## A20Vk




Figure 1A.

## A20VH

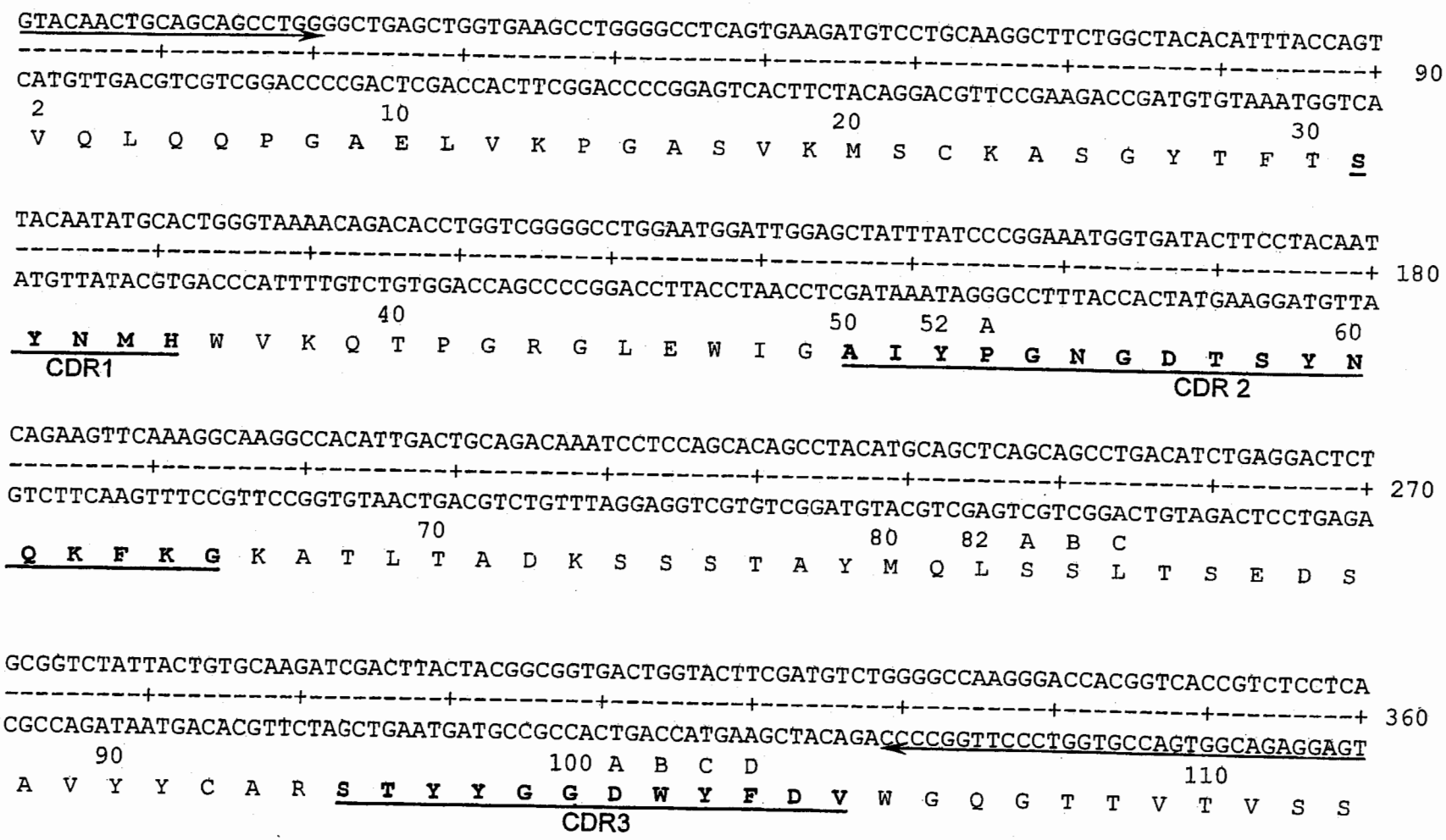



Figure 1B.

**cA20Vk**

```
GACATCCAGCTGACCCAGTCTCCAGCAATCCTGTCTGCATCTCCAGGGGAGAAGGTCACAATGACTTGCAGGGCCAGCTCAAGTGTAAGT    90
1                         10                        20                  27 29 30
D  I  Q  L  T  Q  S  P  A  I  L  S  A  S  P  G  E  K  V  T  M  T  C  R  A  S  S  S  V  S
                                                                      CDR1

TACATCCACTGGTTCCAGCAGAAGCCAGGATCCTCCCCCAAACCCTGGATTTATGCCACATCCAACCTGGCTTCTGGAGTCCCTGTTCGC   180
                  40                        50                        60
Y  I  H  W  F  Q  Q  K  P  G  S  S  P  K  P  W  I  Y  A  T  S  N  L  A  S  G  V  P  V  R
                                                      CDR2

TTCAGTGGCAGTGGGTCTGGGACTTCTTACTCTCTCACAATCAGCAGAGTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAGTGG   270
                  70                        80                        90
F  S  G  S  G  S  G  T  S  Y  S  L  T  I  S  R  V  E  A  E  D  A  A  T  Y  Y  C  Q  Q  W

ACTAGTAACCCACCCACGTTCGGAGGGGGGACCAAGCTGGAGATCAAA                                          318
                  100                       107
T  S  N  P  P  T  F  G  G  G  T  K  L  E  I  K
CDR3
```

Figure 2A.

## cA20VH

```
CAGGTCCAACTGCAGCAGCCTGGGGCTGAGCTGGTGAAGCCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTTCTGGCTACACATTTACC    90
 1                         10                        20                        30
 Q  V  Q  L  Q  Q  P  G  A  E  L  V  K  P  G  A  S  V  K  M  S  C  K  A  S  G  Y  T  F  T

AGTTACAATATGCACTGGGTAAAACAGACACCTGGTCGGGGCCTGGAATGGATTGGAGCTATTTATCCCGGAAATGGTGATACTTCCTAC   180
                          40                        50    52 A
 S  Y  N  M  H  W  V  K  Q  T  P  G  R  G  L  E  W  I  G  A  I  Y  P  G  N  G  D  T  S  Y
    CDR1                                                                    CDR 2

AATCAGAAGTTCAAAGGCAAGGCCACATTGACTGCAGACAAATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGAC   270
60                        70                        80    82 A  B  C
 N  Q  K  F  K  G  K  A  T  L  T  A  D  K  S  S  S  T  A  Y  M  Q  L  S  S  L  T  S  E  D

TCTGCGGTCTATTACTGTGCAAGATCGACTTACTACGGCGGTGACTGGTACTTCGATGTCTGGGGCCAAGGGACCACGGTCACCGTCTCC   360
         90                        100 A  B  C  D                        110
 S  A  V  Y  Y  C  A  R  S  T  Y  Y  G  G  D  W  Y  F  D  V  W  G  Q  G  T  T  V  T  V  S
                         CDR3

TCA                                                                                          363
113
 S
```

Figure 2B.

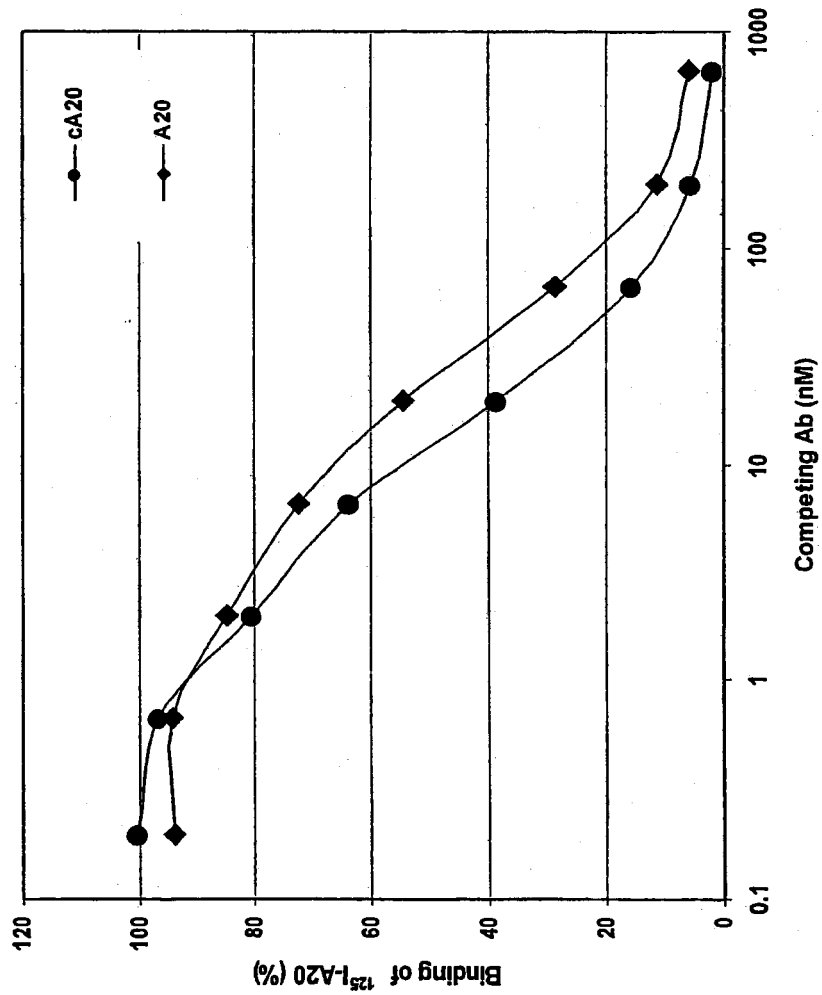

cA20
A20
Binding of $^{125}$I-A20 (%)
120
100
80
60
40
20
0
0.1
1
10
100
1000
Competing Ab (nM)

Figure 3

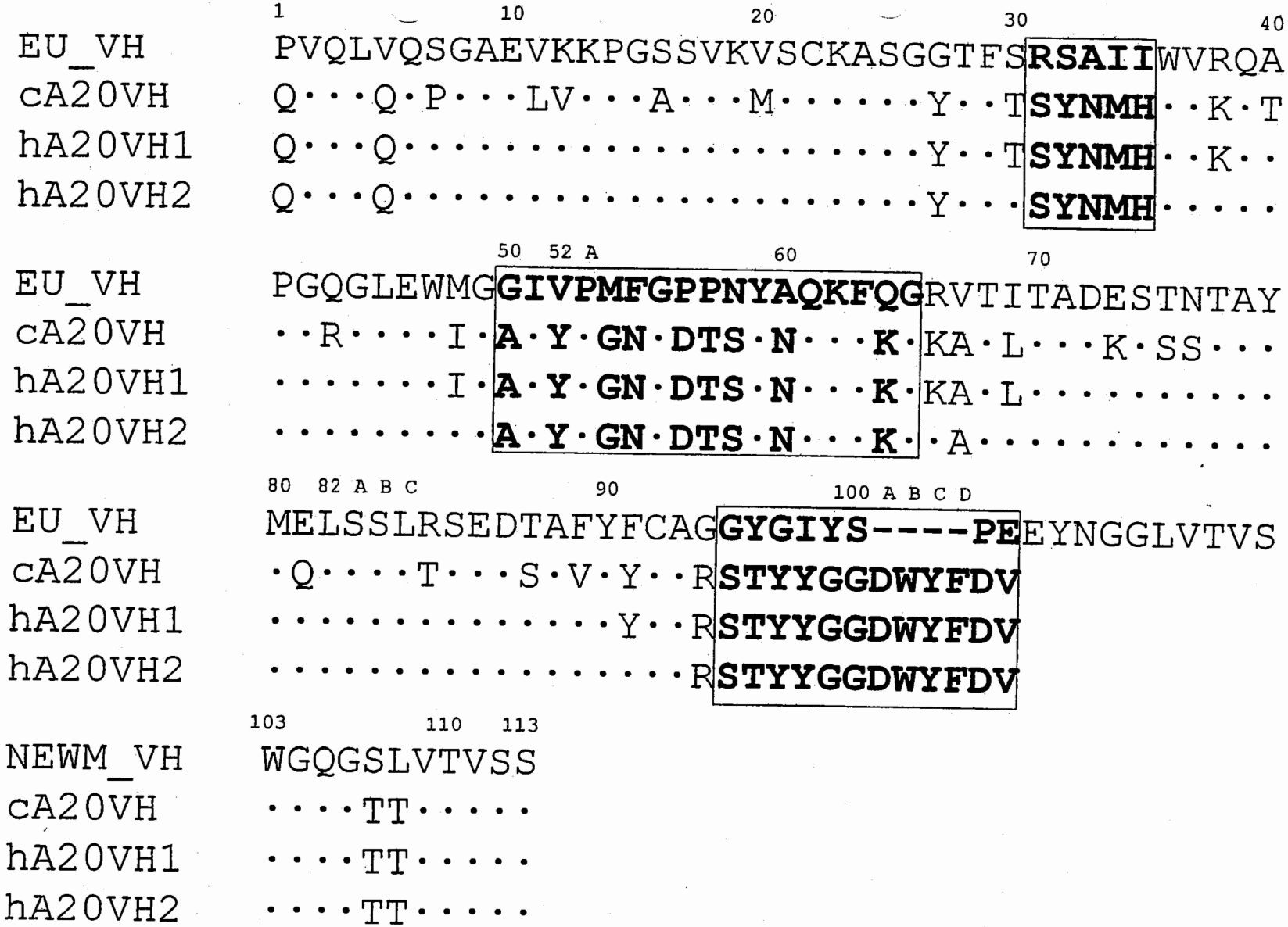

1 10 20 30 40
EU_VH PVQLVQSGAEVKKPGSSVKVSCKASGGTFSRSAIIWVRQA
cA20VH Q···Q·P···LV···A···M·····Y··TSYNMH··K·T
hA20VH1 Q···Q·····Y··TSYNMH··K··
hA20VH2 Q···Q·····Y···SYNMH····
50 52 A 60 70
EU_VH PGQGLEWMGGIVPMFGPPNYAQKFQGRVTITADESTNTAY
cA20VH ··R····I·A·Y·GN·DTS·N···K·KA·L···K·SS···
hA20VH1 ·····I·A·Y·GN·DTS·N···K·KA·L·····
hA20VH2 ·····A·Y·GN·DTS·N···K··A·····
80 82 A B C 90 100 A B C D
EU_VH MELSSLRSEDTAFYFCAGGYGIYS----PEEYNGGLVTVS
cA20VH ·Q····T···S·V·Y··RSTYYGGDWYFDV
hA20VH1 ·····Y··RSTYYGGDWYFDV
hA20VH2 ·····RSTYYGGDWYFDV
103 110 113
NEWM_VH WGQGSLVTVSS
cA20VH ····TT·····
hA20VH1 ····TT·····
hA20VH2 ····TT·····

Figure 4A.

```
                1                   10                  20                  30                  40
REIVk   DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTP
cA20Vk  ···L····AI····P·EK··M··R··S-SVS·IH·F··K·
hA20Vk  ···L··········M··R··S-SVS·IH·F··K·

                50                  60                  70                  80
REIVk   GKAPKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQP
·SS··PW··AT···AS···V··········S·SL···RVEA
····PW··AT···AS···V··········

                90                  100                 107
REIVk   EDIATYYCQQYQSLPYTFGQGTKLQIT
··A·······WT·N·P···G····E·K
···········WT·N·P···G····E·K
```

Figure 4B.

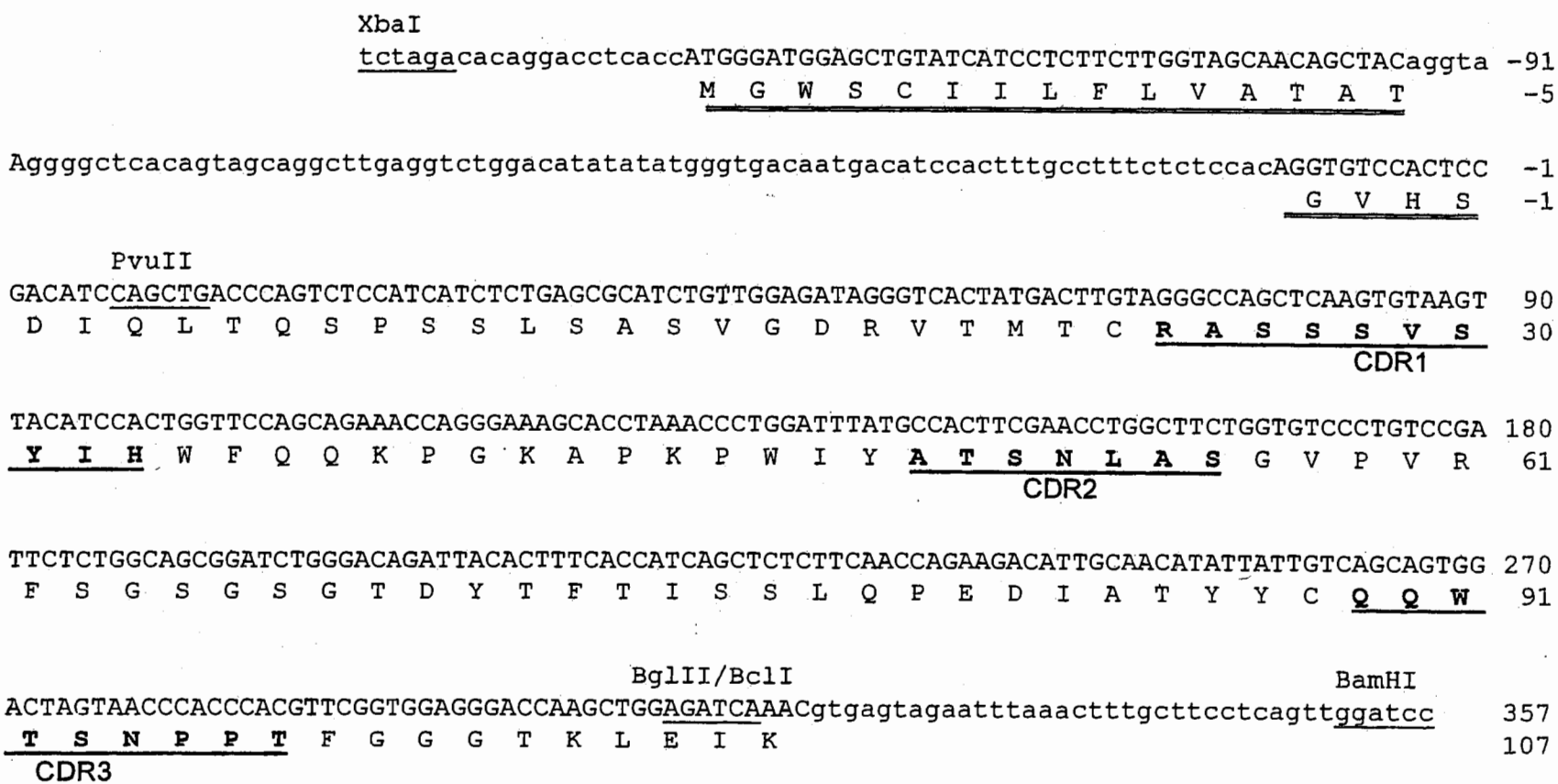

XbaI
tctagacacaggacctcaccATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACaggta -91
M G W S C I I L F L V A T A T -5
AggggctcacagtagcaggcttgaggtctggacatatatatgggtgacaatgacatccactttgcctttctctccacAGGTGTCCACTCC -1
G V H S -1
PvuII
GACATCCAGCTGACCCAGTCTCCATCATCTCTGAGCGCATCTGTTGGAGATAGGGTCACTATGACTTGTAGGGCCAGCTCAAGTGTAAGT 90
D I Q L T Q S P S S L S A S V G D R V T M T C R A S S S V S 30
CDR1
TACATCCACTGGTTCCAGCAGAAACCAGGGAAAGCACCTAAACCCTGGATTTATGCCACTTCGAACCTGGCTTCTGGTGTCCCTGTCCGA 180
Y I H W F Q Q K P G K A P K P W I Y A T S N L A S G V P V R 61
CDR2
TTCTCTGGCAGCGGATCTGGGACAGATTACACTTTCACCATCAGCTCTCTTCAACCAGAAGACATTGCAACATATTATTGTCAGCAGTGG 270
F S G S G S G T D Y T F T I S S L Q P E D I A T Y Y C Q Q W 91
BglII/BclI
BamHI
ACTAGTAACCCACCCACGTTCGGTGGAGGGACCAAGCTGGAGATCAAACgtgagtagaatttaaactttgcttcctcagttggatcc 357
T S N P P T F G G G T K L E I K 107
CDR3

Figure 5A.

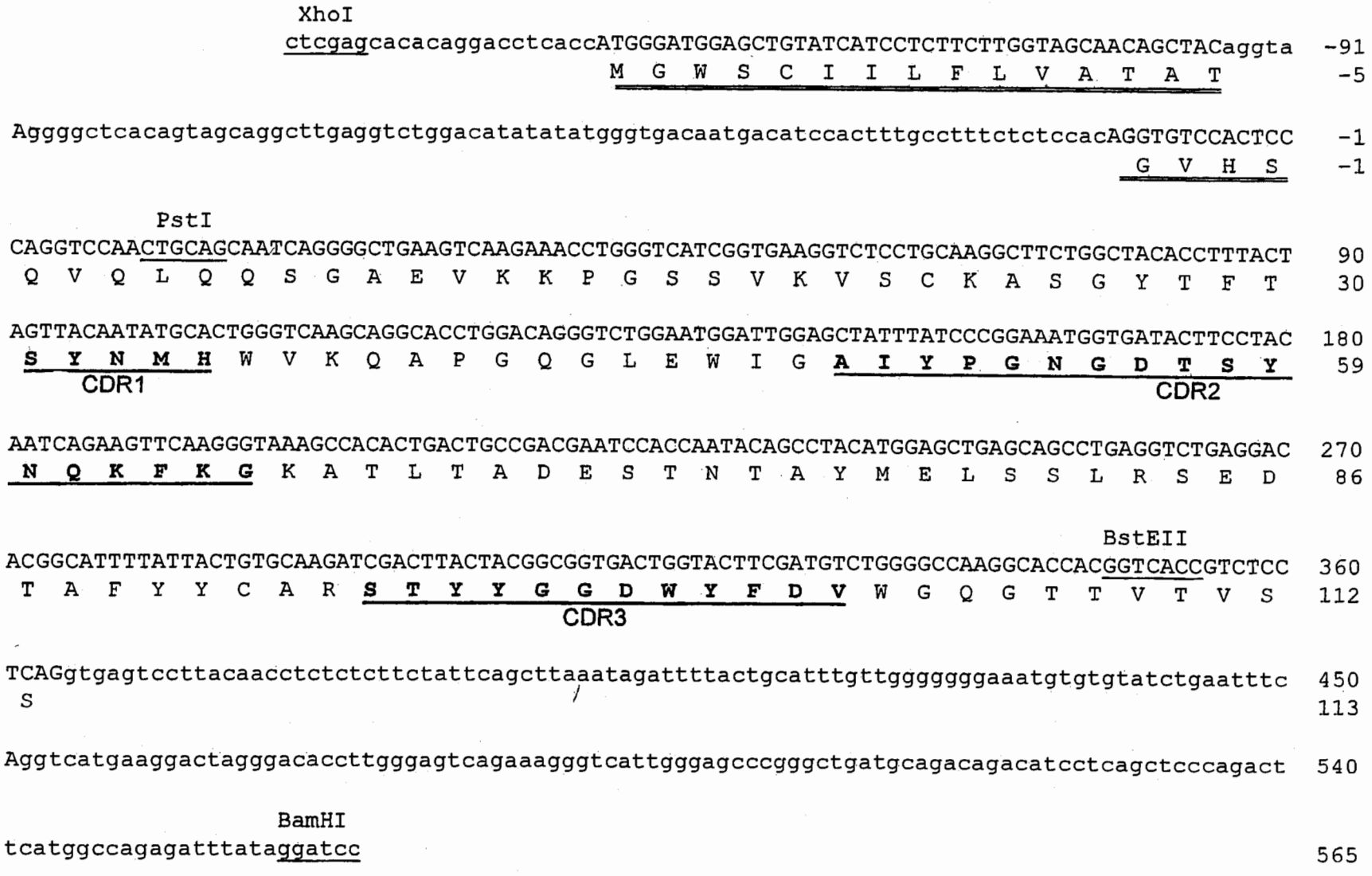

XhoI
ctcgagcacacaggacctcaccATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACaggta -91
M G W S C I I L F L V A T A T -5
AggggctcacagtagcaggcttgaggtctggacatatatatgggtgacaatgacatccactttgcctttctctccacAGGTGTCCACTCC -1
G V H S -1
PstI
CAGGTCCAACTGCAGCAATCAGGGGCTGAAGTCAAGAAACCTGGGTCATCGGTGAAGGTCTCCTGCAAGGCTTCTGGCTACACCTTTACT 90
Q V Q L Q Q S G A E V K K P G S S V K V S C K A S G Y T F T 30
AGTTACAATATGCACTGGGTCAAGCAGGCACCTGGACAGGGTCTGGAATGGATTGGAGCTATTTATCCCGGAAATGGTGATACTTCCTAC 180
S Y N M H W V K Q A P G Q G L E W I G A I Y P G N G D T S Y 59
CDR1
CDR2
AATCAGAAGTTCAAGGGTAAAGCCACACTGACTGCCGACGAATCCACCAATACAGCCTACATGGAGCTGAGCAGCCTGAGGTCTGAGGAC 270
N Q K F K G K A T L T A D E S T N T A Y M E L S S L R S E D 86
BstEII
ACGGCATTTTATTACTGTGCAAGATCGACTTACTACGGCGGTGACTGGTACTTCGATGTCTGGGGCCAAGGCACCACGGTCACCGTCTCC 360
T A F Y Y C A R S T Y Y G G D W Y F D V W G Q G T T V T V S 112
CDR3
TCAGgtgagtccttacaacctctctcttctattcagcttaaatagattttactgcatttgttgggggggaaatgtgtgtatctgaatttc 450
S 113
Aggtcatgaaggactagggacaccttgggagtcagaaagggtcattgggagcccgggctgatgcagacagacatcctcagctcccagact 540
BamHI
tcatggccagagatttataggatcc 565

Figure 5B.

XhoI

ctcgagcacacaggacctcaccATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACaggta -91

M G W S C I I L F L V A T A T -5

AggggctcacagtagcaggcttgaggtctggacatatatatgggtgacaatgacatccactttgcctttctctccacAGGTGTCCACTCC -1

G V H S -1

CAGGTCCAACTGCAGCAATCAGGGGCTGAAGTCAAGAAACCTGGGTCATCAGTGAAGGTCTCCTGCAAGGCTTCTGGCTACACCTTTAGT 90

Q V Q L Q Q S G A E V K K P G S S V K V S C K A S G Y T F S 30

AGTTACAATATGCACTGGGTCAGACAGGCACCTGGACAGGGTCTGGAATGGGATGGGAGCTATTTATCCCGGAAATGGTGATACTTCCTAC 180

**S Y N M H** W V R Q A P G Q G L E W M G **A I Y P G N G D T S Y** 59

CDR1 CDR2

AATCAGAAGTTCAAGGGTAGAGCCACAATAACTGCCGACGAATCCACCACAATACAGCCTACATGGAGCTGAGCAGCCTGAGGTCTGAGGAC 270

**N Q K F K G** R A T I T A D E S T N T A Y M E L S S L R S E D 86

ACGGCATTTTATTTTTGTGCAAGATCGACTTACTACGGCGGTGACTGGTACTTCGATGTCTGGGGCCAAGGCACCACGGTCACCGTCTCC 360

T A F Y F C A R **S T Y Y G G D W Y F D V** W G Q G T T V T V S 112

CDR3

TCAGgtgagtccttacaacctctctcttctattcagcttaaatagattttactgcatttgttgggggggaaatgtgtgtatctgaatttc 450

S 113

Aggtcatgaaggactagggacaccttgggagtcagaaagggtcattgggagcccgggctgatgcagacagacatcctcagctcccagact 540

BamHI

Tcatggccagagatttataggatcc 565

Figure 5C.

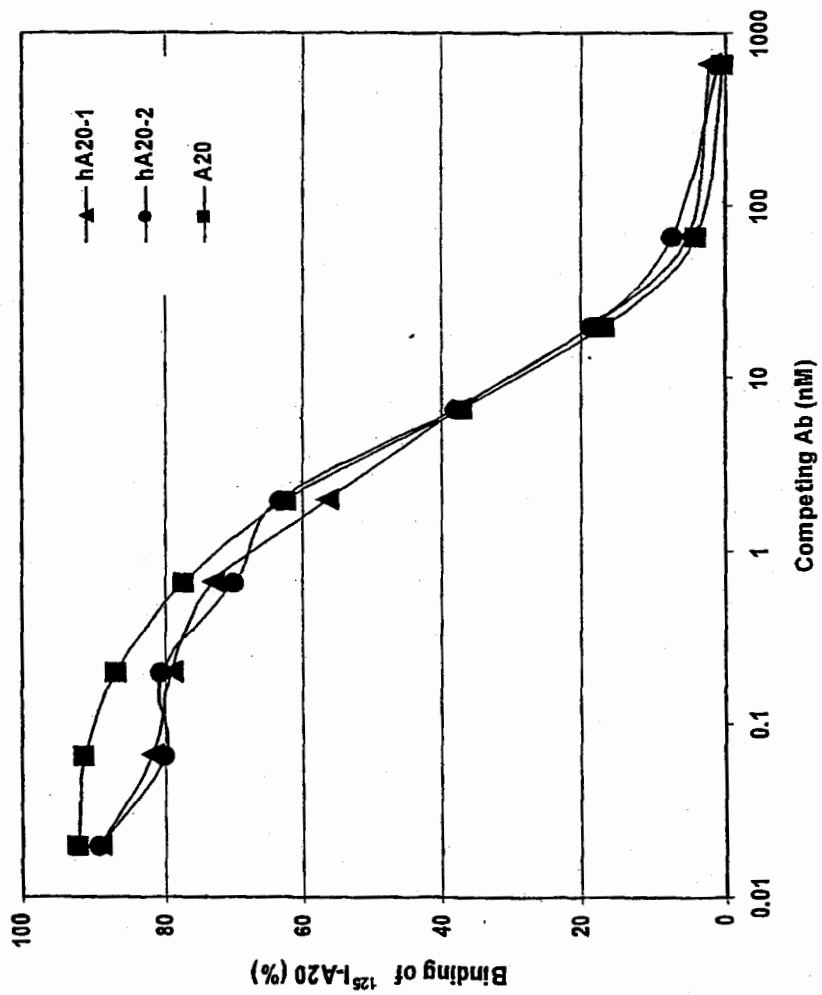

hA20-1
hA20-2
A20
Binding of $^{125}$I-A20 (%)
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
Competing Ab (nM)

Figure 6A.

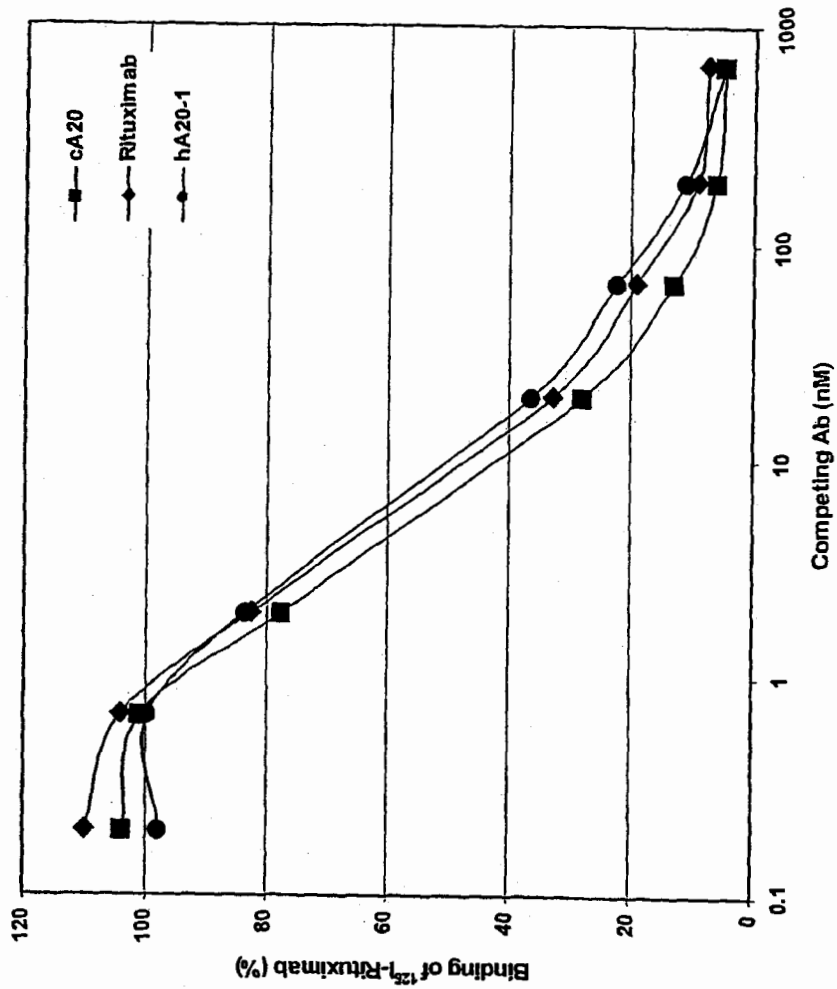

cA20
Rituximab
hA20-1
Binding of 125I-Rituximab (%)
120
100
80
60
40
20
0
0.1
1
10
100
1000
Competing Ab (nM)

Figure 6B.

```
      GCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCG   120
118   A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S
      TGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACC   240
158   W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T
      TACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGGA   360
198   Y  I  C  N  V  N  H  K  P  S  N  T  K  V  D  K  R  V  E  P  K  S  C  D  K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G
      CCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGG   480
238   P  S  V  F  L  F  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D  V  S  H  E  D  P  E  V  K  F  N  W
      TACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAG   600
278   Y  V  D  G  V  E  V  H  N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L  H  Q  D  W  L  N  G  K

      GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAG   720
318   E  Y  K  C  K  V  S  N  K  A  L  P  A  P  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R  E  E
      ATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTG   840
358   M  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V
      CTGGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACG   960
398   L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T
      CAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA                                                                                        993
438   Q  K  S  L  S  L  S  P  G  K  *
```

**Fig. 7A.** The CH sequence of hIgG1.

```
                                                                    120
    CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAG
108 R  T  V  A  A  P  S  V  F  I  F  P  P  S  D  E  Q  L  K  S  G  T  A  S  V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V  Q

                                                                    240
    TGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAG
148 W  K  V  D  N  A  L  Q  S  G  N  S  Q  E  S  V  T  E  Q  D  S  K  D  S  T  Y  S  L  S  S  T  L  T  L  S  K  A  D  Y  E

                                                                    324
    AAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTGA
188 K  H  K  V  Y  A  C  E  V  T  H  Q  G  L  S  S  P  V  T  K  S  F  N  R  G  E  C  *
```

**Fig. 7B.** The constant region sequence of human kappa chain.

# Cell surface competitive binding of CD20 MAbs vs. m2B8 or rituximab (C2B8)

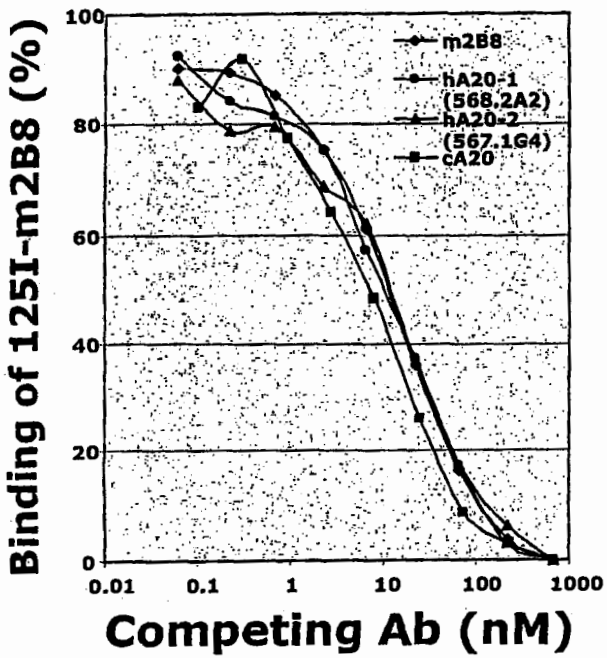



Figure 8A

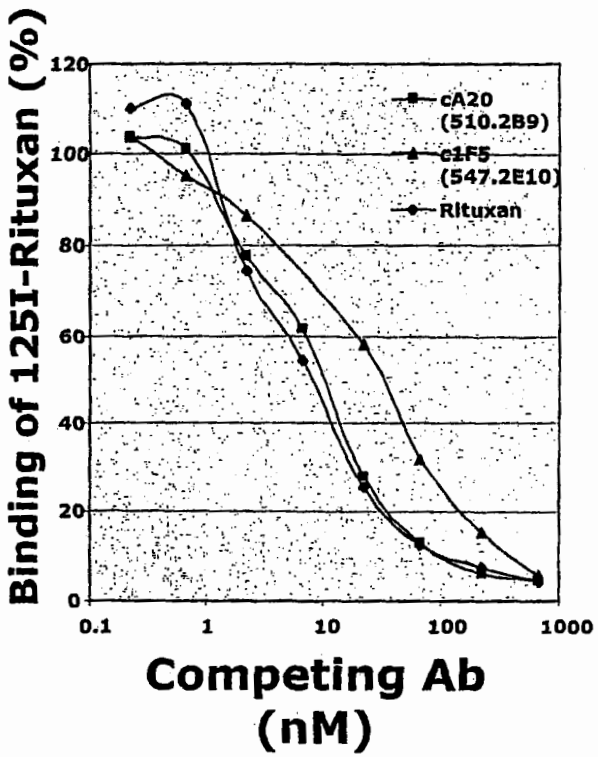



Figure 8B

Figure 8

Competitive Binding to 125I Rituxan
(B1, like 1F5, has lower affinity than hA20-1 or rituximab [C2B8])
page B58
IC50 nM
Rituxan 5
hA20-1 Rohini 6.8
mB1 34
Total Binding (cpm)
3000
2000
1000
0
-12.5
-10.0
-7.5
-5.0
Competitor logM

Figure 9

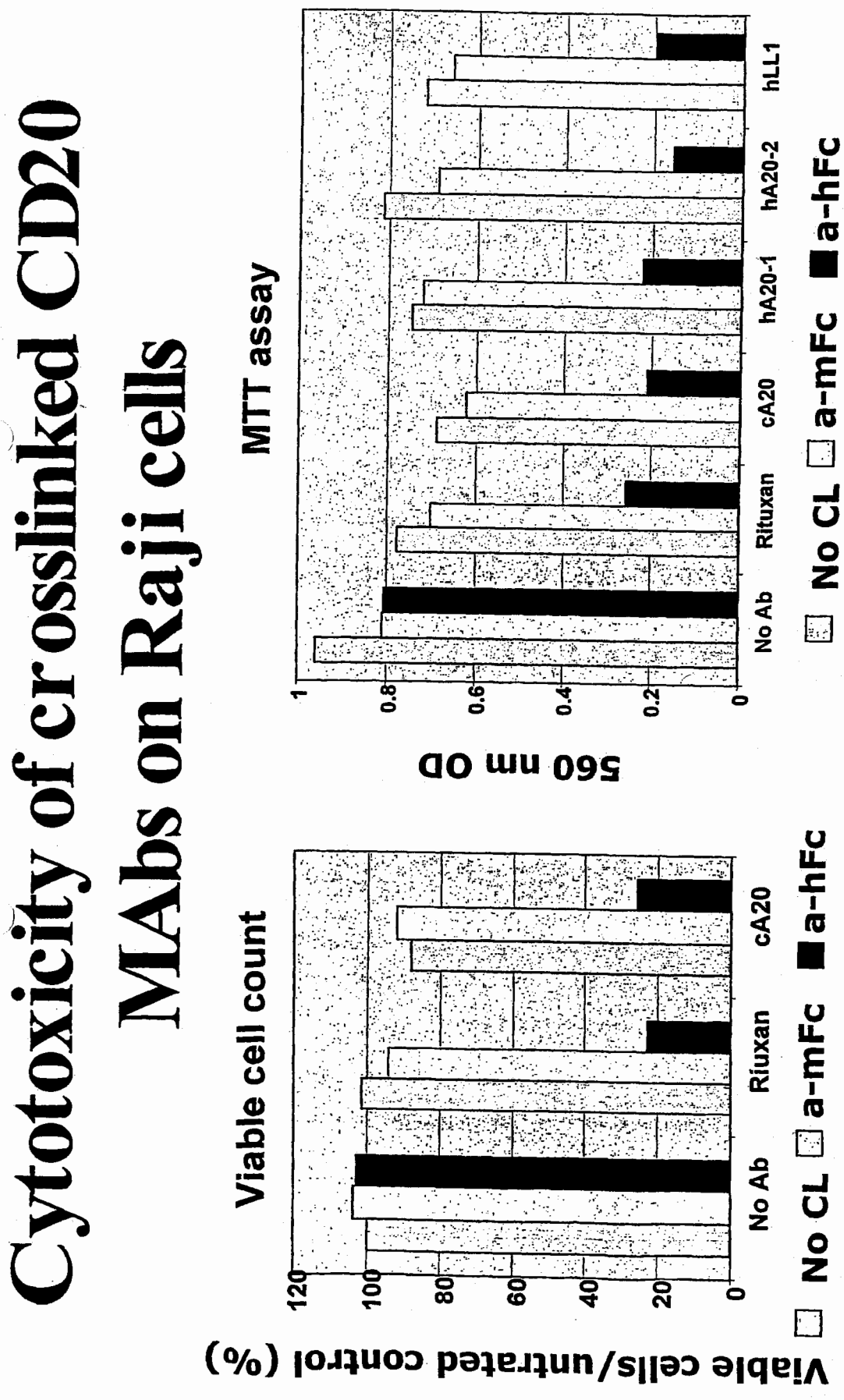



Figure 10A

Figure 10B

Figure 10

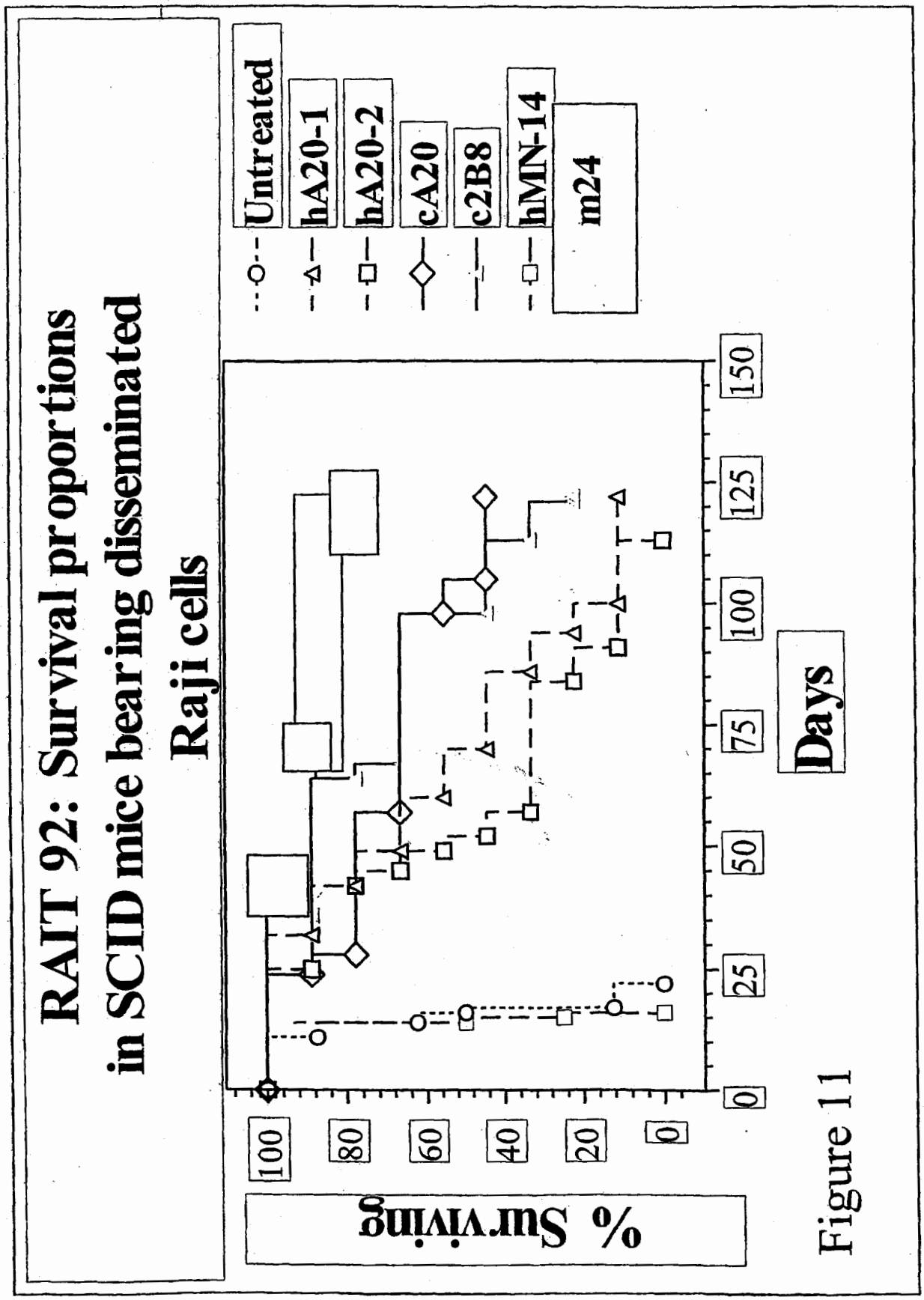

RAIT 92: Survival proportions
in SCID mice bearing disseminated
Raji cells
Untreated
hA20-1
hA20-2
cA20
c2B8
hMN-14
m24
% Surviving
100
80
60
40
20
0
0
25
50
75
100
125
150
Days

Figure 11

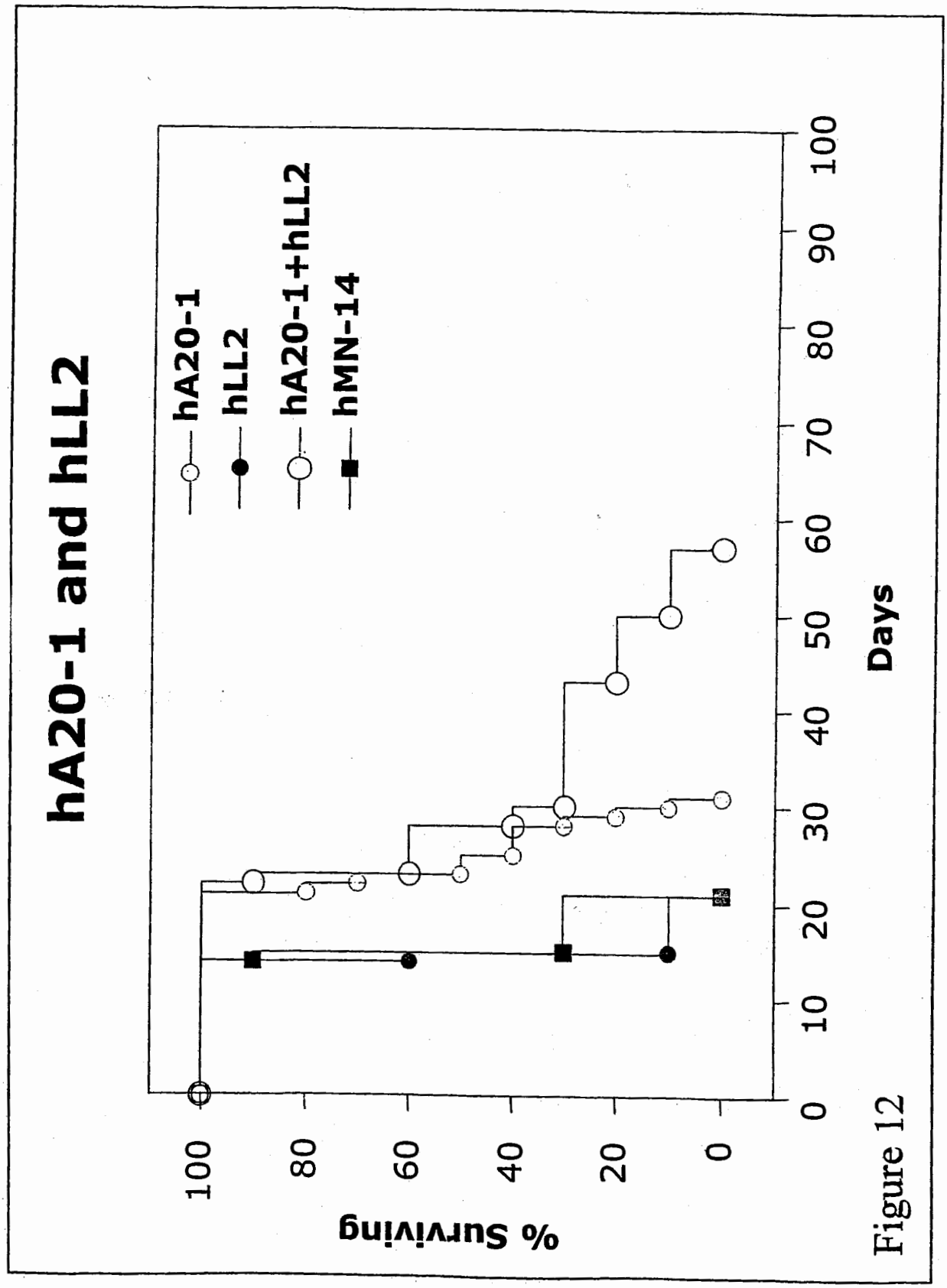

hA20-1 and hLL2
hA20-1
hLL2
hA20-1+hLL2
hMN-14
% Surviving
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
100
Days

Figure 12