LAG3.1 - Anti-LAG3 25F7 VH

V segment: 4-34
D segment: 5-12
J segment: JH5b

```
     Q  V  Q  L  Q  Q  W  G  A  G  L  L  K  P  S  E  T  L
1    CAG GTG CAG CTA CAG CAG TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG ACC CTG

                                                CDR1
                                                ~~~~~~~~~~~~~~~~~~~
     S  L  T  C  A  V  Y  G  G  S  F  S  D  Y  Y  W  N  W
55   TCC CTC ACC TGC GCT GTC TAT GGT GGG TCC TTC AGT GAT TAC TAC TGG AAC TGG

                                                   CDR2
                                                   ~~~~~~~~~~~~~~~~~~~
     I  R  Q  P  P  G  K  G  L  E  W  I  G  E  I  N  H  N
109  ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG TGG ATT GGG GAA ATC AAT CAT AAT

           CDR2
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     G  N  T  N  S  N  P  S  L  K  S  R  V  T  L  S  L  D
163  GGA AAC ACC AAC TCC AAC CCG TCC CTC AAG AGT CGA GTC ACC CTA TCA CTA GAC


     T  S  K  N  Q  F  S  L  K  L  R  S  V  T  A  A  D  T
217  ACG TCC AAG AAC CAG TTC TCC CTG AAG CTG AGG TCT GTG ACC GCC GCG GAC ACG

                                    CDR3
                                    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     A  V  Y  Y  C  A  F  G  Y  S  D  Y  E  Y  N  W  F  D
271  GCT GTG TAT TAC TGT GCG TTT GGA TAT AGT GAC TAC GAG TAC AAC TGG TTC GAC

     CDR3
     ~~~
     P  W  G  Q  G  T  L  V  T  V  S  S
325  CCC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA
```

Fig. 1A

LAG3.1 - Anti-LAG3 25F7 VK

V segment: L6
J segment: JK2

```
     E  I  V  L  T  Q  S  P  A  T  L  S  L  S  P  G  E  R
1    GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                CDR1
                          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     A  T  L  S  C  R  A  S  Q  S  I  S  S  Y  L  A  W  Y
55   GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT ATT AGC AGC TAC TTA GCC TGG TAC

                                                        CDR2
                                                        ~~~~~~~~~~~~~~~~~~~~
     Q  Q  K  P  G  Q  A  P  R  L  L  I  Y  D  A  S  N  R
109  CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG

     CDR2
     ~~~~~~~
     A  T  G  I  P  A  R  F  S  G  S  G  S  G  T  D  F  T
163  GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                              CDR3
                                                              ~~~~~~~
     L  T  I  S  S  L  E  P  E  D  F  A  V  Y  Y  C  Q  Q
217  CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

           CDR3
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~
     R  S  N  W  P  L  T  F  G  Q  G  T  N  L  E  I  K
271  CGT AGC AAC TGG CCT CTC ACT TTT GGC CAG GGG ACC AAC CTG GAG ATC AAA
```

Fig. 1B

LAG3.5 - Anti-LAG VH

Q V Q L Q Q W G A G L L K P S E T L

CDR1

S L T C A V Y G G S F S D Y Y W N W

CDR2

I R Q P P G K G L E W I G E I N H R

CDR2

G S T N S N P S L K S R V T L S L D

T S K N Q F S L K L R S V T A A D T

CDR3

A V Y Y C A F G Y S D Y E Y N W F D

CDR3

P W G Q G T L V T V S S

Fig. 2A

LAG3.5 - Anti- LAG3 VK

V segment: L6
J segment: JK2

```
      E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
1     GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                CDR1
                            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      A   T   L   S   C   R   A   S   Q   S   I   S   S   Y   L   A   W   Y
55    GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT ATT AGC AGC TAC TTA GCC TGG TAC

                                                            CDR2
                                                        ~~~~~~~~~~~~~~~~~~~~
      Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   A   S   N   R
109   CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG

      CDR2
      ~~~~~~~
      A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163   GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                CDR3
                                                            ~~~~~~~~
      L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217   CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

            CDR3
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~
      R   S   N   W   P   L   T   F   G   Q   G   T   N   L   E   I   K
271   CGT AGC AAC TGG CCT CTC ACT TTT GGC CAG GGG ACC AAC CTG GAG ATC AAA
```

Fig. 2B

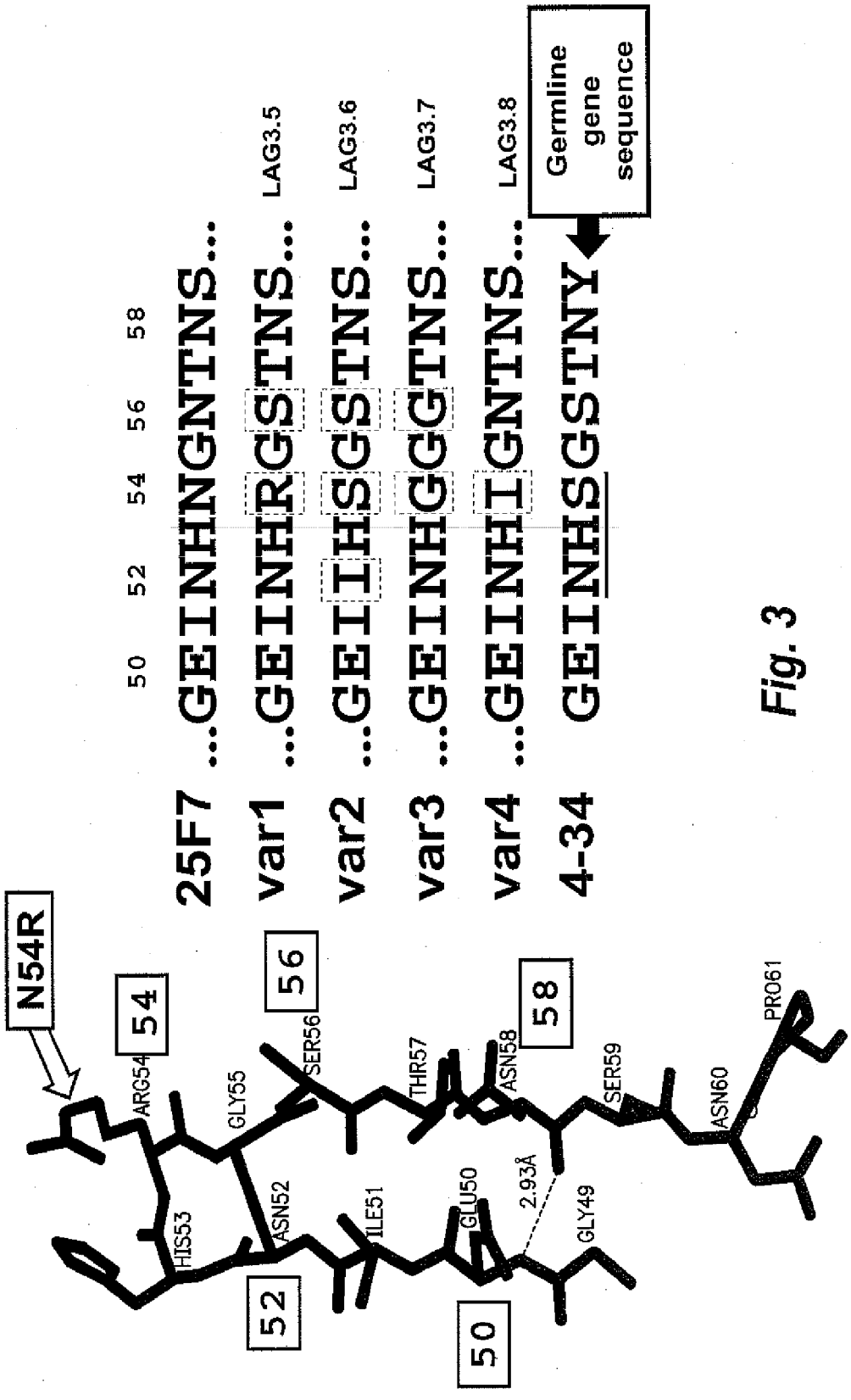

50 52 54 56 58
25F7 ...GEINHNGNTNS...
var1 ...GEINHRGSTNS... LAG3.5
var2 ...GEIIHSGSTNS... LAG3.6
var3 ...GEINHGGGTNS... LAG3.7
var4 ...GEINHIGNTNS... LAG3.8
4-34 GEINHSGSTNY
Germline gene sequence
N54R
54
56
52
50
58
ARG54
GLY55
SER56
THR57
ASN58
SER59
ASN60
PRO61
HIS53
ASN52
ILE51
GLU50
2.93Å
GLY49

Fig. 3

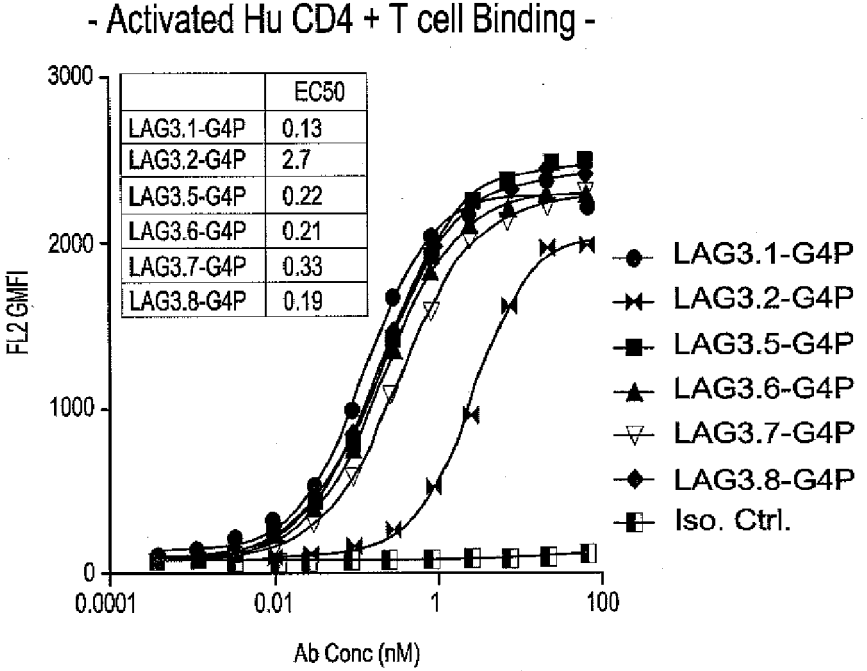
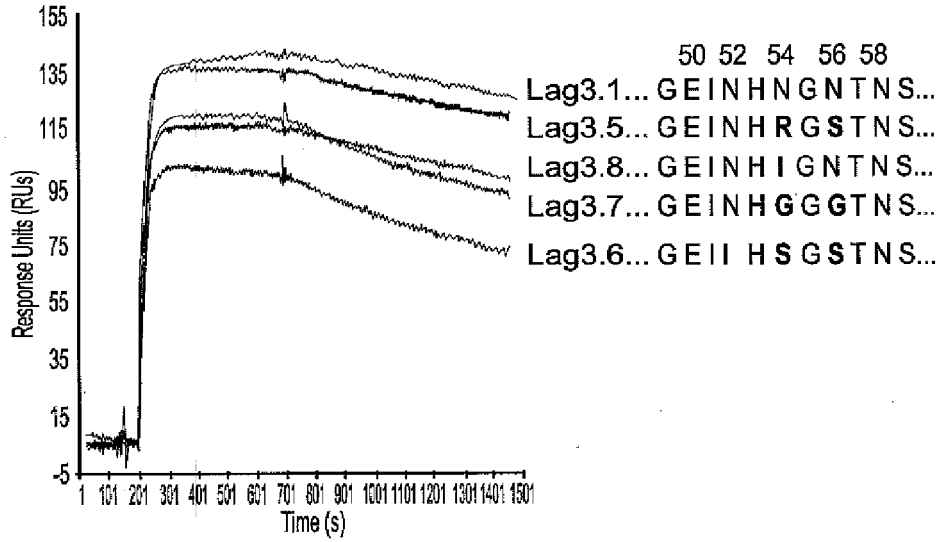


Fig. 4A



Ag: D1D4 construct

Clone ID	$K_D \times 10^{-9}$ (M)	$k_{on} \times 10^4$ (1/Ms)	$k_{off} \times 10^{-4}$ (1/s)
LAG3.1	0.27	102	2.79
LAG3.5	0.27	122	3.27
LAG3.6	0.66	118	7.77
LAG3.7	0.61	104	6.36
LAG3.8	0.30	130	3.36

Fig. 4B

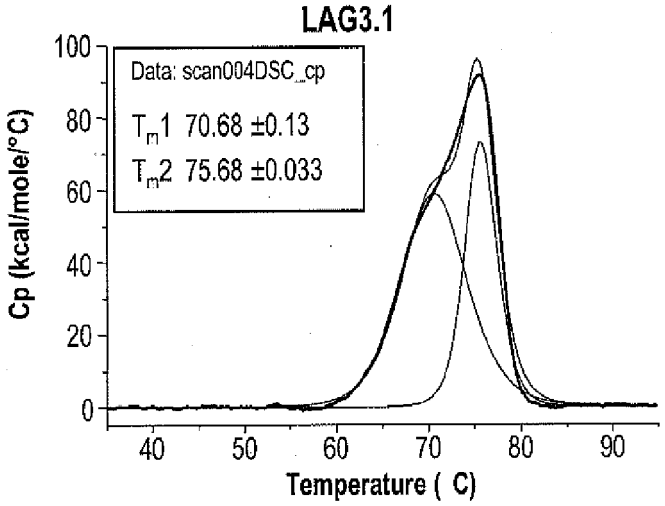


Fig. 5A

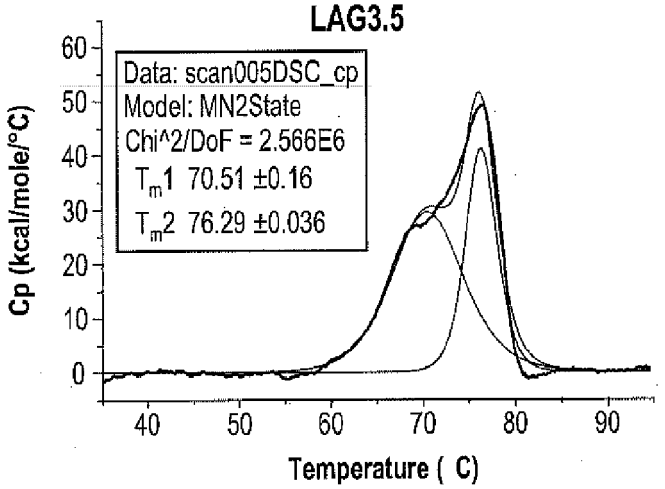


Fig. 5B

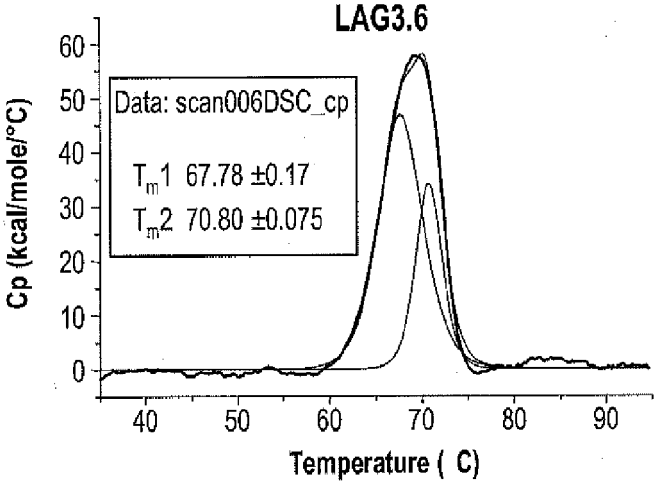


Fig. 5C

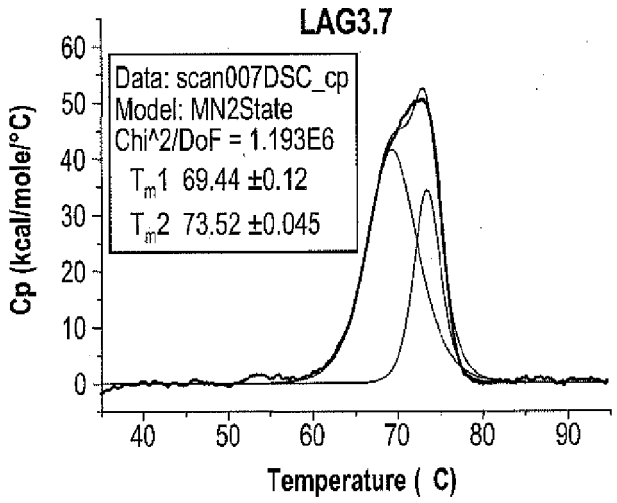

LAG3.7
Data: scan007DSC_cp
Model: MN2State
Chi^2/DoF = 1.193E6
T_m1 69.44 ±0.12
T_m2 73.52 ±0.045
Cp (kcal/mole/°C)
Temperature (C)

Fig. 5D

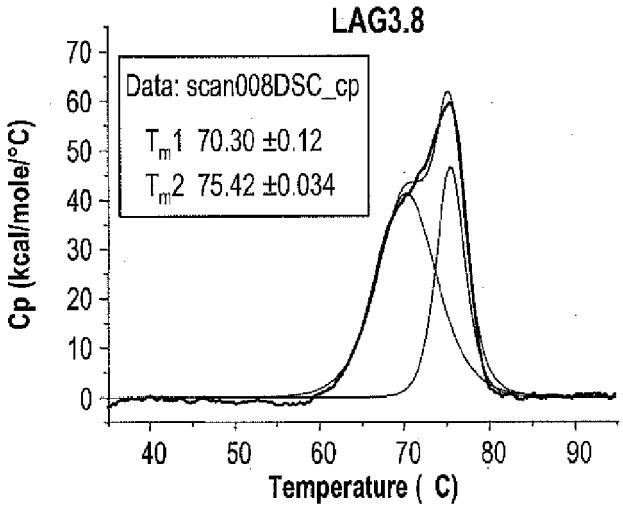

LAG3.8
Data: scan008DSC_cp
T_m1 70.30 ±0.12
T_m2 75.42 ±0.034
Cp (kcal/mole/°C)
Temperature (C)

Fig. 5E

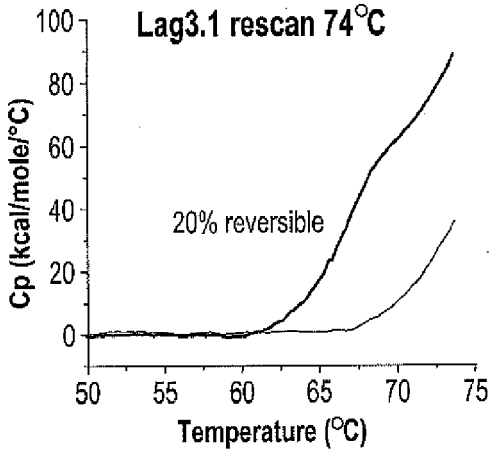


Fig. 6A

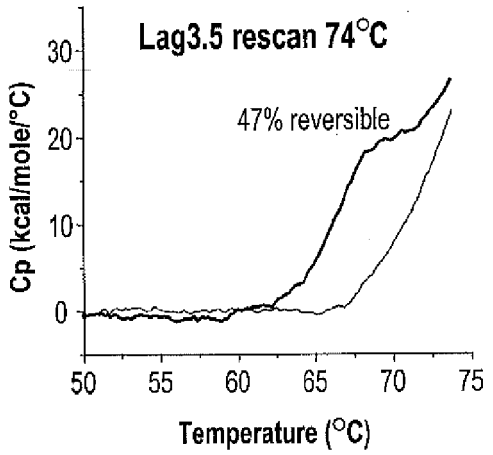


Fig. 6B

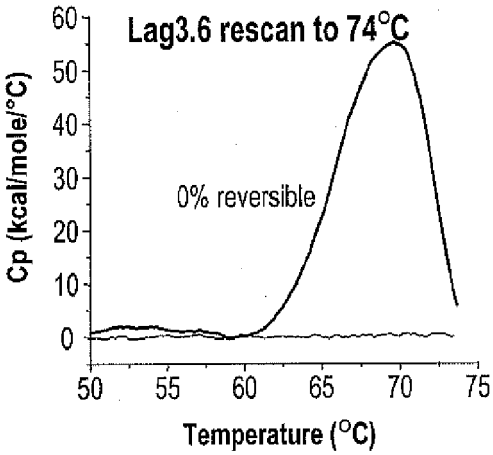


Fig. 6C

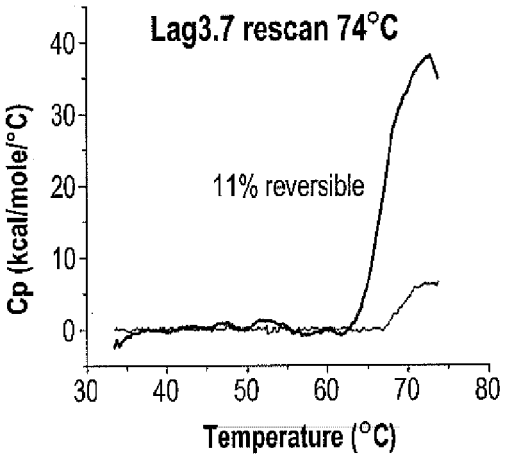

Lag3.7 rescan 74°C
11% reversible
Cp (kcal/mole/°C)
Temperature (°C)
0
10
20
30
40
30
40
50
60
70
80

Fig. 6D

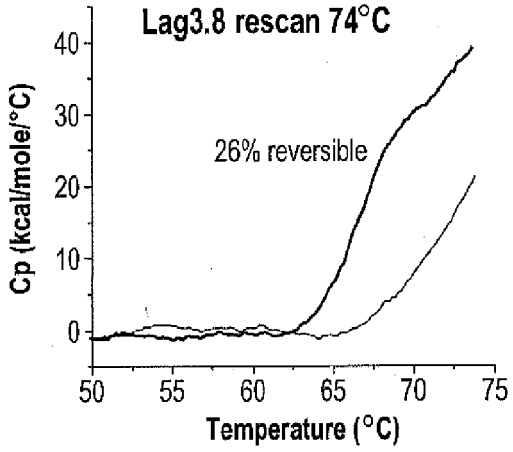

Lag3.8 rescan 74°C
26% reversible
Cp (kcal/mole/°C)
Temperature (°C)
0
10
20
30
40
50
55
60
65
70
75

Fig. 6E

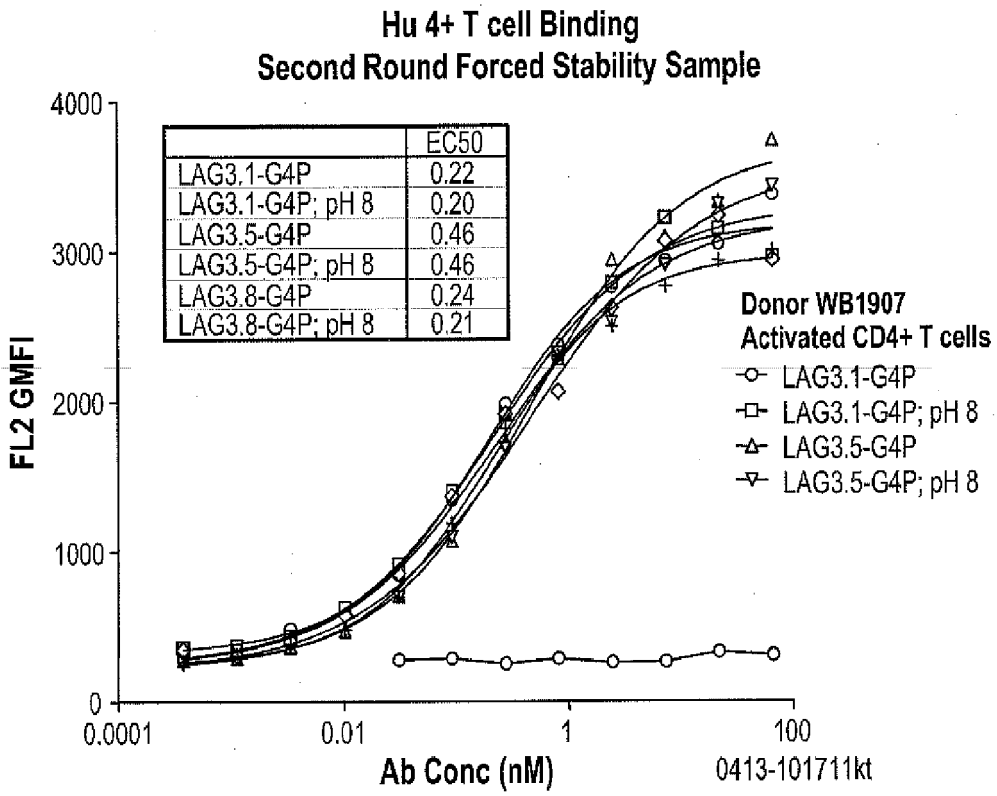

Hu 4+ T cell Binding
Second Round Forced Stability Sample
EC50
LAG3.1-G4P 0.22
LAG3.1-G4P; pH 8 0.20
LAG3.5-G4P 0.46
LAG3.5-G4P; pH 8 0.46
LAG3.8-G4P 0.24
LAG3.8-G4P; pH 8 0.21
Donor WB1907
Activated CD4+ T cells
LAG3.1-G4P
LAG3.1-G4P; pH 8
LAG3.5-G4P
LAG3.5-G4P; pH 8
FL2 GMFI
4000
3000
2000
1000
0
0.0001
0.01
1
100
Ab Conc (nM)
0413-101711kt

Fig. 7

	Mab 5 day incubation	CDR2 peptide alone is shown		CDR2 deamidation
	INHD*GNTNSNPSLK	**INHDGNTNSNPSLK**		
	INHNGNT**D**SNPSLK		I**D**HNGNTNSNPSLK	Total
3.1	**2.5%**	**5.1%**	**1.5%**	**9%**
3.1 pH8	**10.0%**	**15.3%**	**4.9%**	**30%**

	INHRGST**D?**SNPSLK	**INHRGSTNSNPSLK**	I**D?**HRGSTDSNPSLK	
3.5	**2.3%**	**-**	**1.5%**	**4%**
3.5 pH8	**3.1%**	**-**	**1.7%**	**5%**

Fig. 8

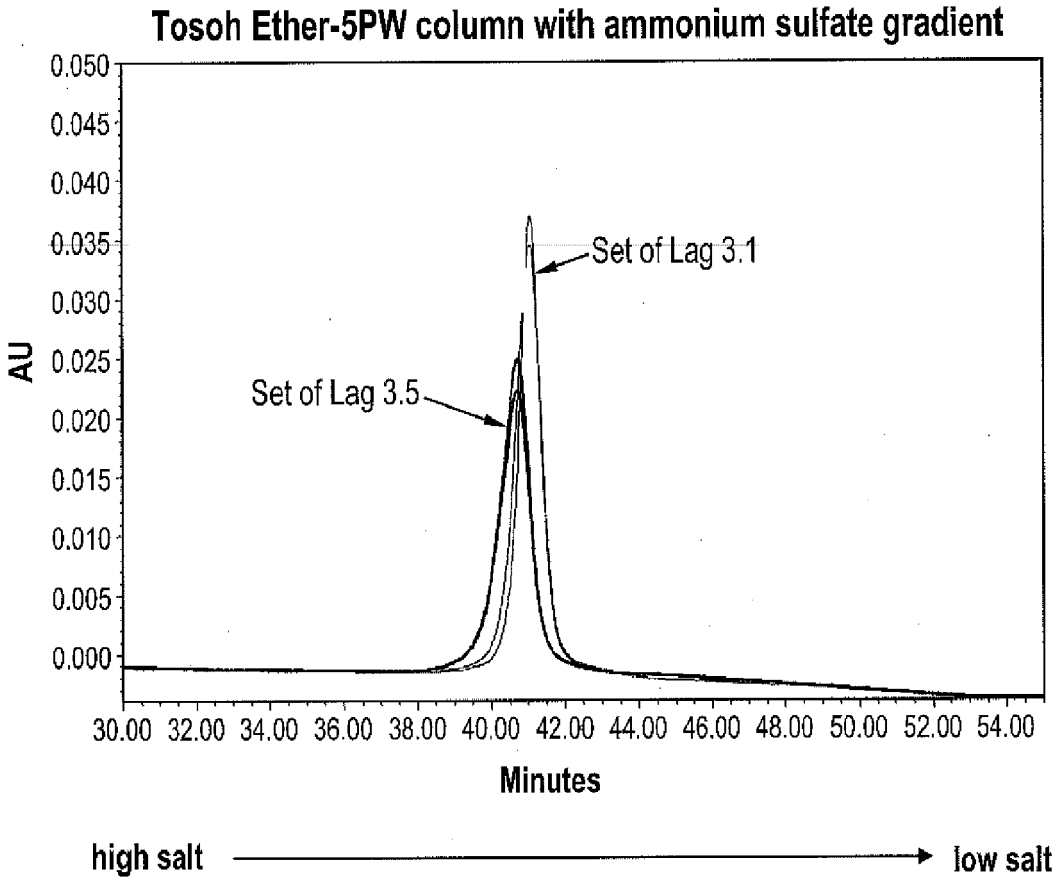

Tosoh Ether-5PW column with ammonium sulfate gradient
AU
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
Set of Lag 3.1
Set of Lag 3.5
30.00 32.00 34.00 36.00 38.00 40.00 42.00 44.00 46.00 48.00 50.00 52.00 54.00
Minutes
high salt
low salt

Fig. 9

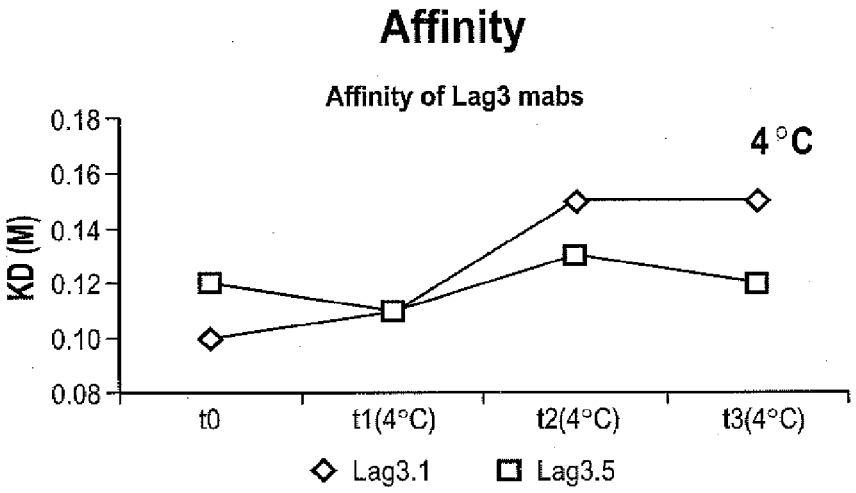


Fig. 10A

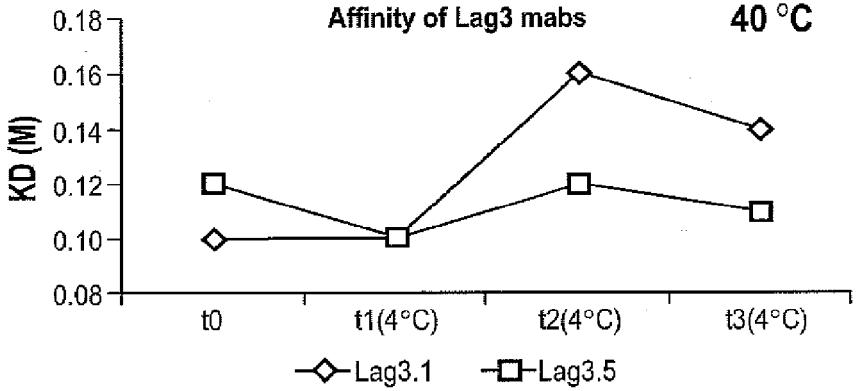


Fig. 10B

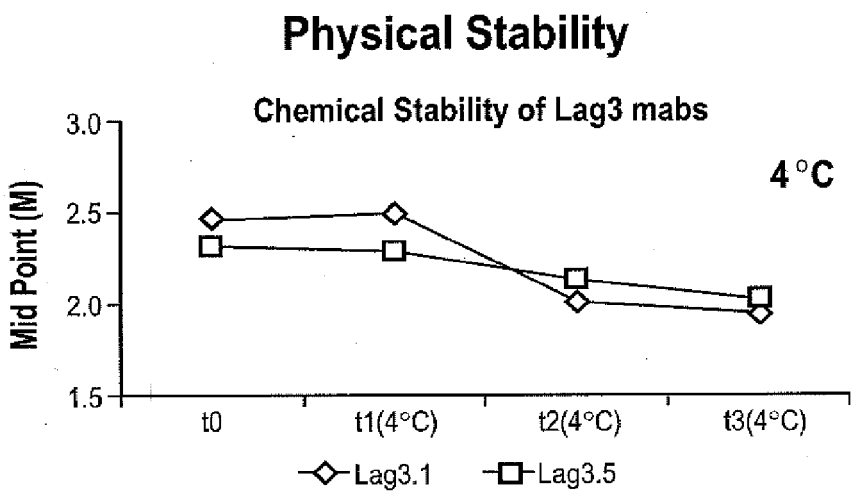


Fig. 10C

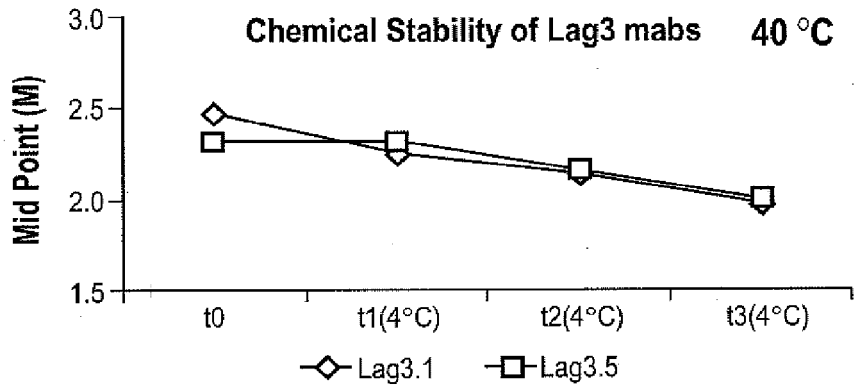


Fig. 10D

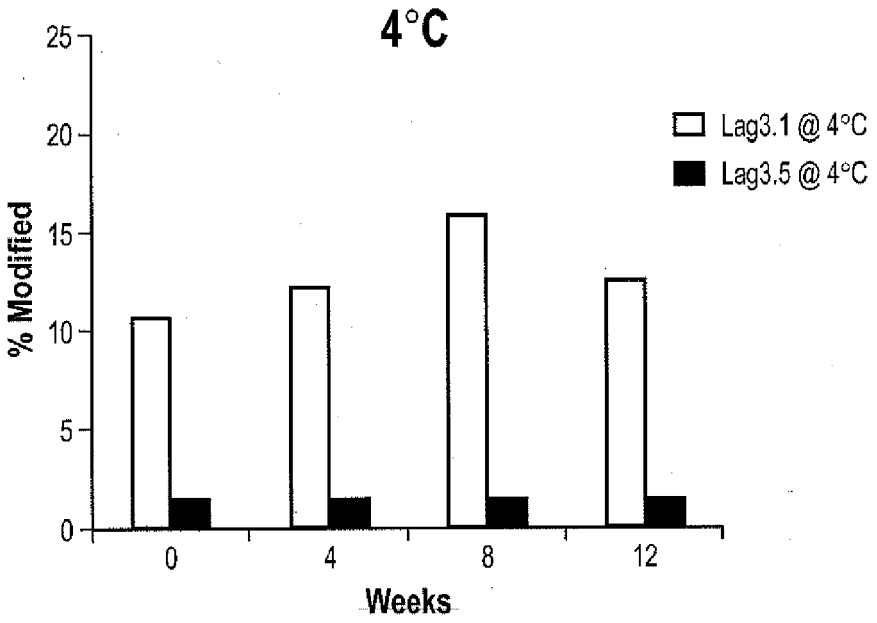


Fig. 11A

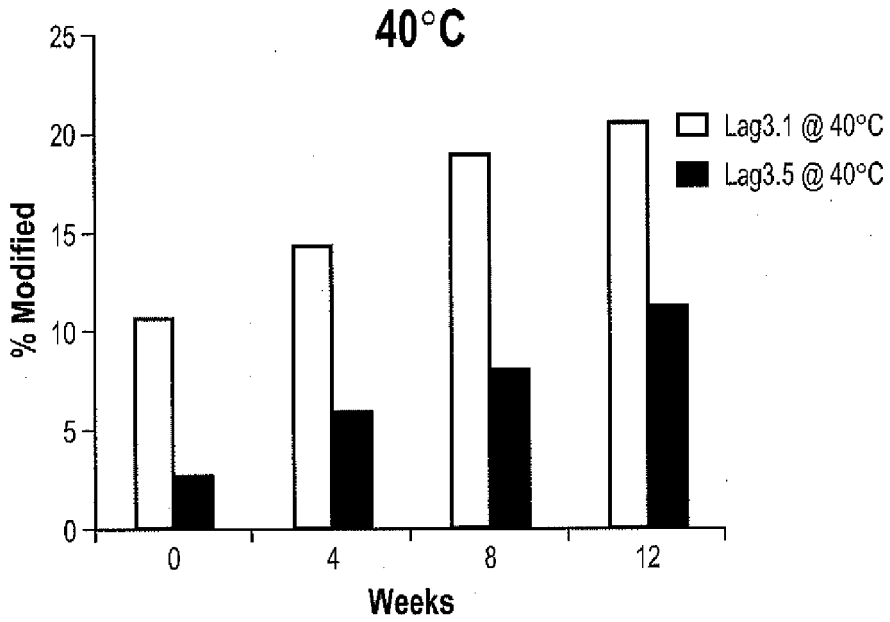


Fig. 11B