Table 1.

Amino acid sequence of variable regions of the heavy chain (VH) and light chain (VL/Vκ) from six different monoclonal antibodies specific for human wild-type Aβ protofibrils.

```
VH-BA1: X731   EVKLVESGGGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTIYYADTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCARVGHYAM-------DYWGQGTSVTVSS
VH-BA2: X736   EVKLVESGGGLVKPGGSLKLSCAASGFTFSSYAMSWVRQTPEKRLEWVATISSGGSYTYYPDSVKGRFTISRDNAKNTLYLQMSSLRSEDTAMYYCARHYGSPRYF------DVWGAGTSVTVSS
VH-BA3: X745   QVHLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGLEWIGYINPYNDGTKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARRVSPLTGYAM---DYWGQGTSVTVSS
VH-BA7: X746   QVQLKESGPGLVAPPQSLSITCTVSGFSLTSYGVHWVRQPPGKGLEWLGVIWAGGSTNYNSALMS-RLSISKDNSKSQVFLKMNSLQTDDTAMYYCARGRYDGKTREA----YWGQGTLVTVSS
VH-BA4: X748   EVKLMESGGGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTIYYADTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCARGRYDGKTREA----YWGQGTLVTVSS
VH-BA9: X758   EVQRVESGGGLVQPGGERKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTIYYGDTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCARGDSF---------DYWGQGTTLTVSS
               FR1            CDR1            FR2            CDR2            FR3                CDR3        FR4

Vκ-BA1: X731   DVVMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLE-WYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPFTFGGGTKLEIK
Vκ-BA2: X736   DIVMTQAPKFLLVSAGDRVTITCKASQSVSNDVA------WYQQKPGQSPKLLIYYASNRYTGVPDRFTGSGYGTDFTFTISTVQAEDLAVYFCQQDYSSPFTFGSGTKLEIK
Vκ-BA3: X745   DIVMTQAPSSLAVSAGEKVTMSCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCKQSYNL-WTFGGGTKLEIK
Vκ-BA7: X746   ENVLTQSPAIMSASPGEKVTMTCRASSSVSSSYLH-----WYQQKSGASPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSVEAEDAATYYCQQYSGYPLTFGAGTKLELK
Vκ-BA4: X748   DIVMTQAPLSLPVSLGDQASISCRSSQSLVHSNGNTYLH-WYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPLTFGAGTKLELK
Vκ-BA9: X758   DIVMTQAPLSLPVSLGDQASISCRSSQSIVHSNGNTYL-EWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSHVPFTFGGGTKLEIK
               FR1            CDR1            FR2            CDR2            FR3                CDR3        FR4
```

*Position of the various CDR regions (1-3) are underlined in VL and VH. The boundaries of the CDR regions (1-3) are shown in Table 3 and Table 4. Antibody BA9, also named 158 in the patent application., is an example of a high affinity protofibril specific antibody according to the invention.

Table 2.

Amino acid sequences of CDR1-2 regions from VH chain from a protofibril selective antibody and amino acid substitutions that are compatible with high affinity binding to human wild-type Aß protofibrils.

VH chain CDR-1 region	
AASGFTFSSFGMHWVR	Antibody 158
---------YA-S---	Substitutions*
VH chain CDR-2 region	
WVAYISSGSSTIYYGDTVKGRFT	Antibody 158
----------A--------	Substitutions*
---T-----G-YT--P-S------	Substitutions*
VH chain CDR-3 region	
CAREG-GYYYGRSYY-TMDYWGQ	Antibody 158
CARYGxxxxxNYxxxxAMDYWGQ	Substitutions and deletions*
CARNYxxxxGSRRxxxYFDVWGA	Substitutions and deletions*

* The amino acid substitutions (other amino acid than in antibody 158) are shown with one amino acid letter code. Deletions are shown with (x).

Table 3.

Amino acid sequences of CDR 1-3 regions from VL chain from a protofibril selective antibody and amino acid substitutions that are compatible with high affinity binding to human wild–type Aß protofibrils

VL chain CDR-1 region	
ISCRSSQSIVHSNGNTYLEWYL	Antibody 158
ITCKASQSVxxSNDxxxVAWYQ	Substitutions and deletions*
VL chain CDR-2 region	
LIYKVSNRFSGVP	Antibody 158
---YA---YT---	Substitutions*
VL chain CDR-3 region	
YYCFQGSHVPPTFGG	Antibody 158
-F-Q-DYSS-F---S	Substitutions*

* The amino acid substitutions (other amino acid than in antibody 158) are shown with one amino acid letter code. Deletions are shown with (x).

Table 4.

Amino acid sequence of mouse framework regions of the mouse and human variable light chain (VL) region from protofibril specific antibodies

Mouse framework* VL regions	
Divmtqaplslpvslgdqasiscwylqkpgqspklliygvpdrfsgsgsgtdftlkisrveaedlgiyyc	antibody 158
.....	BA9_VL_fr123
.v....t.....v...	BA1_VL_fr123
........kf.l..a..rvt.t...q.....t...y.....ft..t.q....av.f.	BA2_VL_fr123
Human framework VL regions	
......t......tp.ep.....q.....v.v...	VKII-3-1-(1)-O11
......s......tp.ep.....q.....v.v...	VKII-4-1-(1)-A19
......t....s.tp.qp.....q.....v.v...	VKII-4-1-(1)-A18
......t....s.tp.qp.....p.q.....v.v...	VKII-4-1-(1)-A2
.v....s......t..qp......fq.r.....rr.....v.v...	VKII-4-1-(1)-A17

* Framework region is the region outside the CDR regions. The CDR regions has been deleted for clarity.

Table 5.

Amino acid sequence of mouse and human framework regions of the mouse and human variable light heavy (VH) region from protofibril specific antibodies

Mouse framework* VH regions	
Evklmesggglvqpggsrklscaaswvrqapekglewvarftisrdnpkntlflqmtslrsedtamyycar	antibody 158
.....	BA9_VH_fr123
....v.....	BA1_VH_fr123
....v.....k....l.....t...r.....a....y...s.....	BA2_VH_fr123
Human framework VH regions	
..q.v.....lr.....g.....a..s.y...n...a....v.....	VH3-7_fr123
..q.v.....i.....lr.....g.....s.....s....y...n...a....v.....	VH3-53_fr123
..q.l.....lr.....g.....s.....s....y...n...a....v....k	VH3-23_fr123
..q.v.....lr.....g.....s.....a..s.y...n...d....v.....	VH3-48_fr123
..q.v.....lr.....g...v..s.....a....y...n...a....v.....	VH3-74_fr123

* Framework region is the region outside the CDR regions. The CDR regions has been deleted for clarity.

Table 6. PCR primers for cloning mouse VK

Name	Sequence (5'→ 3')
MKV1	ATGAAGTTGVVTGTTAGGCTGTTGGTGCTG
MKV2	ATGGAGWCAGACACACTCCTGYTATGGGTG
MKV3	ATGAGTGTGCTCACTCAGGTCCTGGSGTTG
MKV4	ATGAGGRCCCCTGCTCAGWTTYTTGGMWTCTTG
MKV5	ATGGATTTWAGGTGCAGATTWTCAGCTTC
MKV6	ATGAGGTKCKKTGKTSAGSTSCTGRGG
MKV7	ATGGGCWTCAAGATGGAGTCACAKWYYCWGG
MKV8	ATGTGGGGAYCTKTTTYCMMTTTTTCAATTG
MKV9	ATGGTRTCCWCASCTCAGTTCCTTG
MKV10	ATGTATATATGTTTGTTGTCTATTTCT
MKV11	ATGGAAGCCCCAGCTCAGCTTCTCTTCC
MKC	ACTGGATGGTGGGAAGATGG

Table 7. PCR primers for cloning mouse heavy VH

Name	Sequence (5'→ 3')
MHV1	ATGAAATGCAGCTGGGGCATSTTCTTC
MHV2	ATGGGATGGAGCTRTATCATSYTCTT
MHV3	ATGAAGWTGTGGTTAAACTGGGTTTTT
MHV4	ATGRACTTTGGGYTCAGCTTGRTTT
MHV5	ATGGACTCCAGGCTCAATTTAGTTTTCCTT
MHV6	ATGGCTGTCYTRGSGCTRCTCTTCTGC
MHV7	ATGGRATGGAGCKGGRTCTTTMTCTT
MHV8	ATGAGAGTGCTGATTCTTTTGTG
MHV9	ATGGMTTGGGTGTGGAMCTTGCTATTCCTG
MHV10	ATGGGCAGACTTACATTCTCATTCCTG
MHV11	ATGGATTTTGGGCTGATTTTTTTTATTG
MHV12	ATGATGGTGTTAAGTCTTCTGTACCTG
MHCG1	CAGTGGATAGACAGATGGGGG
MHCG2a	CAGTGGATAGACCGATGGGGC
MHCG2b	CAGTGGATAGACTGATGGGGG
MHCG3	CAAGGGATAGACAGATGGGGC

Legend: Wobble bases are defined in Abbreviations (Section 2).

Table 8.

Sequences important for efficient expression of immunoglobulin in mammalian cells

Name	Consensus DNA Sequence (5'→ 3')
Kozak translation initiation site	G C C G C C **R** C C^{-1} A^{+1} U G G
Kappa light chain splice donor site	A C :: **G T** R A G T
Heavy chain splice donor site	M A G :: **G T** R A G T
Immunoglobulin splice acceptor site	Y Y Y Y Y Y Y Y Y Y Y N C A G :: G

Legend: Bases shown in **bold** are considered to be invariant within each consensus sequence. Splice sites are defined by the symbol " : : ". Wobble bases are defined in Abbreviations (see Examples 9-11).

Table 9. Oligonucleotide primers used to generate chimeric 158

Oligonucletotide name	Sequence (5'→ 3')
158vh	AAGCTT**GCCGCCACC**ATGGACTCCAGGCTC
158vhrev	GGGCCCTTGGTGGAGGCTGAGGAGACGGTGACTGAGG
158vl	AAGCTT**GCCGCCACC**ATGAAGTTGCCTGTTAGG
158vlrev	GGATCCACTCACGTTTGATTTCCAGCTTGG

Legend: Restriction sites are underlined. Kozak sequences are in **bold type.**

Table 10. Oligonucleotide primers used for sequencing

Oligonucletotide name	Sequence (5'→ 3')
1212 (17mer)	GTTTTCCCAGTCACGAC
1233 (24mer)	AGCGGATAACAATTTCACACAGGA
Hu-K2 (17mer)	CTCATCAGATGGCGGGA
BDSH61R	CGCTGCTGAGGGAGTAGAGTC

Table 11. DNA sequence of 158 VK, primer MKV1 and the VK sequence derived using primers located within the V region

```
  1 ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGCTTCCAGCAGTGATGTTTTGATGACCCAAACTCCACTCTCCCTG 158 VK
  1 .........VV.....................                                                             MKV1
  1 ------------------------------------------------------...A..G..........GG..............  *** VK

 91 CCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTGTACATAGTAATGGAAACACCTATTTAGAATGGTAC 158 VK
 34 ..........................................................................................  *** VK

181 CTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGA 158 VK
124 ..........................................................................................  *** VK

271 TCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAATTTATTACTGCTTTCAAGGTTCACATGTTCCTCCG 158 VK
214 ..........................................................................................  *** VK

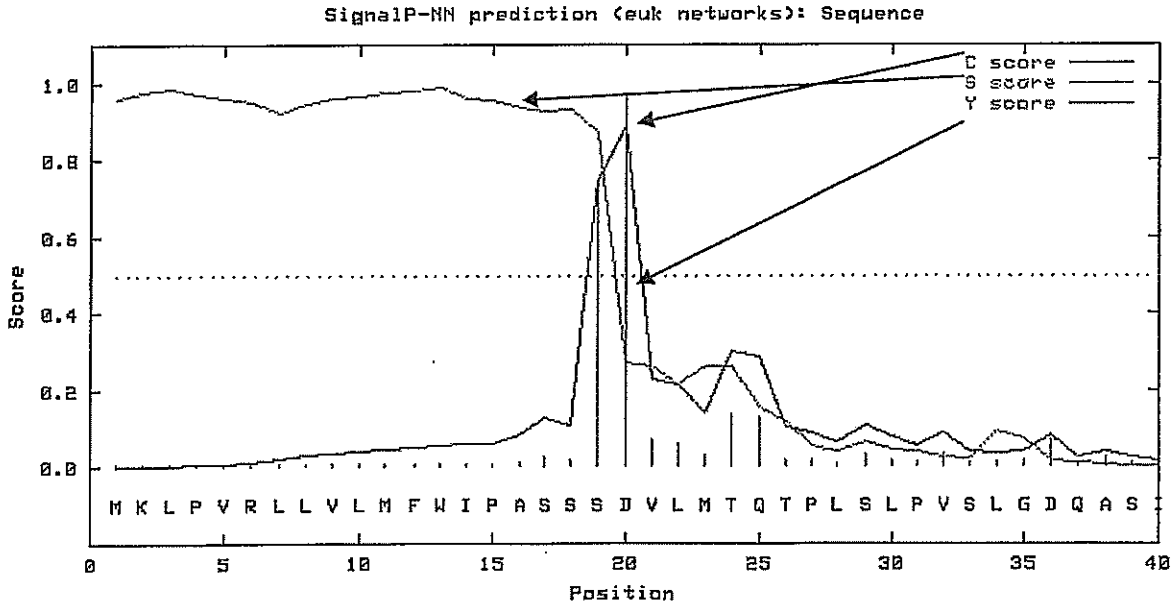
361 ACGTTCGGTGGAGGCACCAAGCTGGAAATCAAACGGGCTG                                                   158 VK
304 ................................                                                            *** VK
```

Legend: Residues identical to 158 VK are indicated by a dot. ***Sequencing using primers located within the V region.

Table 12. Chimeric VK leader sequence selection - K5.1# leader selection for the chimeric VK

158 VK	MKLPVRLLVLMFWIPASSS
K5.1#Protein	MKLPVRLLVLMFWIPASSS
K5.1#DNA	ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCT GCTTCCAGCAGT

Table 13. SignalP result 6 for K5.1# leader




```
>Sequence          length = 40
# Measure Position Value Cut off signal peptide?
 max.   C  20      0.970  0.32  YES
 max.   Y  20      0.890  0.33  YES
 max.   S  13      0.989  0.87  YES
 mean   S  1-19    0.954  0.48  YES
        D  1-19    0.922  0.43  YES
```

Highest probability for cleavage is between amino acid residue 19 and 20 (SSS-DV)

Legend: The signal P algorithm[6] generates the combination score Y ,from the cleavage site score C, and the signal peptide score S.

Table 14. Protein and DNA sequence of chimeric 158 VK construct

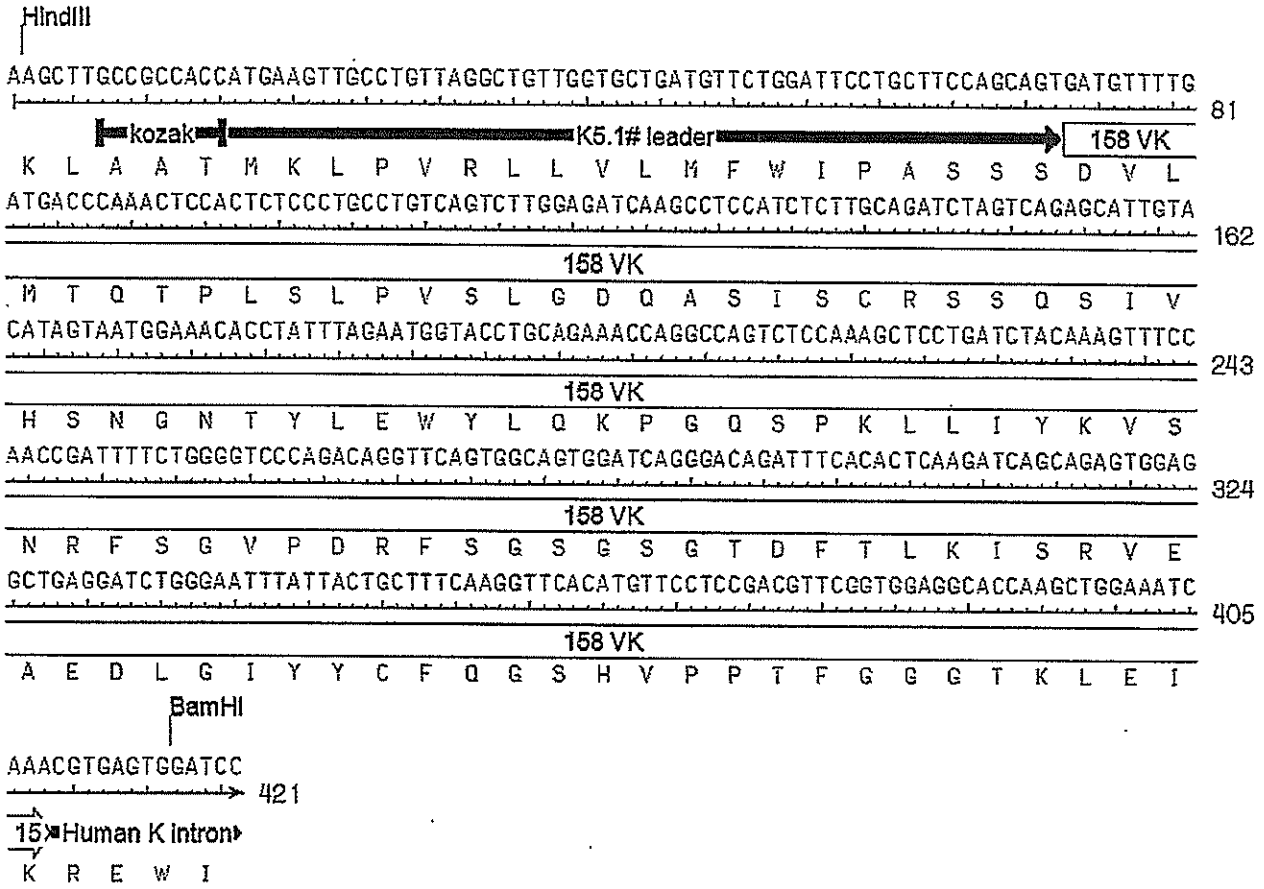


Table 15. DNA sequence of 158 VH, primer MHV5 and the sequence derived using primers located within the V region

```
  1 ATGGACTCCAGGCTCAATTTAGTTTTCCTTGTCCTTATTTTAAAAGGTGTCCAGTGTGATGTGCAGCTGGTGGAGTCTGGGGGAGGCTTA 158 VH
  1 ----------------------------------------------------------..G...A.....A....A.............. *** VH
  1 ............................                                                                  MHV5

 91 GTGCAGCCTGGAGGGTCCCGGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTAGCTTTGGAATGCACTGGGTTCGTCAGGCTCCA 158 VH
 34 ..................................................................................... *** VH

181 GAGAAGGGGCTGGAGTGGGTCGCATACATTAGTAGTGGCAGTAGTACCATCTACTATGGAGACACAGTGAAGGGCCGATTCACCATCTCC 158 VH
124 ..................................................................................... *** VH

271 AGAGACAATCCCAAGAACACCCTGTTCCTGCAAATGACCAGTCTAAGGTCTGAGGACACGGCCATGTATTACTGTGCAAGAGAGGGGGGA 158 VH
214 ..................................................................................... *** VH

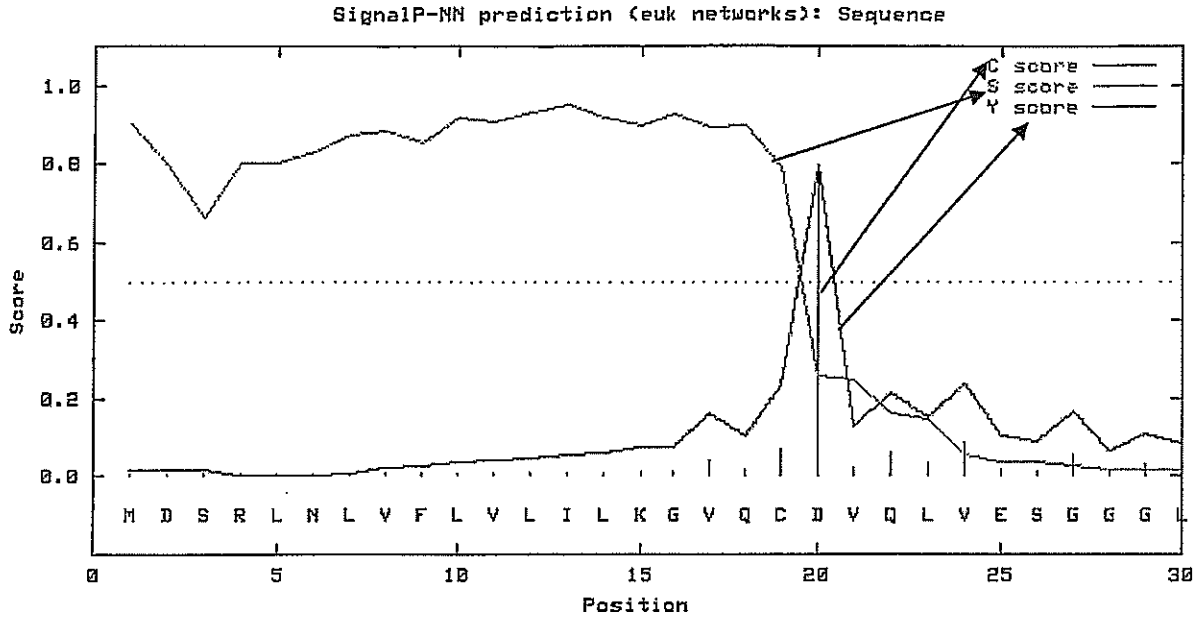
361 TATTACTACGGTAGGAGTTACTATACTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCCAAAACAACAGCCCCA    158 VH
304 ..................................................................................... *** VH
```

Legend: Residues identical to 158 VH are indicated by a dot . ***Sequencing using primers located within the V region.

Table 16. Chimeric VH leader selection - NL-1 VH leader sequence

158 VH leader	MDSRLNLVFLVLILKGVQC
NL-1 protein	MDSRLNLVFLVLILKGVQC
NL-1 DNA	ATGGACTCCAGGCTCAATTTAGTTTTCCTTGTCCTTATTTTAAAGGTGTCCAGTGT

Table 17 - SignalP result 6 for NL-1 VH leader sequence




```
# Measure Position Value Cut off signal peptide?
 max.   C  20     0.775  0.32  YES
 max.   Y  20     0.795  0.33  YES
 max.   S  13     0.953  0.87  YES
 mean   S  1-19   0.866  0.48  YES
        D  1-19   0.830  0.43  YES
```

Highest probability for cleavage is between amino acid residue 19 and 20 (VQC-DV)

```
19 and 20: VQC-DV
```

Legend: The signal P algorithm[6] generates the combination score Y ,from the cleavage site score C, and the signal peptide score S.

Table 18 - Protein and DNA sequence of chimeric 158 VH

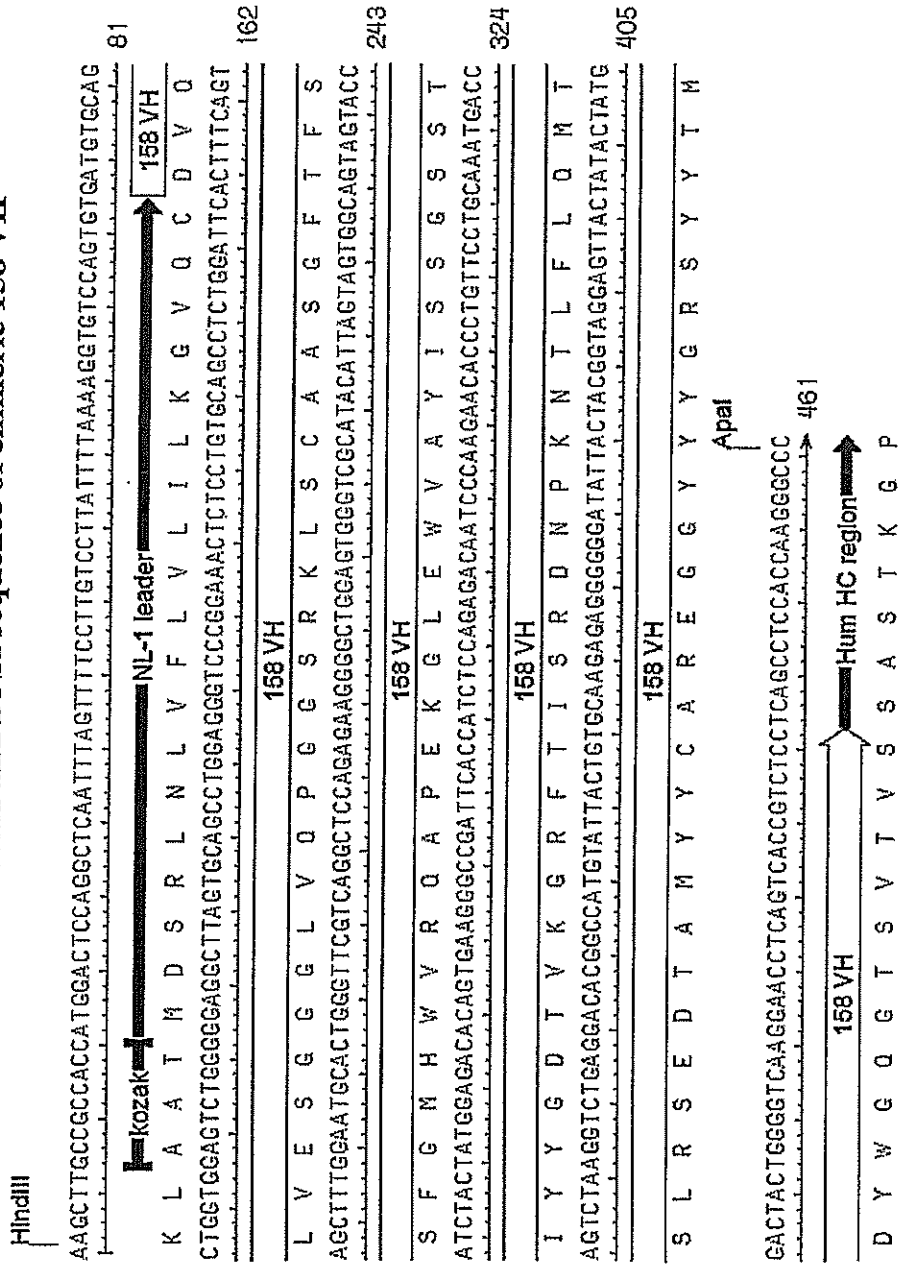


Table 19 - Expression of chimeric 158 antibody in COS cells

Number of Co-transfections	Expression Vector Constructs Co-Transfected	Antibody Concentration (ng/ml)
2 pooled	pG1D200158 and pKN100158	300
2 pooled	pG1D200158 and pKN100158	3700

Legend: Antibody concentration was measured by ELISA in 3-day cultures of transfected COS 7 cells. COS cells were co-transformed with 10μg each of the heavy and light chain chimeric expression vectors pG1D200158 and pKN100158.

Table 20. Amino acid sequence of 158 VH and 158 VK

VH	DVQLVESGGGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTIYYGDTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCAREGGYYYGRSYYTMDYWGQGTSVTVSS
VK	DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSHVPPTFGGGTKLEIK

Table 21. Best human VH framework VCI scores compared with 158 VH

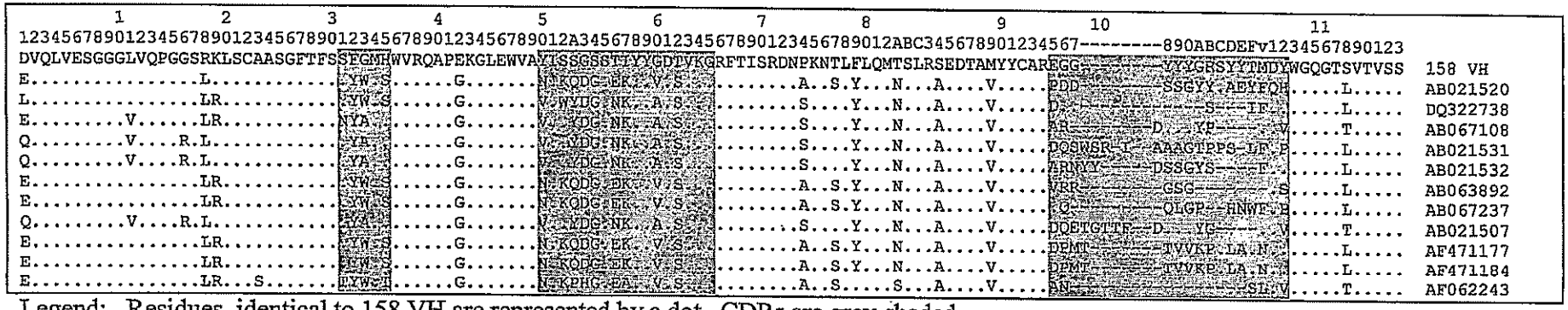
Kabat Number[6]			2 24 26 27 28 29 30 37 39 45 47 48 49 67 69 71 73 78 91 93 94 103
Canonical Residue[8]			-111-1---------2----1-
Vernier Residue[7]			*--****---********-**-
Interface Residue[9]			--------IIII-------II-I
Sequence name	FW score	VCI score	VCI Residues
158 VH	87	22	VAGFTFSVQLWVAFIRNLYARW
38687	79	22	.....
AB021520	77	22	.....
AJ556669	77	22	.....
38672	77	22	.....
38673	77	22	.....
DQ322738	77	22	.....
AB067108	76	22	.....
AB021531	76	22	.....
AB021532	76	22	.....
AB063892	76	22	.....
AB067237	76	22	.....
AB021507	76	22	.....
AF471177	76	22	.....
AF471184	76	22	.....
AF062243	76	22	.....
AF174030	76	22	.....
AF466141	76	22	.....
AF466142	76	22	.....
AJ245279	76	22	.....
AJ579216	76	22	.....

Legend: Canonical residues are numbered in this table according to which CDR they are associated with. FW score and VCI score are the number of residues in the FW or VCI definition respectively, which are identical to their counterpart in 158. Residues identical to those in 158 VH are indicated by a dot.

Table 22. Sequences of best VCI-scoring human VH, compared with 158 VH

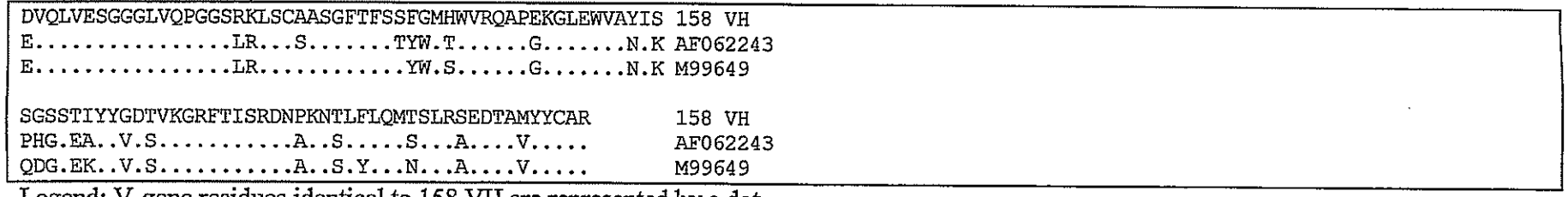
Kabat Number[6]	1 2 3 4 5 6 7 8 9 10 11 -12345678901234567890123456789012345AB6789012345678901 2ABC3456789012345678901234567890123456789012ABC3456789012345678 90ABCDEFGHIJKlmnopqrstuv1234567890123
Canonical	1 11 1 1 2 22 2 1
Vernier	* **** *** * * * * * ** *
Interface	I I I I I I I I I I
Kabat CDR	******* ********** **********
158 VH	-DVQLVESGGGLVQPGGSRKLSCAASGFTFSSFGMH--WVRQAPEKGLEWVAYISS--GSSTIYYGDTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCAREGGYYYGRSYYT----------MDYWGQGTSVTVSS
AB021520	-EVQLVESGGGLVQPGGSLKLSCAASGFTFSSYWMS--WVRQAPGKGLEWVANIKQ--DGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARPDDSSGYYSAEY----------FQHWGQGTLVTVSS
AJ556669	-EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMS--WVRQAPGKGLEWVANIKE--DGGEKFYVDSVKGRFTISRDNPKNSLFLQMNSLRAEDTAVYYCARERGHDFWSIYYTH----------FDYWGQGALVTVSS
DQ322738	PLVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMS--WVRQAPGKGLEWVAVIWY--DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDGGSI----------FDYWGQGTLVTVSS
AB067108	-EVQLVESGGGVVQPGGSLRLSCAASGFTFSNYAMH--WVRQAPGKGLEWVAVISY--DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARARDYYYYP----------MDVWGQGTTVTVSS
AB021531	-QVQLVESGGGVVQPGRSLKLSCAASGFTFSSYAMH--WVRQAPGKGLEWVAVISY--DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDQSWSRIAAAGTPPSL----------FDPWGQGTLVTVSS
AB021532	-QVQLVESGGGVVQPGRSLKLSCAASGFTFSSYAMH--WVRQAPGKGLEWVAVISY--DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARARNYYDSSGYS----------FDYWGQGTLVTVSS
AB063892	-EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMS--WVRQAPGKGLEWVANIKQ--DGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVRRGS----------GDSWGQGTLVTVSS
AB067237	-EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMS--WVRQAPGKGLEWVANIKQ--DGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAREQQLGPHNW----------FDPWGQGTLVTVSS
AB021507	-QVQLVESGGGVVQPGRSLKLSCAASGFTFSSYAMH--WVRQAPGKGLEWVAVISY--DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDQETGTTFDYYYYG----------MDVWGQGTTVTVSS
AF471177	-EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMS--WVRQAPGKGLEWVANIKQ--DGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDPMTTVVKPSLAT----------NDYWGQGTLVTVSS
AF471184	-EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMS--WVRQAPGKGLEWVANIKQ--DGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDCVGALGA----------FDIWGQGTMVTVSS
AF062243	CEVQLVESGGGLVQPGGSLRLSCSASGFTFSTYWMT--WVRQAPGKGLEWVANIKP--HGSEAYYVDSVKGRFTISRDNAKNSLFLQMSSLRAEDTAVYYCARANS----------LDVWGQGTTVTVSS
AF174030	CEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMS--WVRQAPGKGLEWVANIKQ--DGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDGDIGDWW----------FDPWGQGTLVTVSS
AF466141	-QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMH--WVRQAPGKGLEWVAVIWY--DGSNKYYADSAKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCARDKGYYDYVWGSYRSNPKNDA-------FDIWGQGTMVTVSS
AF466142	-QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMH--WVRQAPGKGLEWVAVIWY--DGSNKYYADSAKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCARDKGYYDYVWGSYRSNPKNDA-------FDIWGQGTMVTVSS
AJ245279	-QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMH--WVRQAPGKGLEWVAVIWY--DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRFF----------FDNWGQGTLVTVSS

Table 23. Alignment of 158 VH with the best VCI-scoring human VH



Legend: Residues identical to 158 VH are represented by a dot. CDRs are grey-shaded

Table 24. VH signal peptide selection - 5.5.1 V-gene alignment of 158 VH, human AF062243 and human germline M99649 (VH3-07)

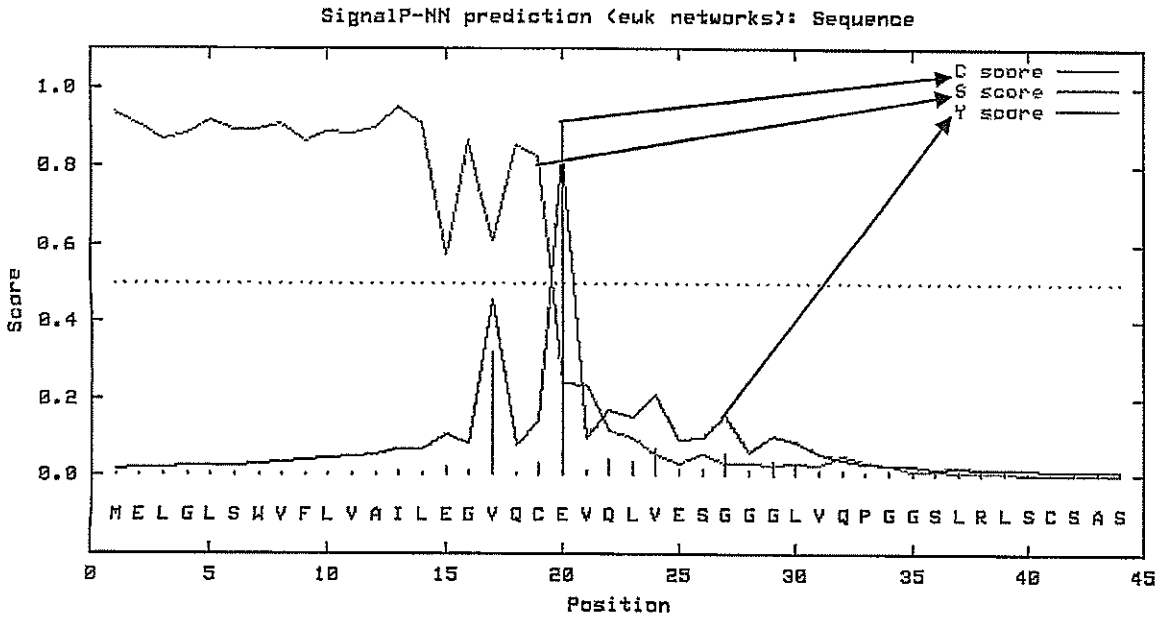


Legend: V-gene residues identical to 158 VH are represented by a dot.

Table 25. Signal peptide of M99649 human germline VH gene

DNA	ATGGAATTGGGGCTGAGCTGGGTTTTCCTTGTTGCTATTTTAGAAGGTGTCCAGTGT
protein	MELGLSWVFLVAILEGVQC

Table 26. M99649 signal peptide cutting prediction



# Measure	Position	Value	Cutoff	signal peptide?
max. C	20	0.909	0.32	YES
max. Y	20	0.836	0.33	YES
max. S	13	0.953	0.87	YES
mean S	1-19	0.859	0.48	YES
D	1-19	0.848	0.43	YES

Highest probability for cleavage is between amino acid residue 19-20: VQC-DV.

Legend: The signal P algorithm[10] generates the combination score Y ,from the cleavage site score C, and the signal peptide score S

Table 27. Generation of 158RHA protein sequence

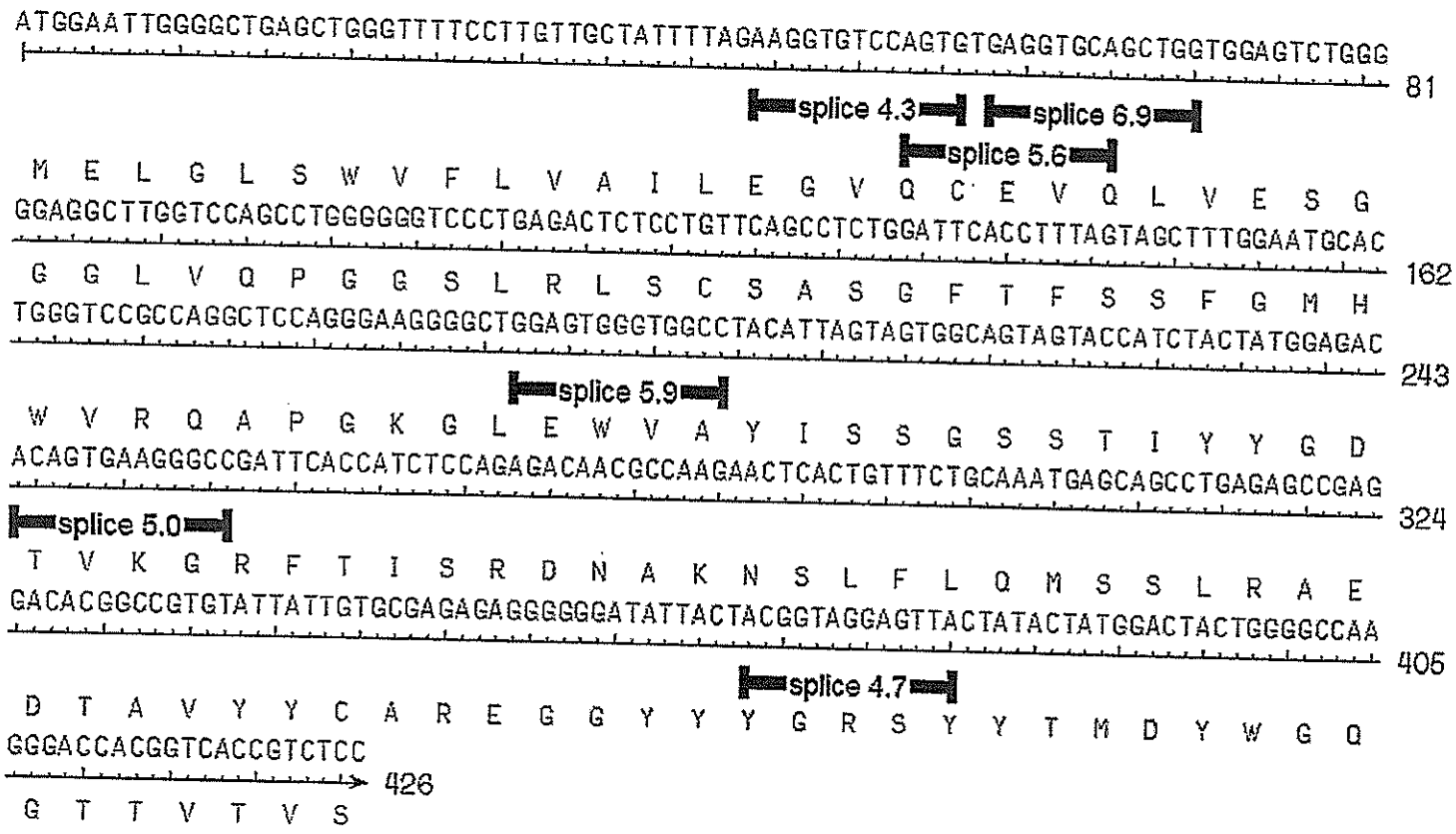
Kabat Number[6]	1 2 3 4 5 6 7 8 9 10 11 01234567890123456789012345678901234567890123456789012345AB6789012345678901ABC3456789012345678901234567890ABC3456789012345678901ABCDEFGHIJK1234567890123
CDR	===H1== =====H2========== =========H3========
158VH	EVQLVESGGGLVQPGGSRKLSCAASGFTFSSFGMH--WVRQAPEKGLEWVAYISS--GSSTIYYGDTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCAREGGYYYGRSYYT---MDYWGQGTSVTVSS
158_CDR	----------SFGMH----------YISS--GSSTIYYGDTVKG----------EGGYYYGRSYYT---MDY
158RHA	-EVQLVESGGGLVQPGGSLRLSCSASGFTFSSFGMH--WVRQAPGKGLEWVAYISS--GSSTIYYGDTVKGRFTISRDNAKNSLFLQMSSLRAEDTAVYYCAREGGYYYGRSYYT---MDY
AF062243 FW	-EVQLVESGGGLVQPGGSLRLSCSASGFTFS WVRQAPGKGLEWVA RFTISRDNAKNSLFLQMSSLRAEDTAVYYCAR----MDYWGQGTTVTVS
AF062243	CEVQLVESGGGLVQPGGSLRLSCSASGFTFSTYWMT--WVRQAPGKGLEWVANIKP--HGSEAYYVDSVKGRFTISRDNAKNSLFLQMSSLRAEDTAVYYCARANS----------LDVWGQGTTVTVSS

Table 28. Generation of 158RHA DNA sequence - Generation of 158RHAss DNA sequence

Region	DNA	Protein
VH3-07 leader	ATGGAATTGGGGCTGAGCTGGGTTTTCCTTGTTGCTATTTTAGAAGGTGTCCAGTGT	MELGLSWVFLVAILEGVQC
AF062243 FW1	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTTCAGCCTCTGGATTCACCTTTAGT	EVQLVESGGGLVQPGGSLRLS CSASGFTFS
158_CDR1	AGGTTTTCCAATGCAC	SFGMH
AF062243 FW2	TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCC	WVRQAPGKGLEWVA
158_CDR2	TACATTAGTAGTGGCAGTAGTACCATCTACTATGCAGACACAGTGAAGGGC	YISSGSSTIYYGDTVKG
AF062243 FW3	CGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTTTCTGCAAATGAGCAGCCTGAGAGCCGAGGACACGGCCGT GTATTATTGTGCGAGA	RFTISRDNAKNSLFLQMSSLR AEDTAVYYCAR
158_CDR3	GAGGGGGCATATTACTACGGTATGGACGTCTGGGGCCAAGGG	EGGYYYGRSYYTMDY
AF062243 FW4	TGGGGCCAAGGGACCACGGTCACCGTCTCC	WGQGTTVTVS

Legend: Human VH3-07 leader and AF062243VH FWs intercalated with 158 VH CDRs (grey-shaded) to generate 158RHAss. (relate to Tables 27 and 28)

Table 29. DNA and protein sequence of 158RHAss



Legend:
Splice donor sites predicted by Lasergene 6.0 GeneQuest analysis, together with their score, using the human_ds_2 matrix with a threshold of 4.2.

Table 30. Mutations in 158RHA removing splice sites in 158RHAss

```
   1 ATGGAATTGGGGCTGAGCTGGGTTTTCCTTGTTGCTATTTTAGAGGGAGT 158RHA
   1 ..........................................A..T..
158RHAss

  51 CCAGTGCGAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTG 158RHA
  51 ......T..G.....................................
158RHAss

 101 GGGGGTCCCTGAGACTCTCCTGTTCAGCCTCTGGATTCACCTTTAGTAGC 158RHA
 101 .............................................
158RHAss

 151 TTTGGAATGCACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGGT 158RHA
 151 .....................................G.....
158RHAss

 201 GGCCTACATTAGTAGTGGCAGTAGTACCATCTACTATGGAGACACCGTGA 158RHA
 201 .........................................A....
158RHAss

 251 AGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTTTCTG 158RHA
 251 .............................................
158RHAss

 301 CAAATGAGCAGCCTGAGAGCCGAGGACACGGCCGTGTATTATTGTGCGAG 158RHA
 301 .............................................
158RHAss

 351 AGAGGGGGGATATTACTACGGAAGGAGTTACTATACTATGGACTACTGGG 158RHA
 351 ..................T..........................
158RHAss

 401 GCCAAGGGACCACGGTCACCGTCTCC                       158RHA
 401 .........................
158RHAss
```

Legend 158RHA DNA sequence compared to 158RHAss (Table 5.7.2) which contains predicted splice sites. Positions identical to 158RHA are identified as a dot.

Table 31. DNA and protein sequence of 158RHA

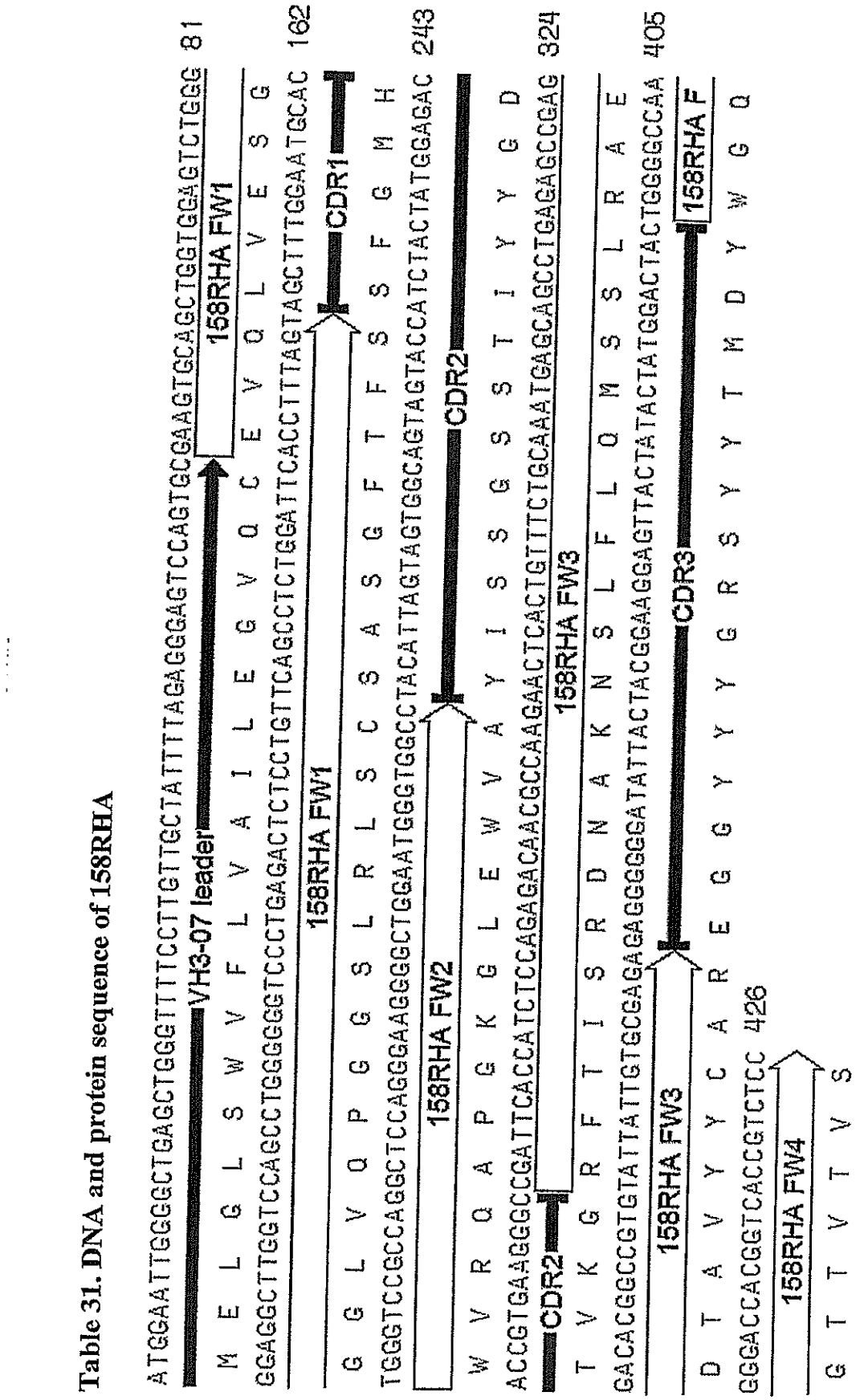


Table 32. Best VCI scores of human VK compared with 158 VK

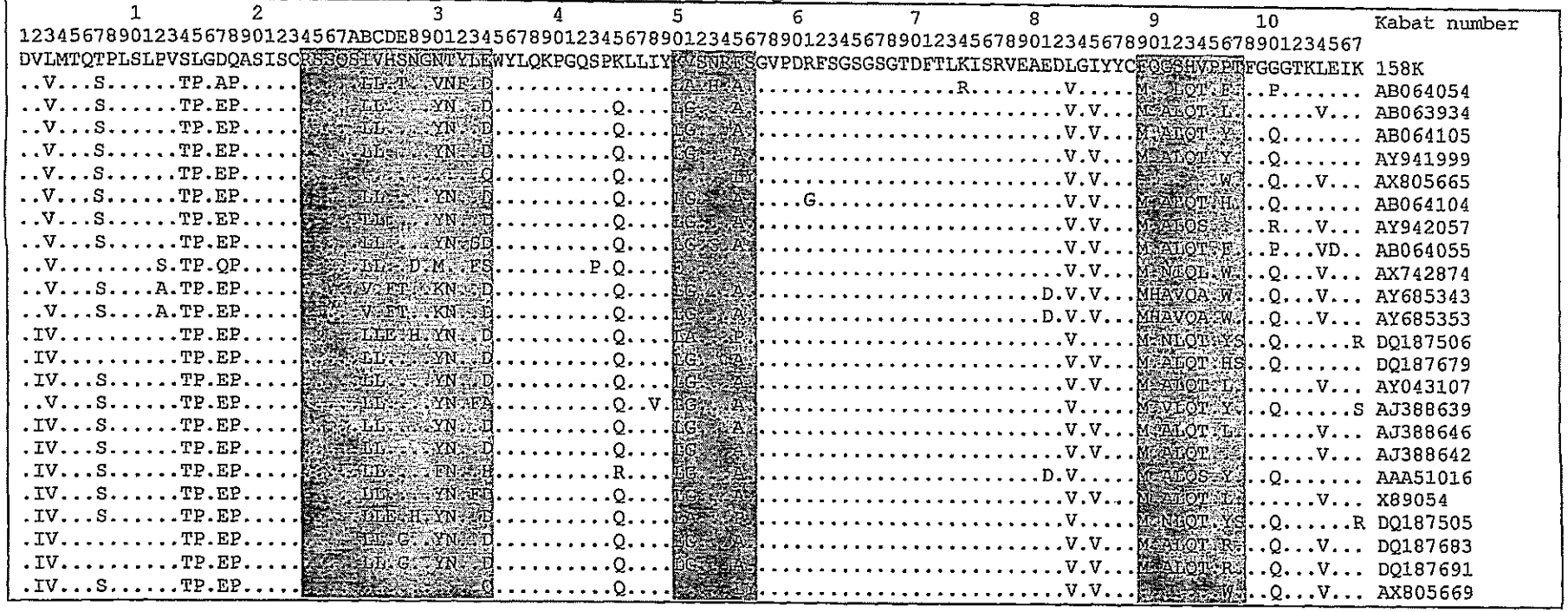
Kabat Number[6]			2 4 35 36 38 44 46 47 48 49 64 66 68 69 71 87 98
Canonical Residue[8]			1 2 2 1
Vernier Residue[7]			**** ********* *
Interface Residue[9]			IIII II
Sequence	Fw score	VCI score	VCI residues
158 VK	80	17	VMWYQPLLIYGGGTFYF
AB064054	71	17	V.
AB063934	70	17	V.
AB064105	70	17	V.
AY941999	70	17	V.
AX805665	69	17	V.
AB064104	69	17	V.
AY942057	69	17	V.
AB064055	68	17	V.
AX742874	68	17	V.
AY685343	67	17	V.
AY685353	67	17	V.
DQ187506	70	16	I.
DQ187679	70	16	I.
AY043107	69	16	I.
AJ388639	69	16	.....V.....
AJ388646	69	16	I.
AJ388642	69	16	I.
M74470	69	16	I.
X72466	69	16	I.
U95244	69	16	I.
AAA51016	69	16	I.
X89054	69	16	I.
DQ187505	69	16	I.
DQ187683	69	16	I.
DQ187691	69	16	I.
AX805669	68	16	I.
AF455562	68	16	I.

Legend: Canonical residues are numbered in this table according to which CDR they are associated. FW score and VCI score are the number of residues in the FW or VCI definition respectively, which are identical to their counterpart in 158. Residues identical to 158 VK are indicated by a dot.

Table 33. Sequences of best VCI-scoring human VK, compared with 158 VK

Kabat number [6]	1 2 3 4 5 6 7 8 9 10 12345678901234567890123456789ABCDEF8901234567890123456789012345678901234567890123456789012345ABCDEF678901234567
Canonical [8]	1 1 1 1 1 2 2 1 3 3 3
Vernier [7]	* * ** **** * * ** * *
Interface [5]	F F F F F F F F
VCI	1 * 1 1 1 1F*F F F **** * * ** * F F3F 33 * F3F
Kabat CDR	********** ******* **********
158 VK	DVLMTQTPLSLPVSLGDQASISCRSSQSIVHS-NGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSHVP------PTFGGGTKLEIK
AB064054	DVVMTQSPLSLPVTPGAPASISCRSSQSLLHT-NGVNFLDWYLQKPGQSPKLLIYLASHRASGVPDRFSGSGSGTDFTLRISRVEAEDVGIYYCMQGLQTP------FTFGPGTKLEIK
AB063934	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------LTFGGGTKVEIK
AB064105 scFv	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------YTFGQGTKLEIK
AY941999 scFv	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------YTFGQGTKLEIK
AX805665 patent	DVVMTQSPLSLPVTPGEPASISCRSSQSIVHS-NGNTYLQWYLQKPGQSPQLLIYKVSNRLYGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVP------WTFGQGTKVEIK
AB064104	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDGFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------HTFGQGTKLEIK
AY942057 scFv	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQSP------PTFGRGTKVEIK
AB064055	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYSDWYLQKPGQSPQLLIYLGSSRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------FTFGPGTKVDIK
AX742874	DVVMTQTPLSLSVTPGQPASISCRSSQSLLHS-DGMTYFSWYLQKPGQPPQLLIYEVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQNIQLP------WTFGQGTKVEIK
AY685343	DVVMTQSPLSLAVTPGEPASISCRSSQSVVFT-NGKNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEADDVGVYYCMHAVQAP------WTFGQGTKVEIK
AY685353	DVVMTQSPLSLAVTPGEPASISCRSSQSVVFT-NGKNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEADDVGVYYCMHAVQAP------WTFGQGTKVEIK
DQ187506	DIVMTQTPLSLPVTPGEPASISCRSSQSLLES-HGYNYLDWYLQKPGQSPQLLIYLASNRPSGVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQNLQTP------YSFGQGTKLEIR
DQ187679	DIVMTQTPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------HSFGQGTKLEIK
AY043107	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------LTFGGGTKVEIK
AJ388639	DVVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYFAWYLQKPGQSPQLLVYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQVLQTP------YTFGQGTKLEIS
AJ388646	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------LTFGGGTKVEIK
AJ388642	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------PTFGGGTKVEIK
M74470	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGFNYLHWYLQKPGQSPRLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEADDVGIYYCMQALQSP------YTFGQGTKLEIK
X72466	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHN-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQVLQIP------LTFGGGTKVEIK
U95244	DIVMTQSPLSLPVTPGEPASISCRSSQSLLYS-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGDYYCMQALQSP------LTFGGGTKVEIK
AAA51016	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGFNYLHWYLQKPGQSPRLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEADDVGIYYCMQALQSP------YTFGQGTKLEIK
X89054	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHS-NGYNYFDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------LTFGGGTKVEIK
DQ187505	DIVMTQSPLSLPVTPGEPASISCRSSQSLLES-HGYNYLDWYLQKPGQSPQLLIYLASNRPSGVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQNLQTP------YSFGQGTKLEIR
DQ187683	DIVMTQTPLSLPVTPGEPASISCRSSQSLLHG-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------RTFGQGTKVEIK
DQ187691	DIVMTQTPLSLPVTPGEPASISCRSSQSLLHG-NGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP------RTFGQGTKVEIK
AX805669	DIVMTQSPLSLPVTPGEPASISCRSSQSIVHS-NGNTYLQWYLQKPGQSPQLLIYKVSNRLYGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVP------WTFGQGTKVEIK

Table 34. Alignment of 158 VK with the best VCI-scoring human VK



Legend: CDR 1, 2 and 3 are grey-shaded.

Table 35. VK signal peptide selection - Alignment of 158 VK with human AB064054 and human germline A19

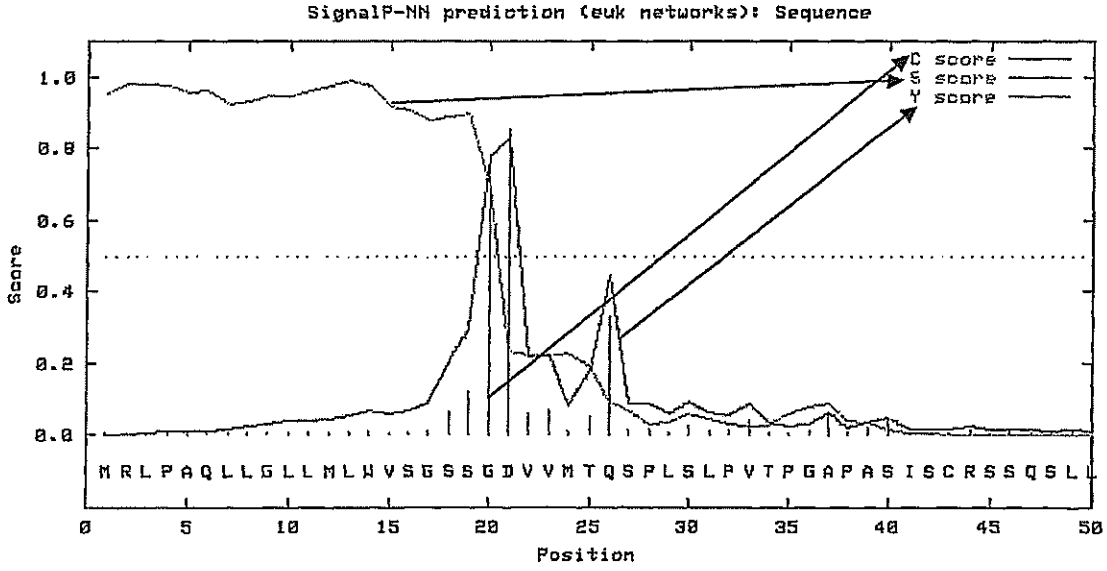
```
DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSN 158 VK
..V...S......TP.AP..........LL.T..VNF.D..............LA.H AB064054
.IV...S......TP.EP..........LL....YN..D..........Q....LG.. A19

RFSGVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSHVPPTFGGGTKLEIK     158 VK
.A..................R........V.....M..LQT.F...P......N     AB064054
.A............................V.V...M.ALQT.                  A19
```

Table 36. Signal peptide of human A19 (VK2-28; X63397) germline VK

	VK A19 leader sequence
DNA	ATGAGGCTCCCTGCTCAGCTCCTGGGGCTGCTAATGCTCTGGGTCTCTGGATCCAGTGGG
protein	MRLPAQLLGLLMLWVSGSSG

Table 37. A19 signal peptide cutting prediction




```
>Sequence                  length = 50
# Measure   Position  Value   Cutoff   signal peptide?
  max. C     21        0.853    0.32    YES
  max. Y     21        0.831    0.33    YES
  max. S     13        0.990    0.87    YES
  mean S      1-20     0.932    0.48    YES
       D      1-20     0.881    0.43    YES
# Most likely cleavage site between pos. 20 and 21: SSG-DV
```

Legend: The signal P algorithm[10] generates the combination score Y ,from the cleavage site score C, and the signal peptide score S.

Table 38. Generation of 158RKA Protein Sequence

Kabat number [6]	1 2 3 4 5 6 7 8 9 10 12345678901234567890123456789ABCDEF8901234567890123456789012345678901234567890123456789012345ABCDEF67890123456
CDR	=======L1========== ==L2=== ======L3=======
158 VK	DVLMTQTPLSLPVSLGDQASISCRSSQSLVHS-NGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSHVP-------PTFGGGTKLEIK
CDR	L1 L2 L3 RSSQSLVHS-NGNTYLE KVSNRFS FQGSHVP-------PT
158RKA	DVVMTQSPLSLPVTPGAPASISCRSSQSLVHS-NGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLRISRVEAEDVGIYYCFQGSHVP-------PTFGPGTKLEIK
FW	DVVMTQSPLSLPVTPGAPASISC WYLQKPGQSPKLLIY GVPDRFSGSGSGTDFTLRISRVEAEDVGIYYC FGPGTKLEIK
AB064054	DVVMTQSPLSLPVTPGAPASISCRSSQSLLHT-NGVNFLDWYLQKPGQSPKLLIYLASHRASGVPDRFSGSGSGTDFTLRISRVEAEDVGIYYCMQGLQTP------FTFGPGTKLEIK

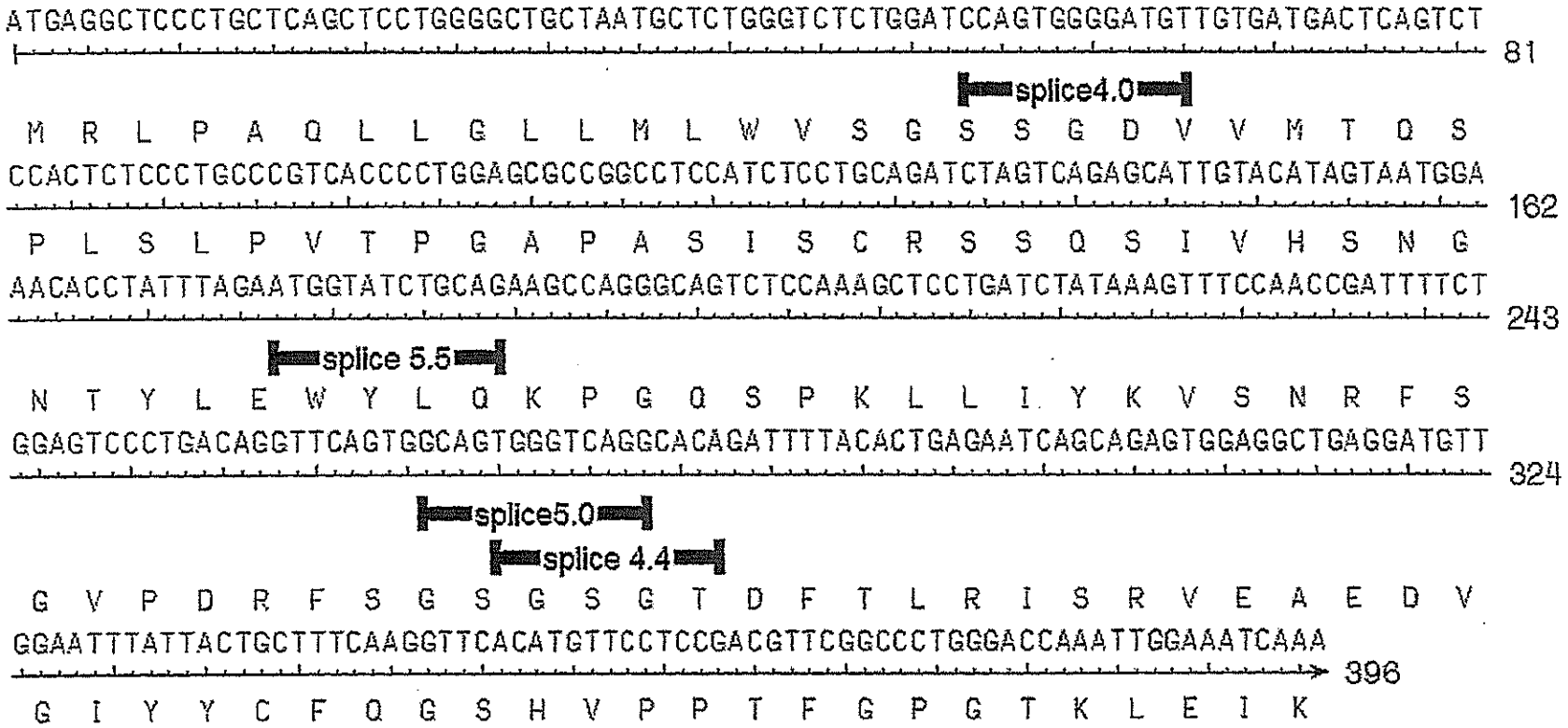
Legend: 158 VK (grey-shaded) CDRs intercalated in human AB064054 FW to generate 158RKA

Table 39 - Generation of 158RKAss DNA Sequence - Intercalation of 158VK into AB064054 FW

Region	DNA	Protein
A19 leader	ATGAGGCTCCCTGCTCAGCTCCTGGGGCTGCTAATGCTCTGGGTCTCTGGATCCAGTGGG	MRLPAQLLGLLMLWVSGSSG
AB064054 FW1	GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGCGCCGGCCTCCATCTCCTGC	DVVMTQSPLSLPVTPGAPASISC
158 CDR1	AGAATCTAGTCAGAGCATTGTACATAGTAATGGAAACACCTATTTAGAA	RSSQSLVHSNGNTYLE
AB064054 FW2	TGGTATCTGCAGAAGCCAGGGCAGTCTCCAAAGCTCCTGATCTAT	WYLQKPGQSPKLLIY
158 CDR2	AAAGTTTCCAACCGATTTTCT	KVSNRFS
AB064054 FW3	GGAGTCCCTGACAGGTTCAGTGGCAGTGGGTCAGGCACAGATTTTACACTGAGAATCAGCAGAGTGGAGGC TGAGGATGTTGGAATTTATTACTGC	GVPDRFSGSGSGTDFTLRISRVEAEDVGIYYC
158 CDR3	TTTGGAGGCTCAGGTTCCCATGTTCCG	FQGSHVP-T
AB064054 FW4	TTCGGCCCTGGGACCAAATTGGAAATCAAA	FGPGTKLEIK

Legend: A19 leader and AB064054 FW with intercalated 158 VK (grey-shaded) CDRs to generate 158RKAss

Table 40. 158RKAss DNA sequence



Legend: Splice donor sites predicted by Lasergene 6.0 GeneQuest analysis, together with their score, using the human_ds_2 matrix with a threshold of 4.2.

Table 41. Mutations in 158RKA removing splice sites in 158RKA

```
1   ATGAGGCTCCCTGCTCAGCTCCTGGGGCTGCTAATGCTCTGGGTCTCTGGAAGCAGTGGG 158RKA
1   ..................................................TC....... 158RKAss

61  GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGCGCCGGCCTCC 158RKA
61  ..................................................... 158RKAss

121 ATCTCCTGCAGATCTAGTCAGAGCATTGTACATAGTAATGGAAACACCTATTTAGAGTGG 158RKA
121 .....................................................A... 158RKAss

181 TATCTTCAAAAGCCAGGGCAGTCTCCAAAGCTCCTGATCTATAAAGTTTCCAACCGATTT 158RKA
181 .....G..G..... 158RKAss

241 TCTGGAGTCCCTGACAGGTTCAGTGGAAGTGGATCAGGCACAGATTTTACACTGAGAATC 158RKA
241 ..........................C.....G..... 158RKAss

301 AGCAGAGTGGAGGCTGAGGATGTTGGAATTTATTACTGCTTTCAAGGTTCACATGTTCCT 158RKA
301 ..... 158RKAss

361 CCGACGTTCGGCCCTGGGACCAAATTGGAAATCAAA 158RKA
361 ..... 158RKAss
```

Legend: 158RKA DNA sequence compared to 158RKAss (Table 5.13.2) which contains predicted splice sites. Residues identical to 158RKA are identified by a dot.

Table 42. DNA and protein sequence of 158RKA



Table 43. Further version of humanized 158VHA (158RHB,158RHC,158RHD)

Kabat number[6]	1 2 3 4 5 6 7 8 9 10 11 12345678901234567890123456789012345678901234567890123456789012A3456789012345678901234567890123456789012ABC3456789012345678901234567890ABCDEFv1234567890123
158RHA	EVQLVESGGGLVQPGGSLRLSCSASGFTFSSFGMHWVRQAPGKGLEWVAYISSGSSTIYYGDTVKGRFTISRDNAKNSLFLQMSSLRAEDTAVYYCAREGGYYYGRSYYTMDYWGQGTTVTVS
158RHB	EVQLVESGGGLVQPGGSLRLSCSASGFTFSSFGMHWVRQAPGKGLEWVAYISSGSSTIYYGDTVKGRFTISRDNAKNSLFLQMSSLRAEDTAVYYCAREGGYYYGRSYYTMDYWGQGTTVTVS
158RHC	EVQLVESGGGLVQPGGSLRLSCSASGFTFSSFGMHWVRQAPGKGLEWVAYISSGSSTIYYGDTVKGRFTISRDNEKNSLFLQMSSLRAEDTAVYYCAREGGYYYGRSYYTMDYWGQGTTVTVS
158RHD	EVQLVESGGGLVQPGGSLRLSCSASGFTFSSFGMHWVRQAPGKGLEWVAYISSGSSTIYYGDTVKGRFTISRDNAKNSLFLQMESLRAEDTAVYYCAREGGYYYGRSYYTMDYWGQGTTVTVS

Table 44. Further version of humanized 158VKA (158RKB,158RKC)

Kabat number	1 2 3 4 5 6 7 8 9 10 12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567
158RKA	DVVMTQSPLSLPVTPGAPASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLRISRVEAEDVGIYYCFQGSHVPPTFGPGTKLEIK
158RKB	DVEMTQSPLSLPVTPGAPASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLRISRVEAEDVGIYYCFQGSHVPPTFGPGTKLEIK
158RKC	DVVMTQSPLSLPVTPGAPASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLRISRVEAEDVGIYYCFQGSHVPPTFGEGTKLEIK

Figure 1

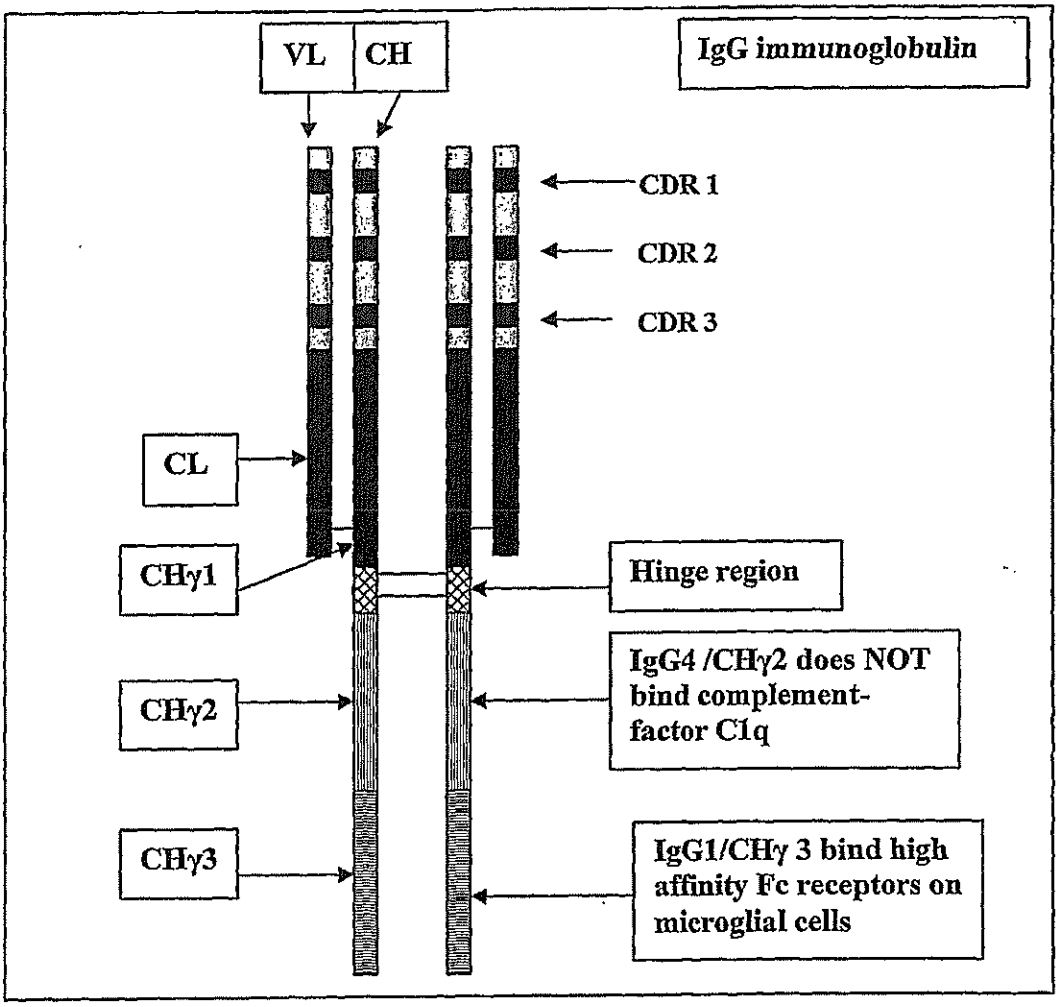
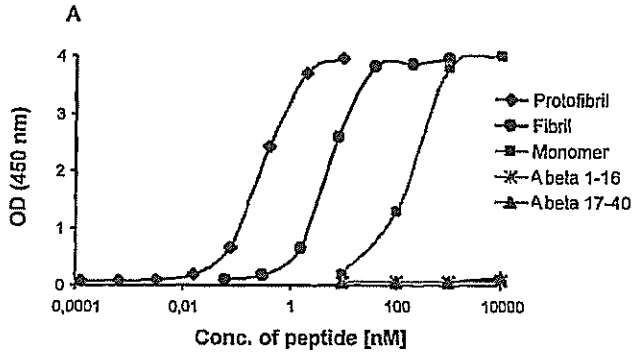

VL
CH
IgG immunoglobulin
CDR 1
CDR 2
CDR 3
CL
CHγ1
Hinge region
IgG4 /CHγ2 does NOT bind complement-factor C1q
CHγ2
CHγ3
IgG1/CHγ 3 bind high affinity Fc receptors on microglial cells

Figure 2: Characterization of a high affinity protofibril selective monoclonal antibody.



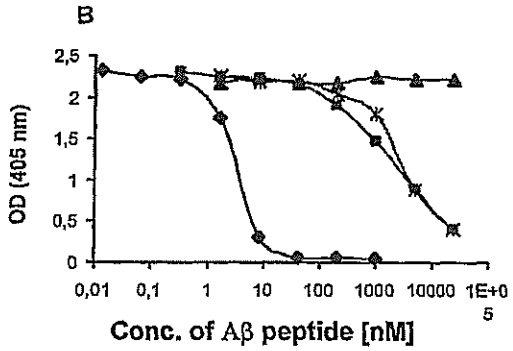


Figure 3. Therapeutic efficacy of a high affinity protofibril selective antibody in transgenic mouse model (APPswe)

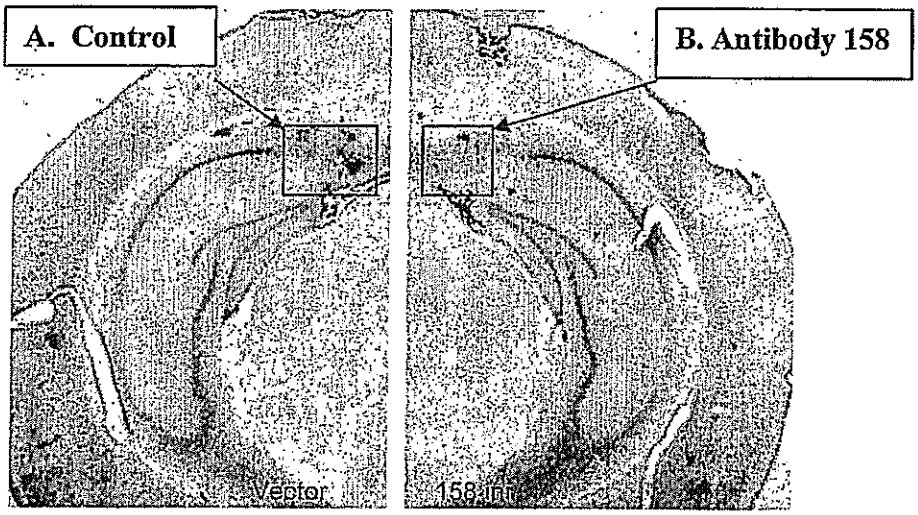


Figure 4
Human Aß protofibrils are measured at pM levels by proximity ligation technique.

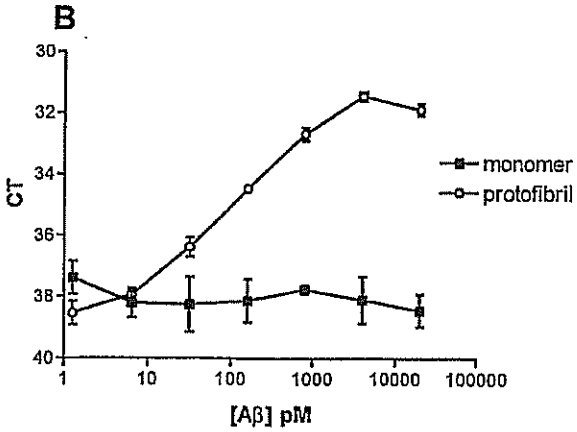


Figure 5

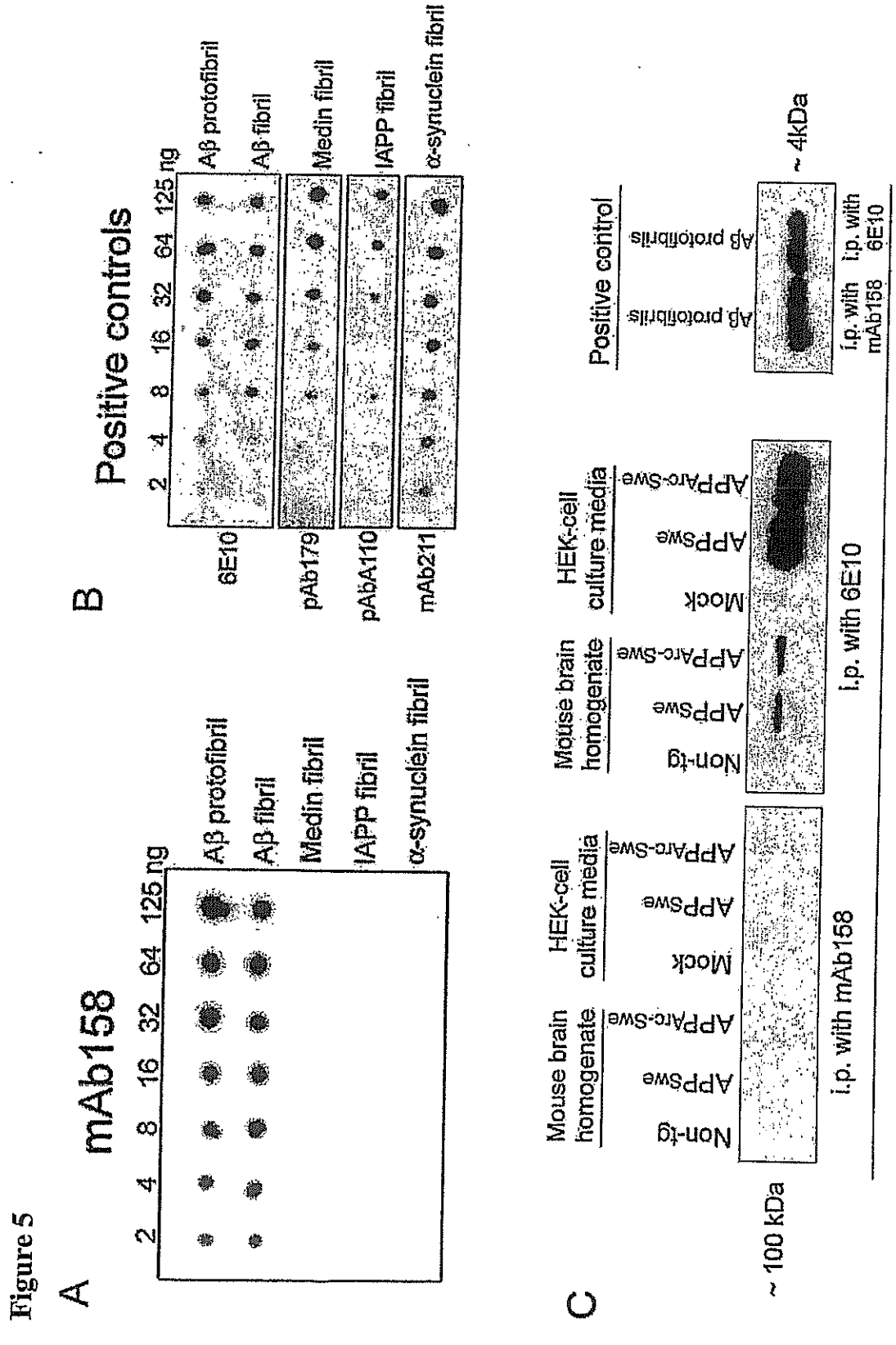
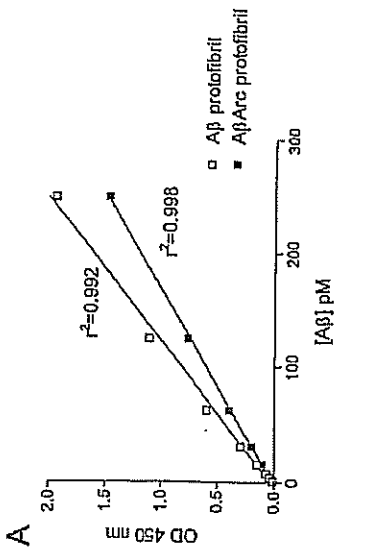
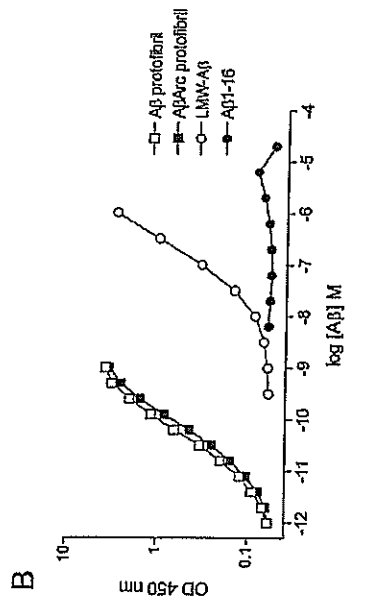

A
mAb158
2 4 8 16 32 64 125 ng
Aβ protofibril
Aβ fibril
Medin fibril
IAPP fibril
α-synuclein fibril
B
Positive controls
2 4 8 16 32 64 125 ng
Aβ protofibril
Aβ fibril
Medin fibril
IAPP fibril
α-synuclein fibril
6E10
pAb179
pAbA110
mAb211
C
Mouse brain homogenate
HEK-cell culture media
Non-tg
APPSwe
APPArc-Swe
Mock
APPSwe
APPArc-Swe
~ 100 kDa
i.p. with mAb158
i.p. with 6E10
Positive control
Aβ protofibrils
Aβ protofibrils
~ 4kDa
i.p. with mAb158
i.p. with 6E10
Western blot with 6E10

Figure 6


A
OD 450 nm
2.0
1.5
1.0
0.5
0.0
0
100
200
300
[Aβ] pM
r²=0.992
r²=0.998
Aβ protofibril
AβArc protofibril


B
OD 450 nm
10
1
0.1
-12
-11
-10
-9
-8
-7
-6
-5
-4
log [Aβ] M
Aβ protofibril
AβArc protofibril
LMW-Aβ
Aβ1-16

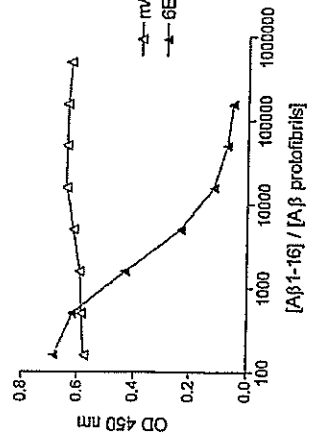

C
OD 450 nm
0.8
0.6
0.4
0.2
0.0
100
1000
10000
100000
1000000
[Aβ1-16] / [Aβ protofibrils]
mAb158 sandwich ELISA
6E10 sandwich ELISA

Figure 7

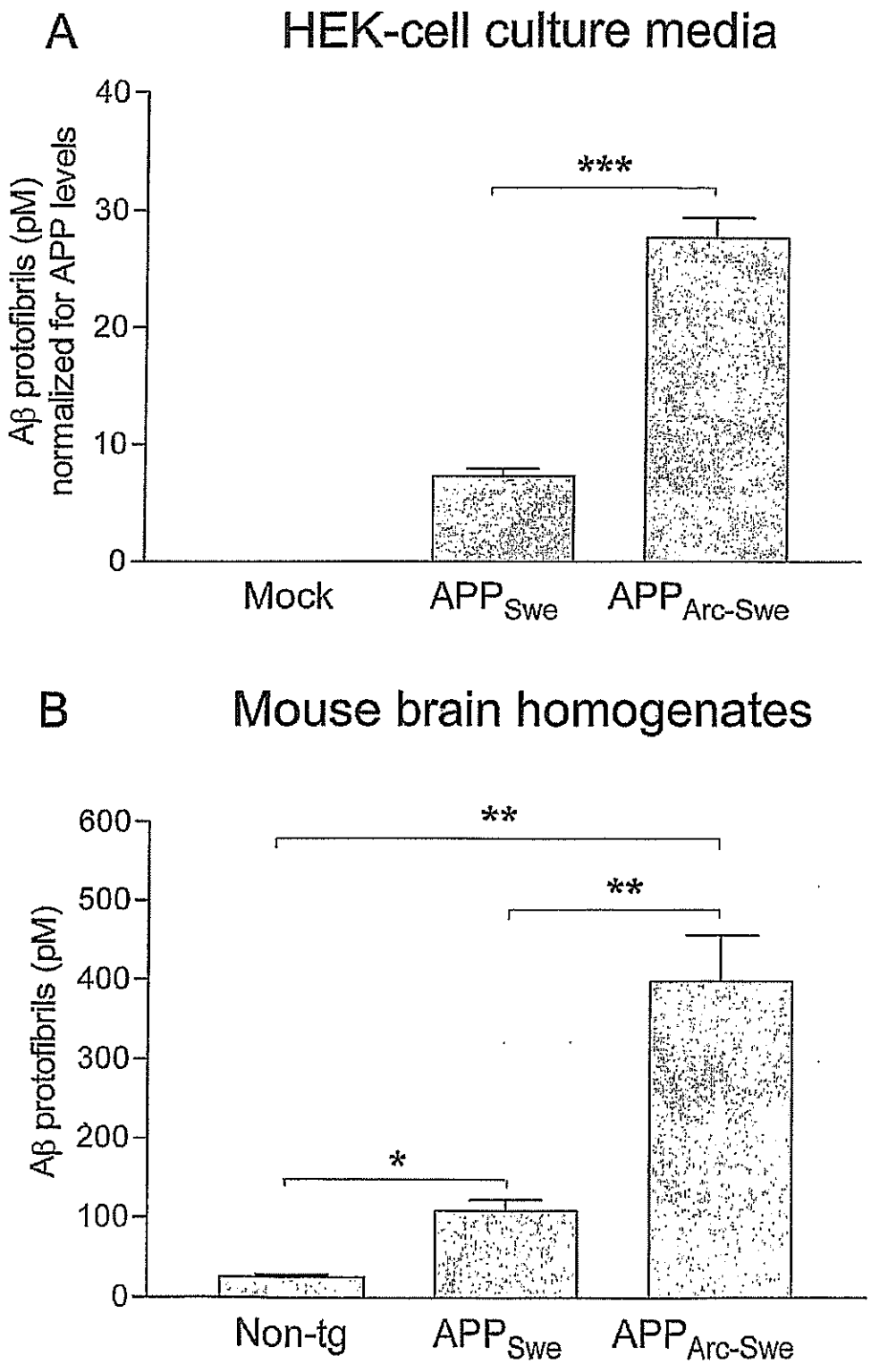

A
HEK-cell culture media
Aβ protofibrils (pM) normalized for APP levels
40
30
20
10
0
***
Mock
APPSwe
APPArc-Swe
B
Mouse brain homogenates
Aβ protofibrils (pM)
600
500
400
300
200
100
0
**
**
*
Non-tg
APPSwe
APPArc-Swe

Fig. 8. Aβ protofibril levels in APP_{swearc} transgenic mouse brain TBS extracts after 4 months treatment with either mAb158 or placebo.

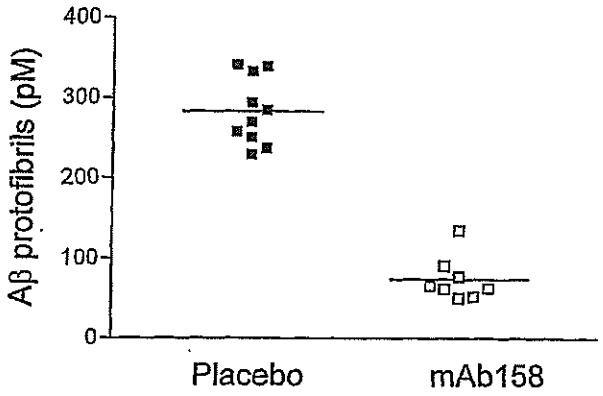


Fig. 9. Total Aβ levels in APP_{swearc} transgenic mouse brain formic acid extracts after 4 months

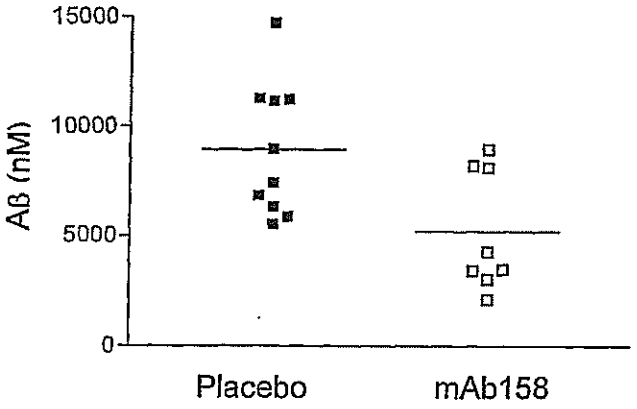


Fig. 10. pKN100 vector

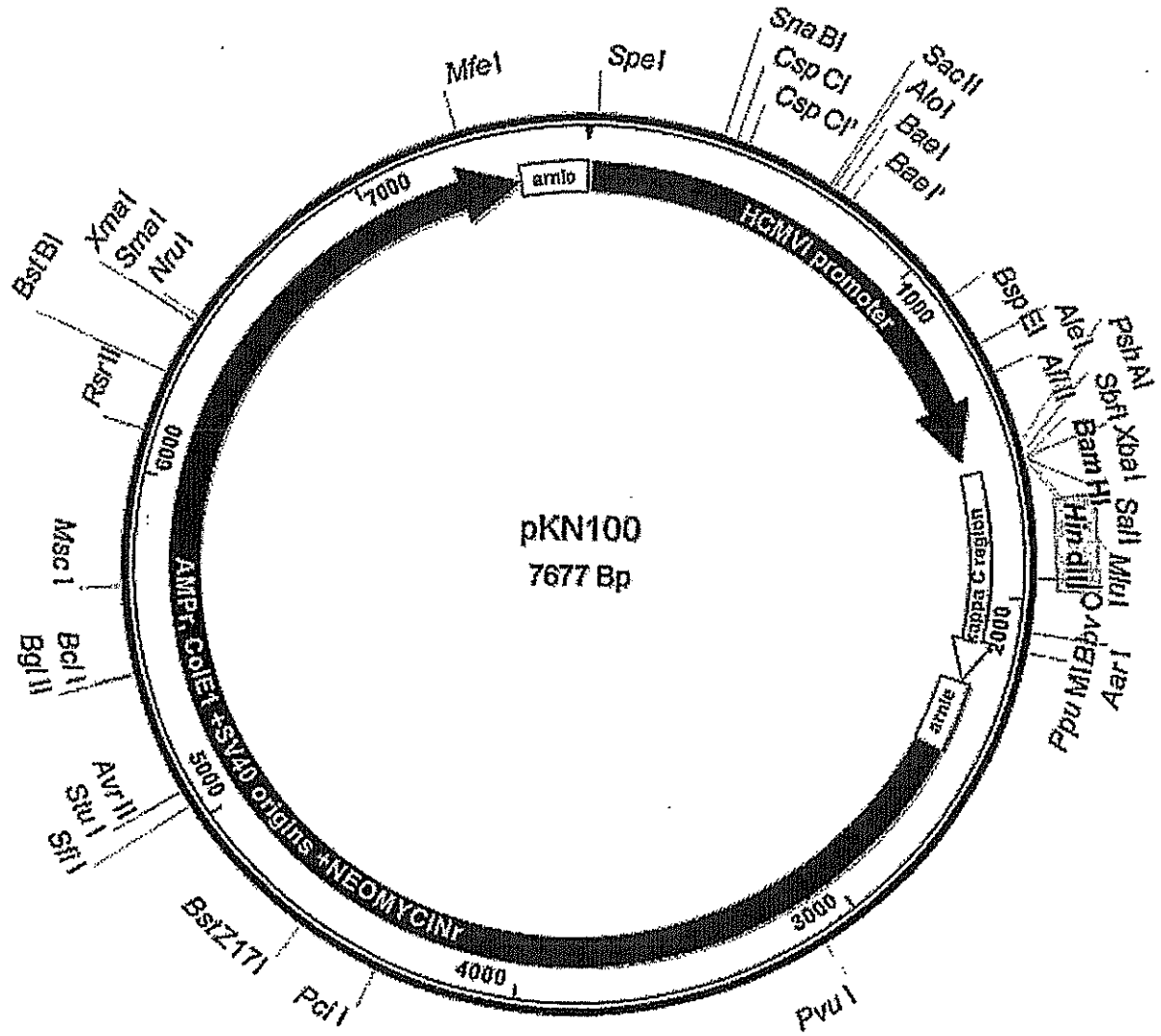


Fig. 11. pG1D200 vector

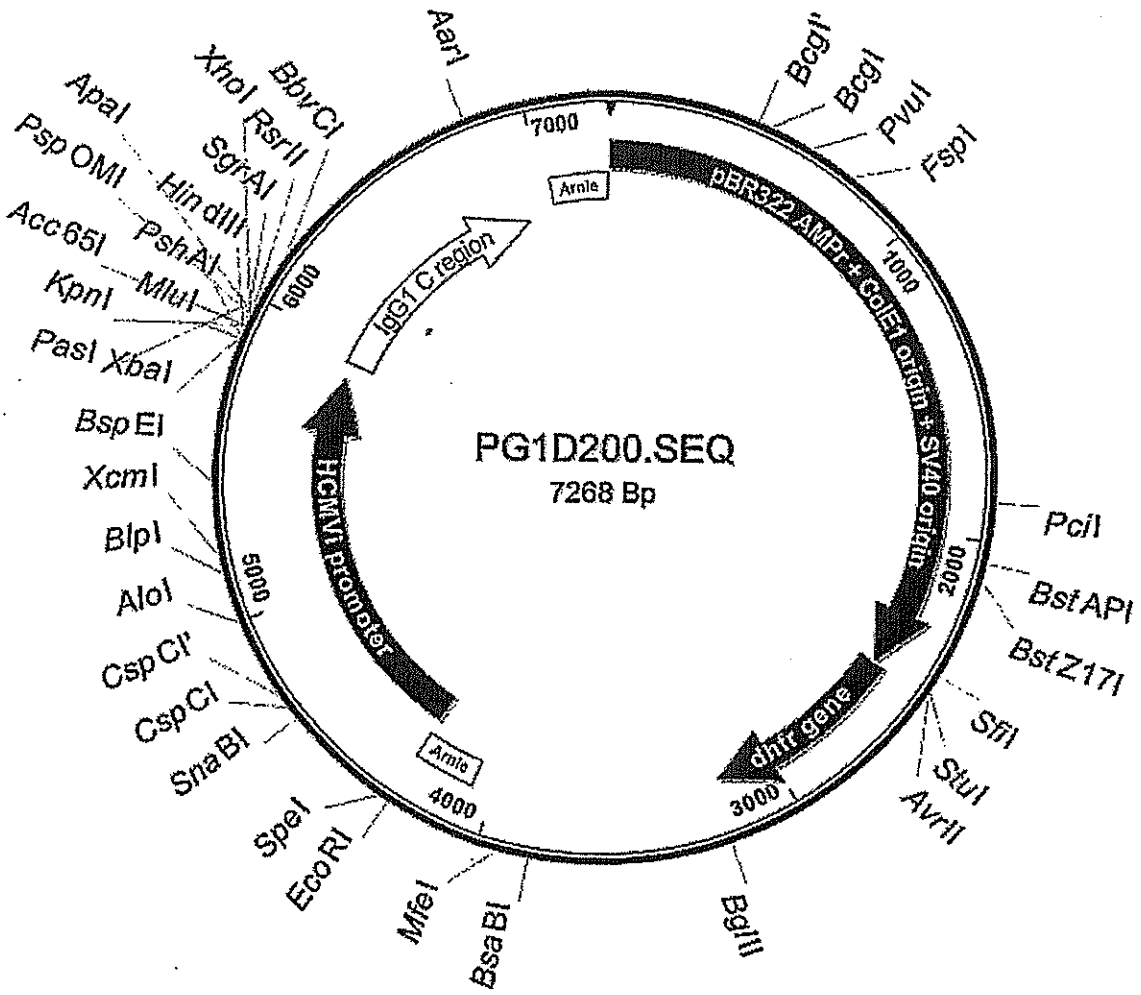
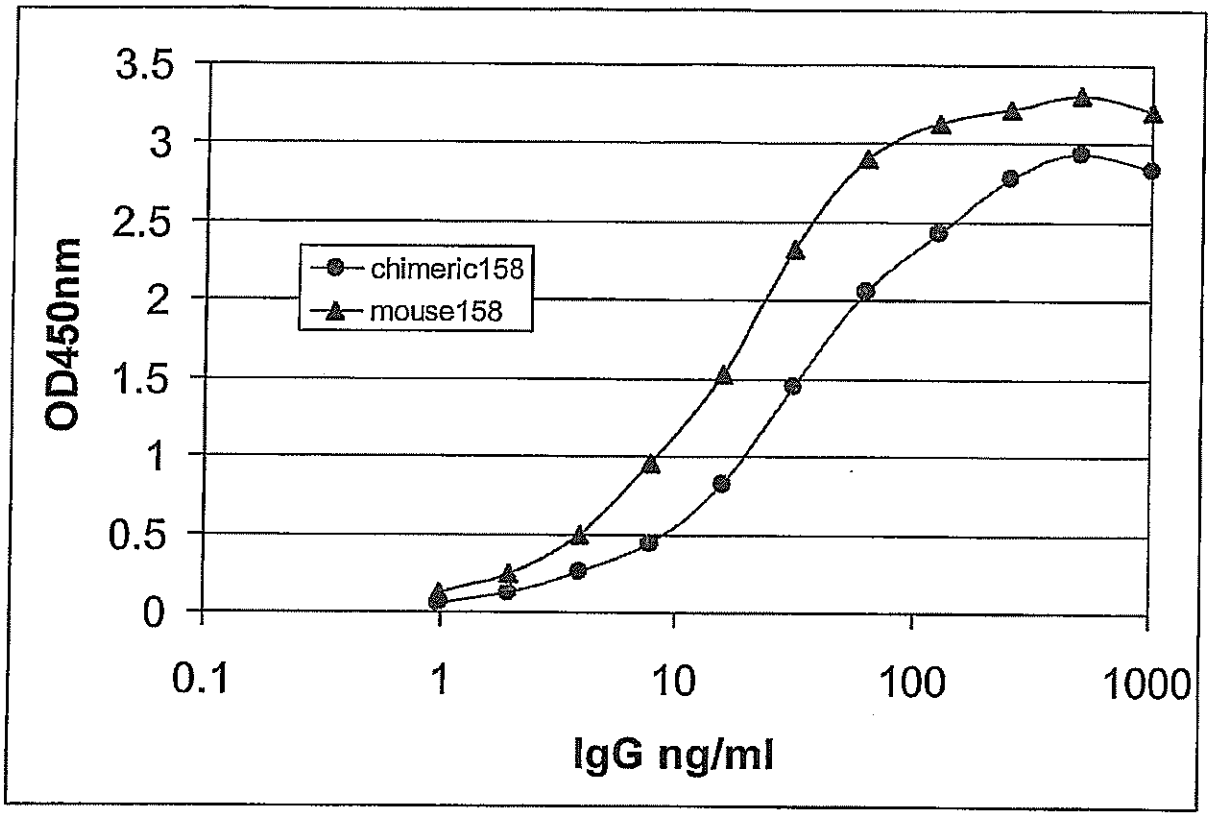
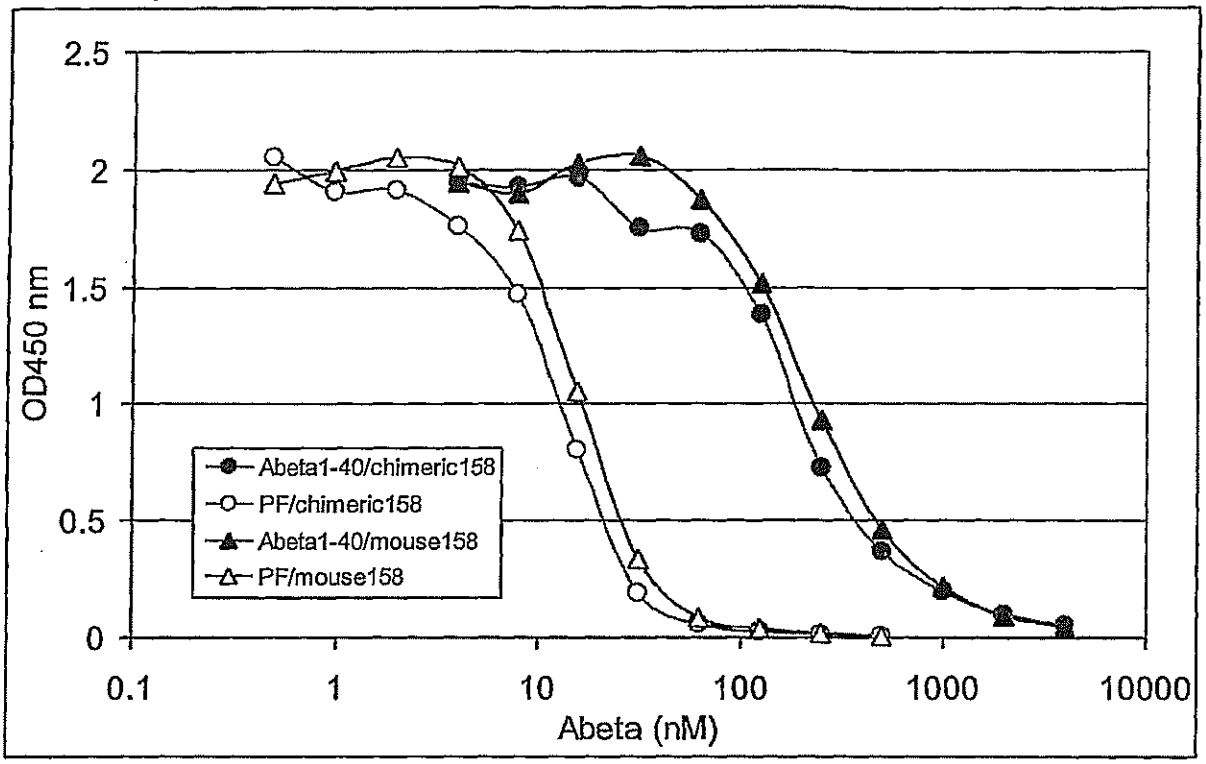


Fig. 12. Aβ monomer binding by chimeric and mouse 158 antibodies



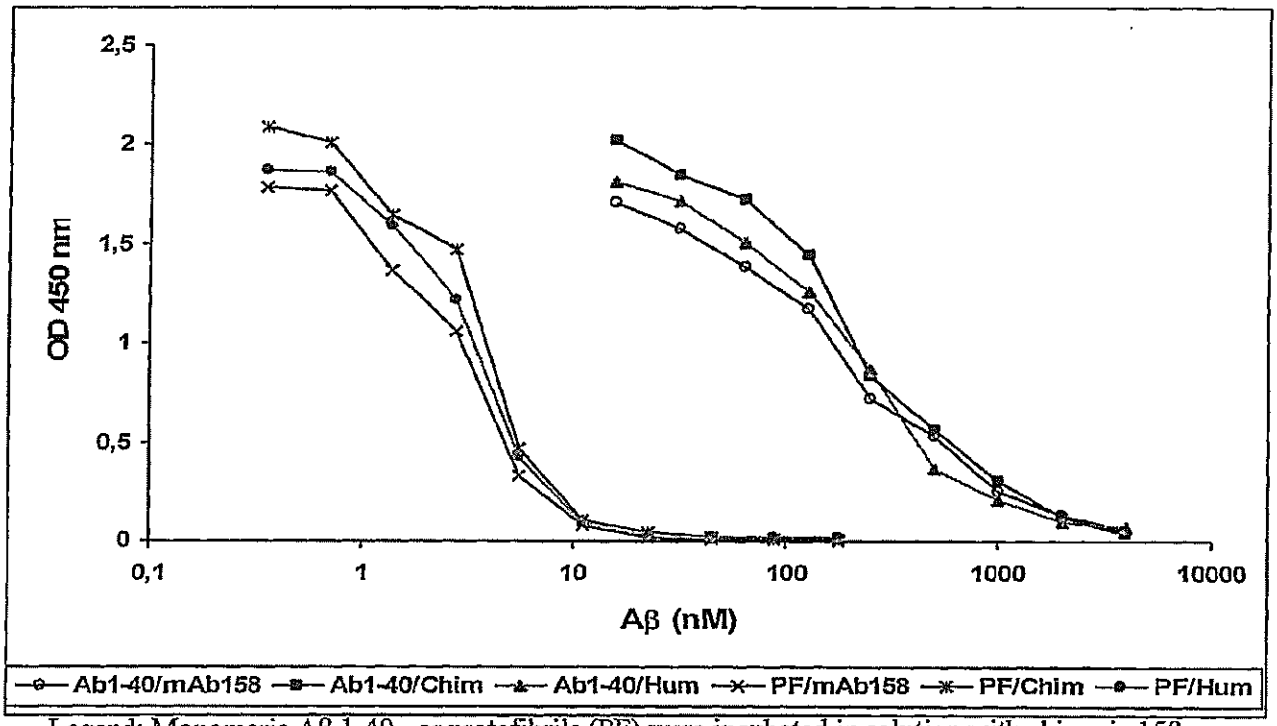
Legend: Direct ELISA coated with Aβ 1-40 (217 ng/well) and using serial dilutions of mAb. Amount of FCS at highest concentration (1 μg/ml) is 2.7%. Detection with anti-mouse k or anti-human k light chain conjugates.

Fig. 13. Competition of monomeric or protofibrillar Aβ for binding to chimeric 158 or mouse 158 antibody



Legend: Monomeric Aβ 1-40 (●▲) or protofibrils (PF) (○△) were incubated in solution with chimeric 158 (○●) or mouse 158 (△▲). The final concentration of FCS was 0.3%. After an incubation for 1 h, the mixture was added to a plate coated with Aβ monomers. The binding of antibody to the plate was detected by anti-mouse κ or anti-human κ light chain conjugates.

Fig. 14. Competition of monomeric or protofibrillar Aβ for binding to chimeric 158, mouse 158 antibody and humanized 158 antibody (BAN2401).



Legend: Monomeric Aβ 1-40 , or protofibrils (PF) were incubated in solution with chimeric 158 antibody (chim), mouse 158 antibody (mAb158) or humanized 158 antibody BAN2401 (Hum). The final concentration of FCS was 0.3%. After an incubation for 1 h, the mixture was added to a plate coated with Aβ monomers. The binding of antibody to the plate was detected by anti-mouse κ or anti-human κ light chain conjugates.