSLKDDPSQSANLLAEAKKL  
NDAQAPKHHHHHHH

**Figure 6b**

E24Y (SEQ ID NO: 23)

MADNKFNKEQQNAFYEILHLPNLN~~Y~~EQRNGFIQSLKDDPSQSANLLAEAKKL  
NDAQAPKHHHHHH

E24H (SEQ ID NO: 24)

MADNKFNKEQQNAFYEILHLPNLN~~H~~EQRNGFIQSLKDDPSQSANLLAEAKKL  
NDAQAPKHHHHHH

E24K (SEQ ID NO: 25)

MADNKFNKEQQNAFYEILHLPNLN~~K~~EQRNGFIQSLKDDPSQSANLLAEAKKL  
NDAQAPKHHHHHH

E24D (SEQ ID NO: 26)

MADNKFNKEQQNAFYEILHLPNLN~~D~~EQRNGFIQSLKDDPSQSANLLAEAKKL  
NDAQAPKHHHHHH

**Figure 7a**Wildtype B domain 6 Histidine (SEQ ID NO:27)

GAATTCTAATACGACTCACTATAACGCGTCCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTT  
 AAGAAGGAGATATACATATGGCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCC  
 TGCTCTGCCGAACCTGAACGAAGAACAACGCAACGGTTTCATTTCAGAGCCTGAAAGACGACCCATCTCA  
 GTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAACATCATCACCATCA  
 CCACTAATAAGGATCC

E24M (SEQ ID NO: 28)

GAATTCTAATACGACTCACTATAACGCGTCCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTT  
 AAGAAGGAGATATACATATGGCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCC  
 TGCTCTGCCGAACCTGATGGAAGAACAACGCAACGGTTTCATTTCAGAGCCTGAAAGACGACCCATCTCA  
 GTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAACATCATCACCATCA  
 CCACTAATAAGGATCC

E24I (SEQ ID NO: 29)

GAATTCTAATACGACTCACTATAACGCGTCCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTT  
 AAGAAGGAGATATACATATGGCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCC  
 TGCTCTGCCGAACCTGATAGAAGAACAACGCAACGGTTTCATTTCAGAGCCTGAAAGACGACCCATCTCA  
 GTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAACATCATCACCATCA  
 CCACTAATAAGGATCC

E24F (SEQ ID NO: 30)

GAATTCTAATACGACTCACTATAACGCGTCCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTT  
 AAGAAGGAGATATACATATGGCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCC  
 TGCTCTGCCGAACCTGTTTGAAGAACAACGCAACGGTTTCATTTCAGAGCCTGAAAGACGACCCATCTCA  
 GTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAACATCATCACCATCA  
 CCACTAATAAGGATCC

E24T (SEQ ID NO: 31)

GAATTCTAATACGACTCACTATAACGCGTCCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTT  
 AAGAAGGAGATATACATATGGCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCC  
 TGCTCTGCCGAACCTGACAGAAGAACAACGCAACGGTTTCATTTCAGAGCCTGAAAGACGACCCATCTCA  
 GTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAACATCATCACCATCA  
 CCACTAATAAGGATCC

E24P (SEQ ID NO: 32)

GAATTCTAATACGACTCACTATAACGCGTCCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTT  
 AAGAAGGAGATATACATATGGCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCC  
 TGCTCTGCCGAACCTGCCAGAAGAACAACGCAACGGTTTCATTTCAGAGCCTGAAAGACGACCCATCTCA  
 GTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAACATCATCACCATCA  
 CCACTAATAAGGATCC

E24W (SEQ ID NO: 33)

GAATTCTAATACGACTCACTATAACGCGTCCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTT  
 AAGAAGGAGATATACATATGGCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCC  
 TGCTCTGCCGAACCTGTGGGAAGAACAACGCAACGGTTTCATTTCAGAGCCTGAAAGACGACCCATCTCA  
 GTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAACATCATCACCATCA  
 CCACTAATAAGGATCC

E24R (SEQ ID NO: 34)

GAATTCTAATACGACTCACTATAACGCGTCCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTT  
 AAGAAGGAGATATACATATGGCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCC  
 TGCTCTGCCGAACCTGCGCGAAGAACAACGCAACGGTTTCATTTCAGAGCCTGAAAGACGACCCATCTCA  
 GTCCGC

**Figure 7b**

TAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAACATCATCACCATCACCCTA  
ATAAGGATCC

E24V (SEQ ID NO: 35)

GAATTCTAATACGACTCACTATAACGCGTCCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTT  
AAGAAGGAGATATACATATGGCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCC  
TGCTCTGCCGAACCTGGTGAAGAACAACGCAACGGTTTCATTCAGAGCCTGAAAGACGACCCATCTCA  
GTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAACATCATCACCATCA  
CCTAATAAGGATCC

E24L (SEQ ID NO: 36)

GAATTCTAATACGACTCACTATAACGCGTCCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTT  
AAGAAGGAGATATACATATGGCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCC  
TGCTCTGCCGAACCTGTTGAAGAACAACGCAACGGTTTCATTCAGAGCCTGAAAGACGACCCATCTCA  
GTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAACATCATCACCATCA  
CCTAATAAGGATCC

E24Y (SEQ ID NO: 37)

GAATTCTAATACGACTCACTATAACGCGTCCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTT  
AAGAAGGAGATATACATATGGCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCC  
TGCTCTGCCGAACCTGTACGAAGAACAACGCAACGGTTTCATTCAGAGCCTGAAAGACGACCCATCTCA  
GTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAACATCATCACCATCA  
CCTAATAAGGATCC

E24H (SEQ ID NO: 38)

GAATTCTAATACGACTCACTATAACGCGTCCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTT  
AAGAAGGAGATATACATATGGCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCC  
TGCTCTGCCGAACCTGCACGAAGAACAACGCAACGGTTTCATTCAGAGCCTGAAAGACGACCCATCTCA  
GTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAACATCATCACCATCA  
CCTAATAAGGATCC

E24K (SEQ ID NO: 39)

GAATTCTAATACGACTCACTATAACGCGTCCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTT  
AAGAAGGAGATATACATATGGCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCC  
TGCTCTGCCGAACCTGAAAGAAGAACAACGCAACGGTTTCATTCAGAGCCTGAAAGACGACCCATCTCA  
GTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAACATCATCACCATCA  
CCTAATAAGGATCC

E24D (SEQ ID NO: 40)

GAATTCTAATACGACTCACTATAACGCGTCCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTT  
AAGAAGGAGATATACATATGGCAGACAATAAGTTCAATAAAGAGCAGCAGAACGCATTTTACGAGATCC  
TGCTCTGCCGAACCTGGACGAAGAACAACGCAACGGTTTCATTCAGAGCCTGAAAGACGACCCATCTCA  
GTCCGCTAACCTGCTGGCGGAAGCAAAGAAGCTGAACGATGCACAGGCGCCGAAACATCATCACCATCA  
CCTAATAAGGATCC

**Figure 8**