Figure 1a

IgG1 wild type CH1 & hinge

(A)STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV

DKKV(E)PKSCDKTHTCPPCPAPELGGP (SEQ ID NO:1)

IgG4 wild type CH1 & hinge

(A)STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV

DKRV(E)SKYGPPCPSCPAPEFLGGP (SEQ ID NO:2)

Ig wild type kappa constant light chain

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP

VTKSFNRGEC (SEQ ID NO:66)

Figure 1b

Light chain	**C_L**			
Human κ	FNRGEC (SEQ ID NO:3)			
Heavy chain	**C_H1 (N-term)**	**UPPER**	**CORE**	**LOWER**
Human IgGγ1	LAPSSKSTS (SEQ ID NO:4)	EPKSCDKTHT (SEQ ID NO:5)	CPPCP	APELLGGP
Human IgGγ2	LAPCSRSTS (SEQ ID NO:6)	ERK (SEQ ID NO:7)	CCVECPPCP	APPVA GP
Human IgGγ3	LAPCSRSTS (SEQ ID NO:8)	ELKTPLGDTTHT (SEQ ID NO:9)	CPRCP(EPKSCDTPPPCPRCP)$_3$	APELLGGP
Human IgGγ4	LAPCSRSTS ↑ C127 (SEQ ID NO:10)	ESKYGPP ↑ G230 (SEQ ID NO:11)	CPSCP ↑ C239 ↑ C242	APEFLGGP
Heavy chain	**C_H1 (N-term)**	**Hinge**		
Human IgD	IISGCRHPK (SEQ ID NO:67)	(E)SPKAQASSVPTAQPQAEGSLAKATTAPATTRNT (SEQ ID NO:68)		
Heavy chain	**C_H1 (N-term)**	**C_H1 (C-term)**	**C_H2 (N-term)**	
Human IgM	LVSCENSPS (SEQ ID NO:69)	EKNVPLP (SEQ ID NO:70)	(V)IAELPPKVSV (SEQ ID NO:71)	

Figure 2a

	CH1	HINGE														
EU Numbering for IgG1	131	216	217	218	221			222	223	224	225	226	227	228	229	230
Kabat Numbering for IgG1	127	226	227	228	232	233	234	235	236	237	238	239	240	241	242	243
IMGT Numbering for IgG1	10	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
IgG1 wt residues	S	E	P	K	S	C	D	K	T	H	T	C	P	P	C	P
EU Numbering for IgG4	131	216	217	218	219	220	224	225	-	-	-	226	227	228	229	230
Kabat Numbering for IgG4	127	226	227	228	229	230	237	238	-	-	-	239	240	241	242	243
IMGT Numbering for IgG4	10	1	2	3	4	5	6	7				8	9	10	11	12
IgG4 wt residues	C	E	S	K	Y	G	P	P	-	-	-	C	P	S	C	P
Mutations to IgG4	S		C or P	C	C or S	C	D or A	K or A	A or T or G	A or H or G	A or T or G	S		P	S	

Figure 2b

	CH1	HINGE						
Kabat Numbering	**127**	**226**	**227**	**228**	**229**	**230**	**232**	**233**
IMGT Numbering for IgG3	**10**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
IgG3 wt residues	C	E	L	K	T	P	L	G
Mutations to IgG3	S	C	C	C	C	C	C	C

Figure 2c

	CH1					CH2		
Kabat numbering for IgM	**127**	**223**	**223A**	**223B**	**223C**	**243G**	**243H**	**243I**
IMGT Numbering for IgM	**10**	**121**	**122**	**123**	**124**	**1.5**	**1.4**	**1.3**
IgM wt residues	C	V	P	L	P	V	I	A
Mutations to IgM	S	C	C	C	C	C	C	C

Figure 2d

	CH1	Hinge						
Kabat numbering for IgD	**128**	**227**	**228**	**229**	**230**	**231**	**232**	**233**
IMGT Numbering for IgD	**11**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
IgD wt residues	C	E	S	P	K	A	Q	A
Mutations to IgD	S	C	C	C	C	C	C	C

Figure 3a

	ANTIBODIES											
Mutations to HC IgG4	**G4**	**G1**	**1**	**2**	**3**	**6**	**7**	**8**	**12**	**13**	**15**	**16**
C127S			•	•	•	•	•	•			•	•
G230C						•	•	•			•	•
C239S				•		•		•	•			
S241P												•
C242S					•		•	•		•		

Figure 3b

	ANTIBODIES											
HC Cys position	**G4**	**G1**	**1**	**2**	**3**	**6**	**7**	**8**	**12**	**13**	**15**	**16**
127	LC								LC	LC		
230 (G4) 233 (G1)		LC				LC	LC	LC			LC	LC
239	HC	HC	LC or HC		LC		HC			HC	HC or LC	HC or LC
242	HC	HC	LC or HC	LC		HC			HC		HC or LC	HC or LC

Figure 4a

		ANTIBODIES																
Mutations to G4	**G4**	**G1**	**28**	**28P**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**44**	**45**	**46**	**47**
C127S			•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
S227C												•	•	•	•				
K228C								•	•	•	•								
Y229C			•	•	•	•	•												
G230C																•	•	•	•
P238PAAA																•	•	•	•
C239S					•		•		•		•		•		•		•		•
S241P				•															
C242S						•	•			•	•			•	•			•	•

Figure 4b

	ANTIBODIES																	
HC Cys position	**G4**	**G1**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**44**	**45**	**46**	**47**
127	LC																	
227											LC or HC	LC	LC	LC				
228							LC or HC	LC	LC	LC								
229 G4)			LC or HC	LC	LC	LC												
230 (G4) 233 (G1)		LC													LC or HC	LC	LC	LC
239	HC	HC	HC or LC		HC		HC or LC		HC		HC or LC		HC		HC or LC		HC	
242	HC	HC	HC or LC	HC			HC or LC	HC			HC or LC	HC			HC or LC	HC		

Figure 5

(Ab 6) (SEQ ID NO:12)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKYCPPSPSCPAPEFLGGP

(Ab 7) (SEQ ID NO:13)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKYCPPCPSSPAPEFLGGP

(Ab 8) (SEQ ID NO:14)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKYCPPSPSSPAPEFLGGP

(Ab 15) (SEQ ID NO:15)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKYCPPCPSCPAPEFLGGP

(Ab 16) (SEQ ID NO:16)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKYCPPCPPCPAPEFLGGP

(Ab 28) (SEQ ID NO:17)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKCGPPCPSCPAPEFLGGP

(Ab 29) (SEQ ID NO:18)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKCGPPSPSCPAPEFLGGP

(Ab 30) (SEQ ID NO:19)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKCGPPCPSSPAPEFLGGP

(Ab 31) (SEQ ID NO:20)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKCGPPSPSSPAPEFLGGP

(Ab 32) (SEQ ID NO:21)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SCYGPPCPSCPAPEFLGGP

(Ab 33) (SEQ ID NO:22)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SCYGPPSPSCPAPEFLGGP

(Ab 34) (SEQ ID NO:23)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SCYGPPCPSSPAPEFLGGP

(Ab 35) (SEQ ID NO:24)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SCYGPPSPSSPAPEFLGGP

(Ab 36) (SEQ ID NO:25)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) CKYGPPCPSCPAPEFLGGP

(Ab 37) (SEQ ID NO:26)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) CKYGPPSPSCPAPEFLGGP

(Ab 38) (SEQ ID NO:27)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) CKYGPPCPSSPAPEFLGGP

(Ab 39) (SEQ ID NO:28)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) CKYGPPSPSSPAPEFLGGP

(Ab 44) (SEQ ID NO:29)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKYCPPAAACPSCPAPEFLGGP

(Ab 45) (SEQ ID NO:30)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKYCPPAAASPSCPAPEFLGGP

(Ab 46) (SEQ ID NO:31)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKYCPPAAACPSSPAPEFLGGP

(Ab 47) (SEQ ID NO:32)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKYCPPAAASPSSPAPEFLGGP

(Ab 2) (SEQ ID NO:33)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKYGPPSPSCPAPEFLGGP

(Ab 3) (SEQ ID NO:34)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKYGPPCPSSPAPEFLGGP

(Ab 48) (SEQ ID NO:35)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) PKSCDKTHTCPPCPAPEFLGGP

(Ab 28P) (SEQ ID NO:36)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKCGPPCPPCPAPEFLGGP

(Ab 44P) (SEQ ID NO:37)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKYCPPAAACPPCPAPEFLGGP

Figure 6

IgG4 CH2 and CH3: (SEQ ID NO:64)
SVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
LPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR
WQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

IgG4 CH2 IgG1 CH3: (SEQ ID NO:65)
SVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
LPSSIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

(Ab 6) (SEQ ID NO:38)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)SKYCPPSPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 7) (SEQ ID NO:39)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)SKYCPPCPSSPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 8) (SEQ ID NO:40)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)SKYCPPSPSSPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 15) (SEQ ID NO:41)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKYCPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 16) (SEQ ID NO:42)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKYCPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 28) (SEQ ID NO:43)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKCGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 29) (SEQ ID NO:44)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKCGPPSPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 30) (SEQ ID NO:45)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKCGPPCPSSPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 31) (SEQ ID NO:46)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)SKCGPPSPSSPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 32) (SEQ ID NO:47)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)SCYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 33) (SEQ ID NO:48)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)SCYGPPSPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 34) (SEQ ID NO:49)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)SCYGPPCPSSPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 35) (SEQ ID NO:50)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)SCYGPPSPSSPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 36) (SEQ ID NO:51)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)CKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 37) (SEQ ID NO:52)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)CKYGPPSPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 38) (SEQ ID NO:53)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)CKYGPPCPSSPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 39) (SEQ ID NO:54)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)CKYGPPSPSSPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 44) (SEQ ID NO:55)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)SKYCPPAAACPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 45) (SEQ ID NO:56)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)SKYCPPAAASPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 46) (SEQ ID NO:57)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)SKYCPPAAACPSSPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 47) (SEQ ID NO:58)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)SKYCPPAAASPSSPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 2) (SEQ ID NO:59)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)SKYGPPSPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 3) (SEQ ID NO:60)
(A)STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV(E)SKYGPPCPSSPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 48) (SEQ ID NO:61)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
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REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 28P) (SEQ ID NO:62)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKCGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

(Ab 44P) (SEQ ID NO:63)
(A) STKGPSVFPLAPSSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH
KPSNTKVDKRV (E) SKYCPPAAACPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Figure 7

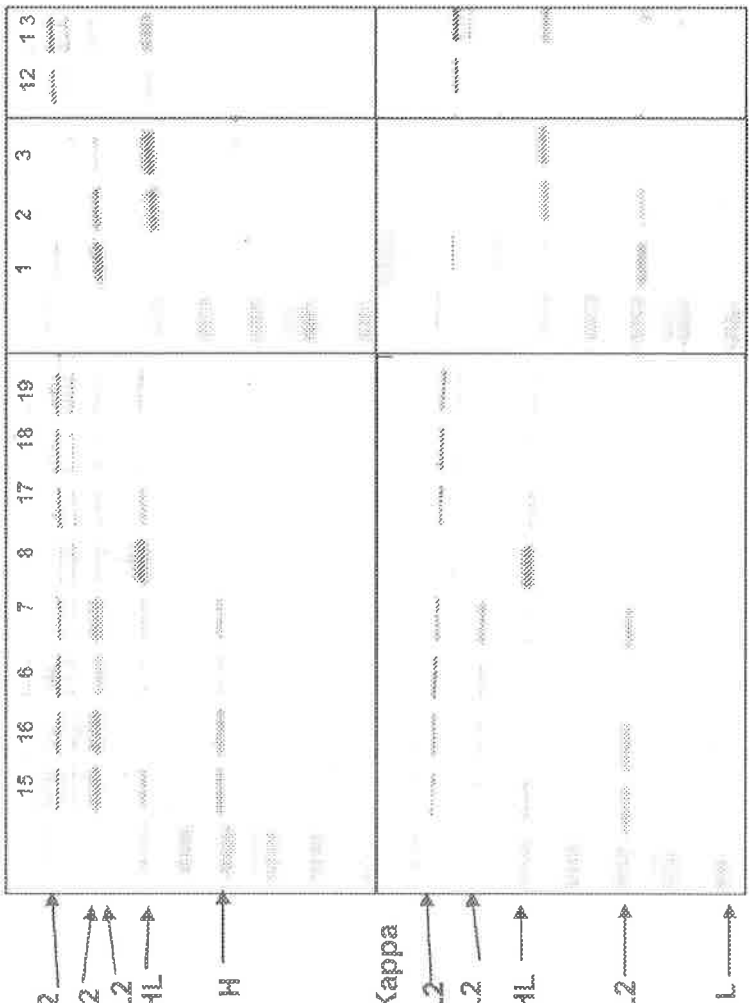

Anti-hFc
H2L2
H2
HL2
H1
H
Anti-Kappa
H2L2
HL2
H1
L2
L
15
16
6
7
8
17
18
19
1
2
3
12
13

Figure 8

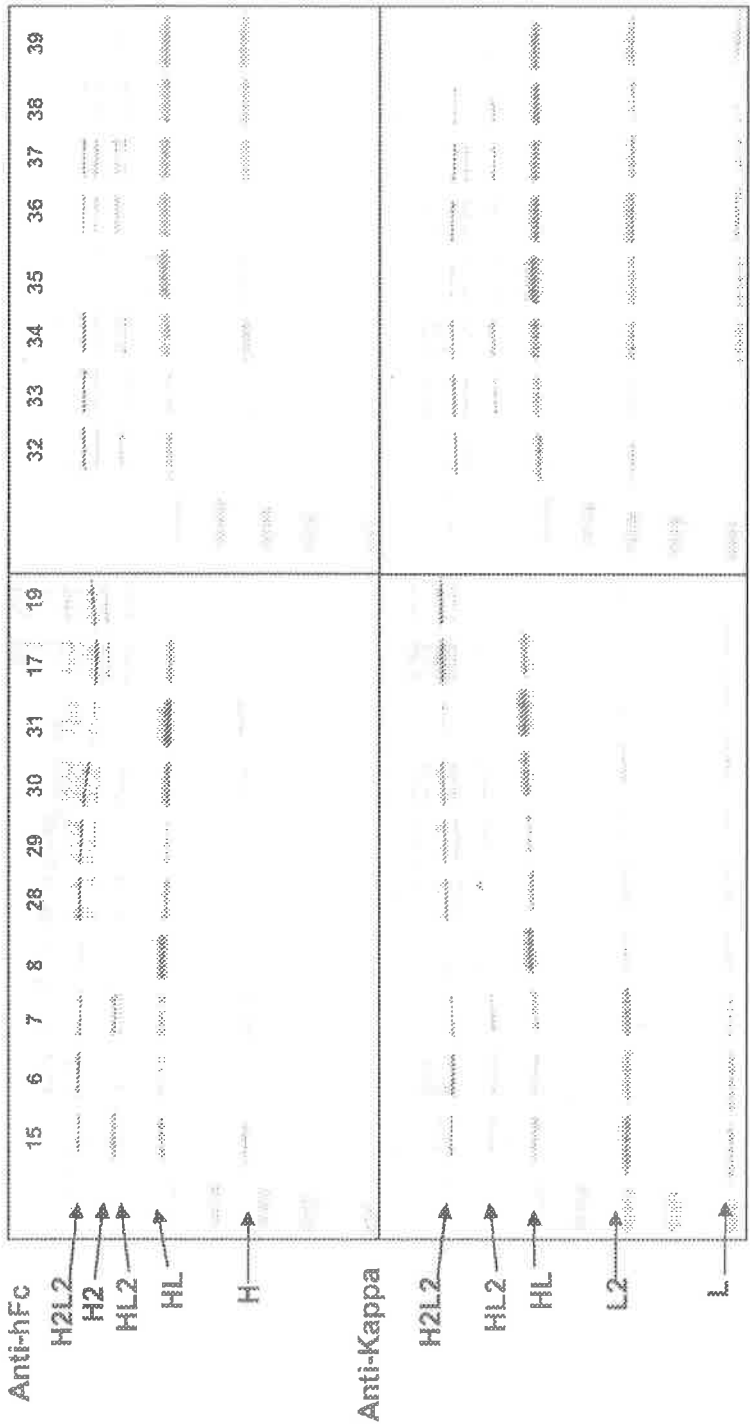

Anti-hFc
H2L2
H2
HL2
HL
H
Anti-Kappa
H2L2
HL2
HL
L2
L
15
6
7
8
28
29
30
31
17
19
32
33
34
35
36
37
38
39

Figure 9

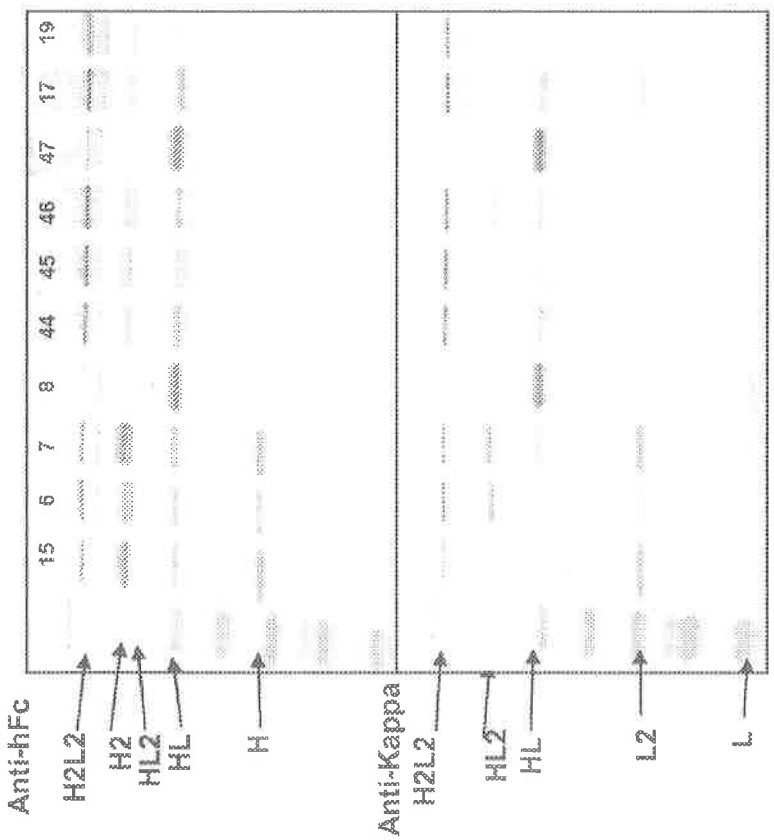

Anti-hFc
H2L2
H2
HL2
HL
H
Anti-Kappa
H2L2
HL2
HL
L2
L
15
6
7
8
44
45
46
47
17
19

Figure 10

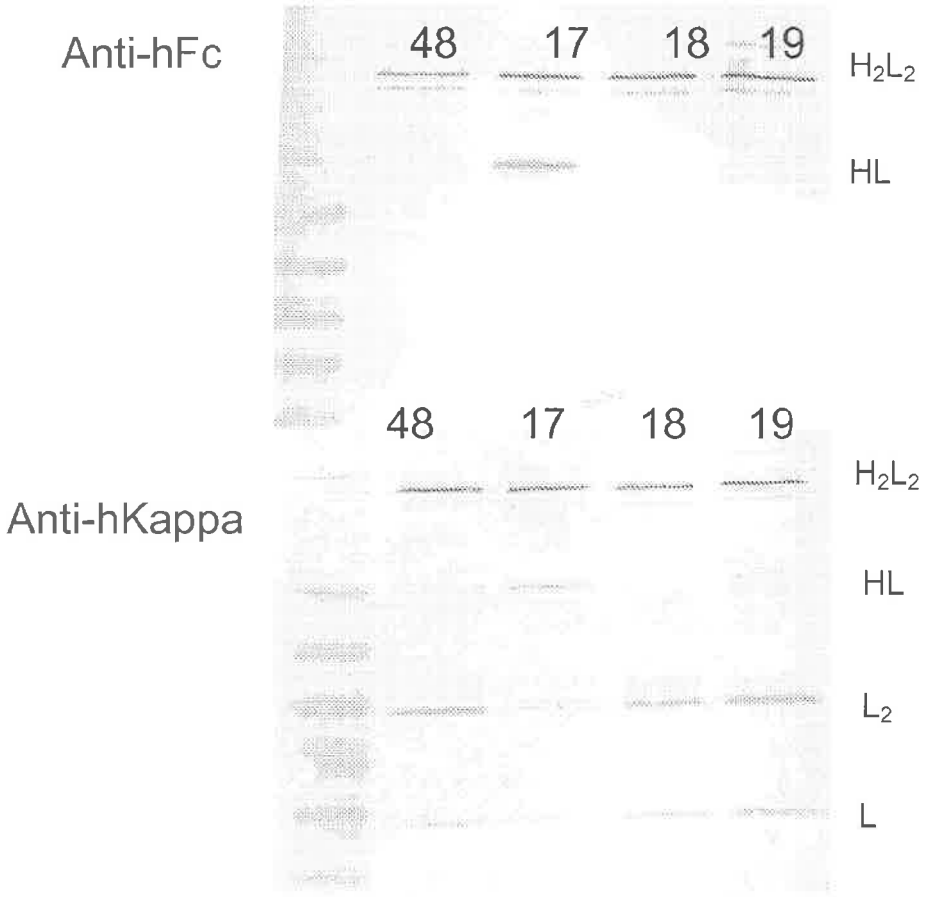


Figure 11


Temperature (degrees Celsius)
90
85
80
75
70
65
60
55
◆ Fab
□ CH2
15 C127S G230C
16 C127S G230C S241P
6 C127S G230C C239S
7 C127S G230C C242S
8 C127S G230C C239S C242S
17 hG4 wt
18 hG4 S241P
19 hG1 wt
1 C127S
2 C127S C239S
3 C127S C242S
12 C239S
13 C242S

Figure 12


Temperature (degrees Celsius)
90
85
80
75
70
65
60
55
◆ Fab
□ CH2
15 C127S G230C
6 C127S G230C C239S
7 C127S G230C C242S
8 C127S G230C C239S C242S
28 C127S Y229C
29 C127S Y229C C239S
30 C127S Y229C C242S
31 C127S Y229C C239S C242S
32 C127S K228C
33 C127S K228C C239S
34 C127S K228C C242S
35 C127S K228C C239S C242S
36 C127S S227C
37 C127S S227C C239S
38 C127S S227C C242S
39 C127S S227C C239S C242S
17 IgG4 wt
19 IgG1 wt

Figure 13

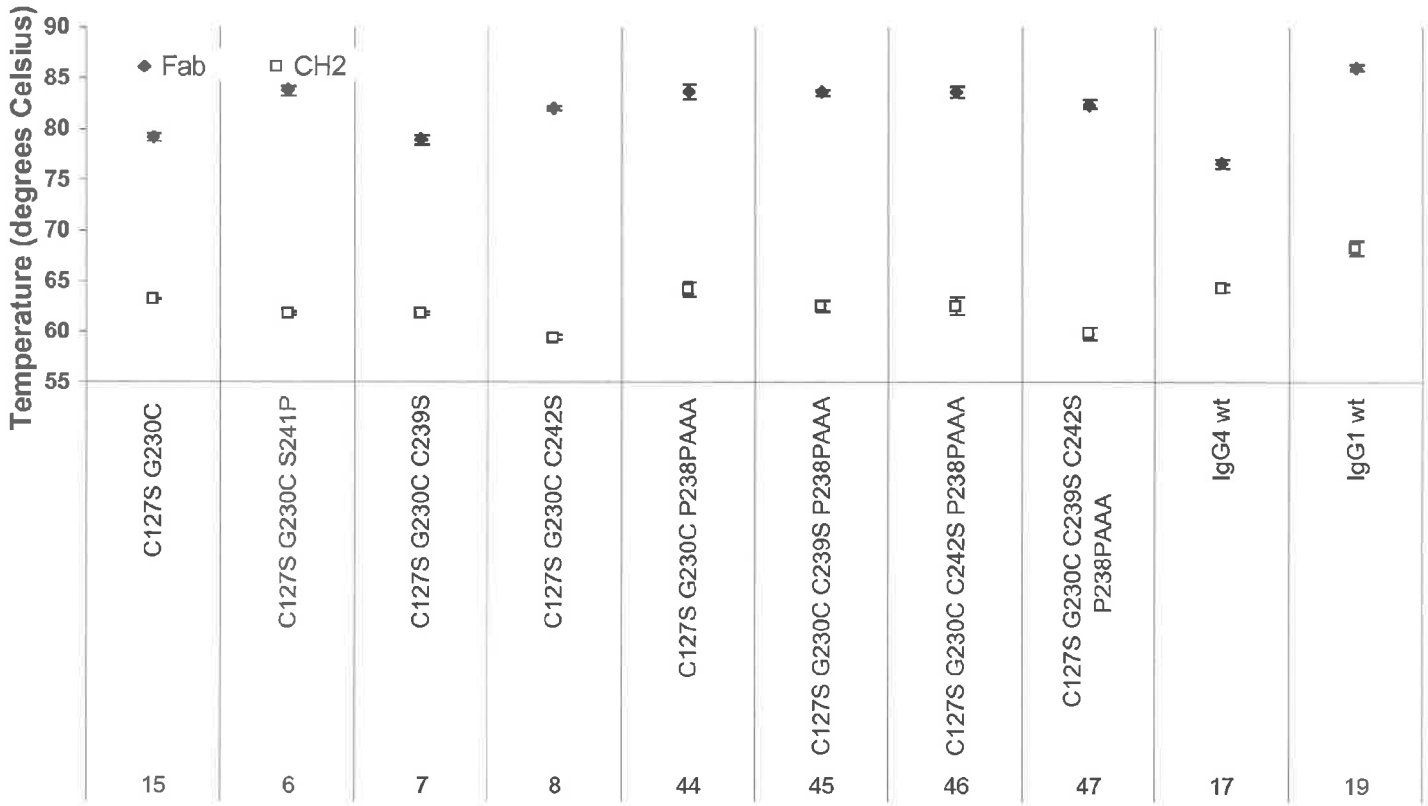

Temperature (degrees Celsius)
90
85
80
75
70
65
60
55
Fab
CH2
C127S G230C
C127S G230C S241P
C127S G230C C239S
C127S G230C C242S
C127S G230C P238PAAA
C127S G230C C239S P238PAAA
C127S G230C C242S P238PAAA
C127S G230C C239S C242S P238PAAA
IgG4 wt
IgG1 wt
15
6
7
8
44
45
46
47
17
19

Figure 14

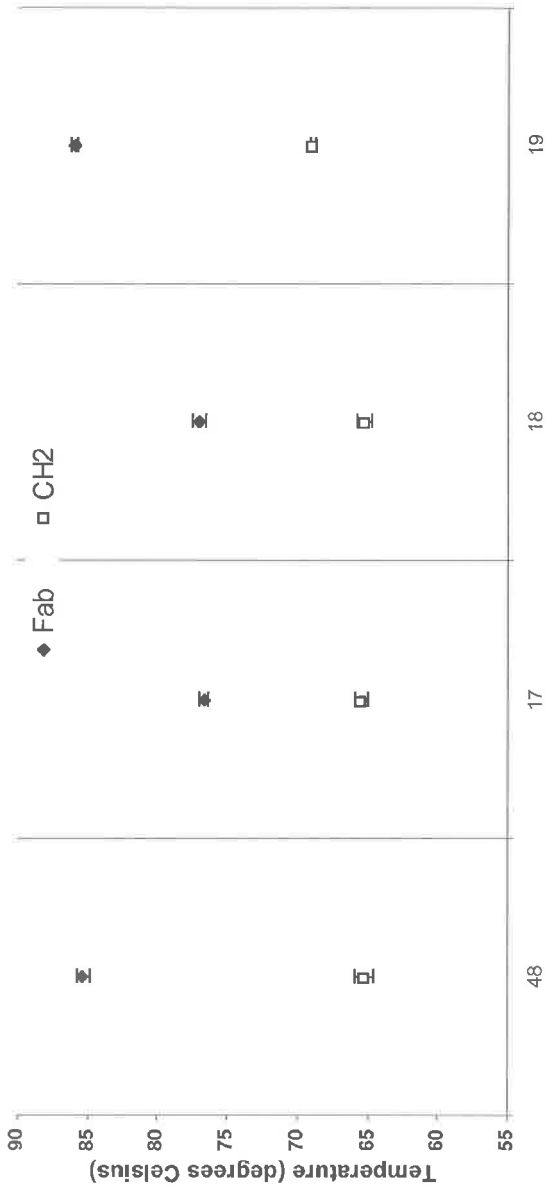

Temperature (degrees Celsius)
90
85
80
75
70
65
60
55
48
17
18
19
Fab
CH2

Figure 15

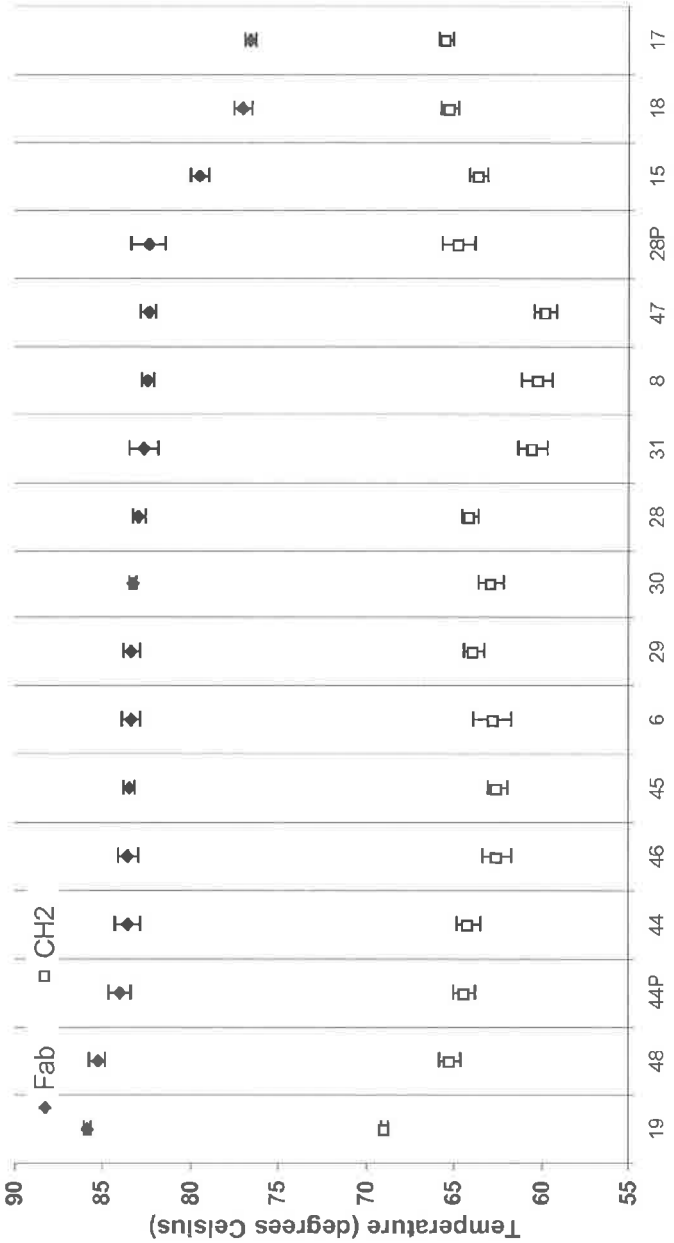

Temperature (degrees Celsius)
90
85
80
75
70
65
60
55
Fab
CH2
19
48
44P
44
46
45
6
29
30
28
31
8
47
28P
15
18
17

Figure 16

Antibody	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	KD (pM)
Control				
17 (hg4 wt)	3.03E+06	2.41E-04	7.95E-11	**80**
18 (hg4 S241P)	2.65E+06	2.51E-04	9.47E-11	**95**
19 (hG1 wt)	2.77E+06	3.54E-04	1.28E-10	**128**
17 (hg4 wt)	2.87E+06	3.26E-04	1.14E-10	**114**
Mutants				
15 (C127S G233C)	3.13E+06	2.73E-04	8.72E-11	**87**
16 (C127S G233C S241P)	2.83E+06	3.54E-04	1.25E-10	**125**
6 (C127S G233C C239S)	2.89E+06	3.32E-04	1.15E-10	**115**
7 (C127S G233C C242S)	2.98E+06	2.95E-04	9.90E-11	**99**
8 (C127S G233C C239S C242S)	2.71E+06	2.62E-04	9.67E-11	**97**

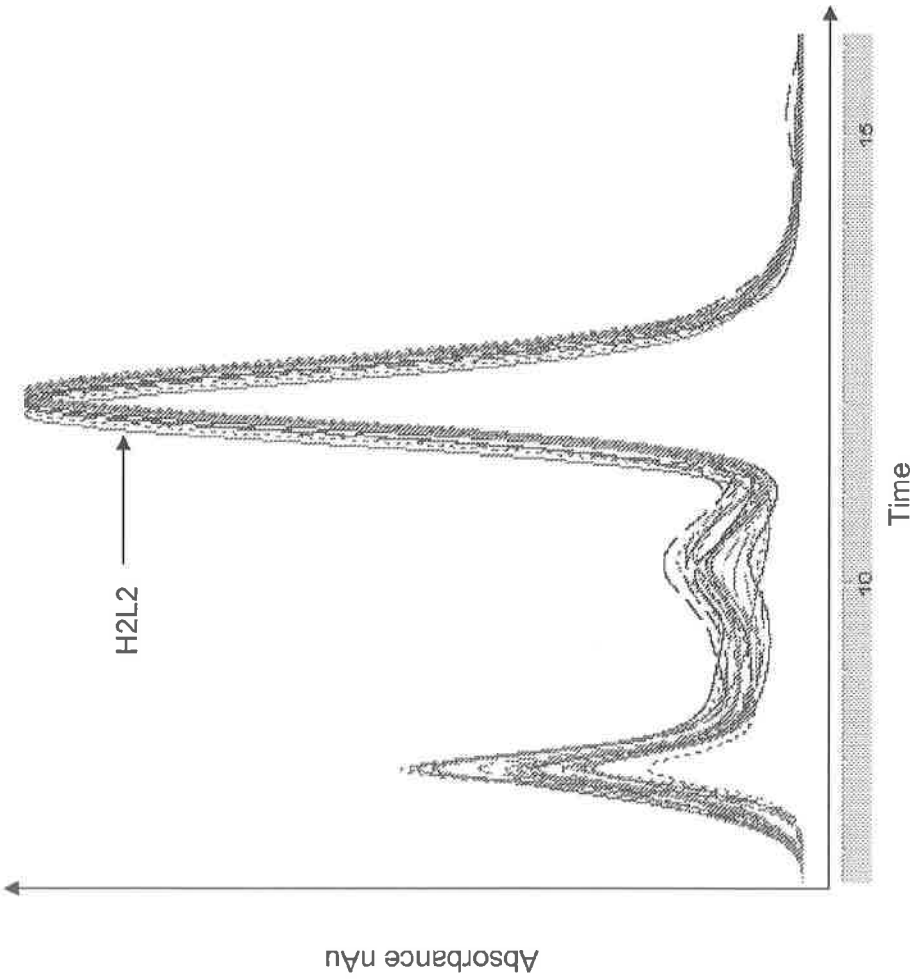
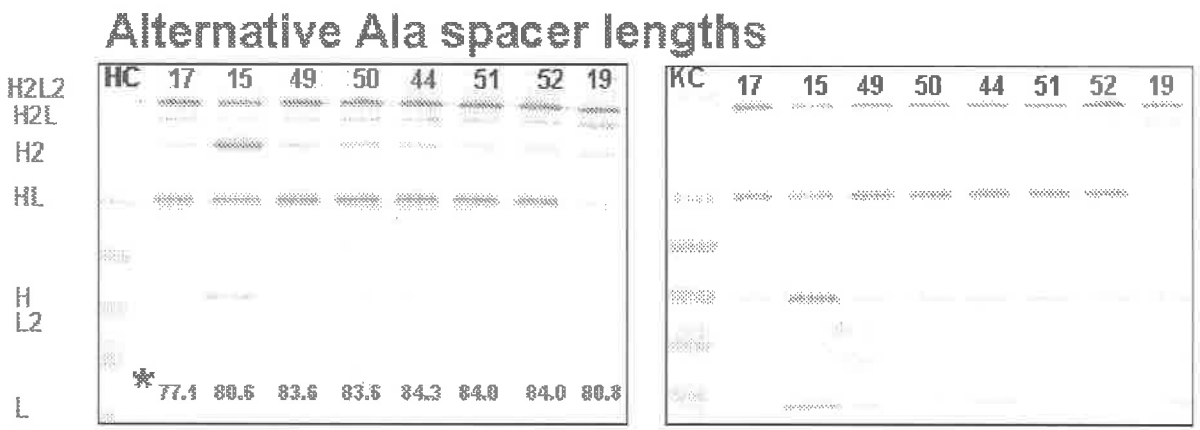


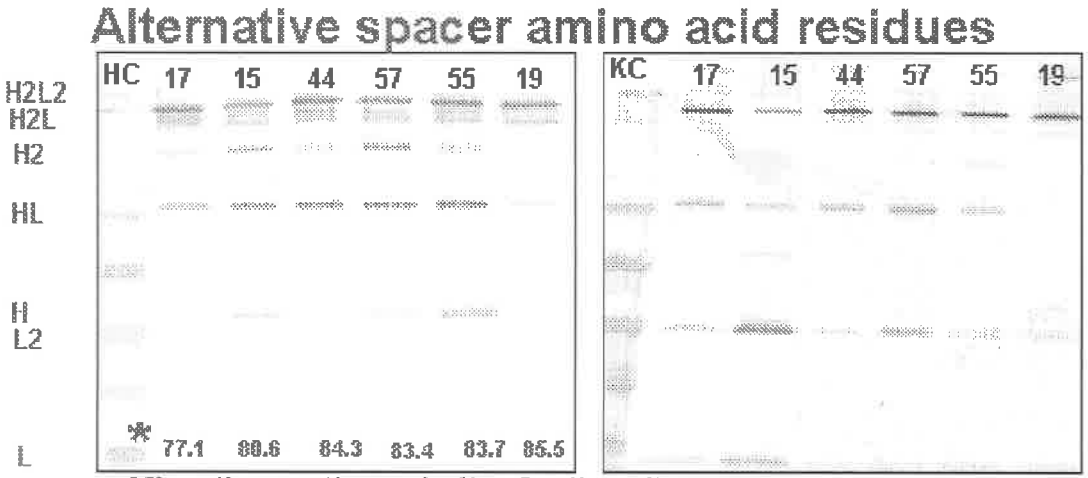
Figure 17

Figure 18



* Fab stability ◦C

Figure 19



Migration pattern similar for the alternate spacer residues compared to AAA, with slight differences in band intensities.

17. IgG4 wt

15. C127S G230C

44. C127S G230C ^AAA

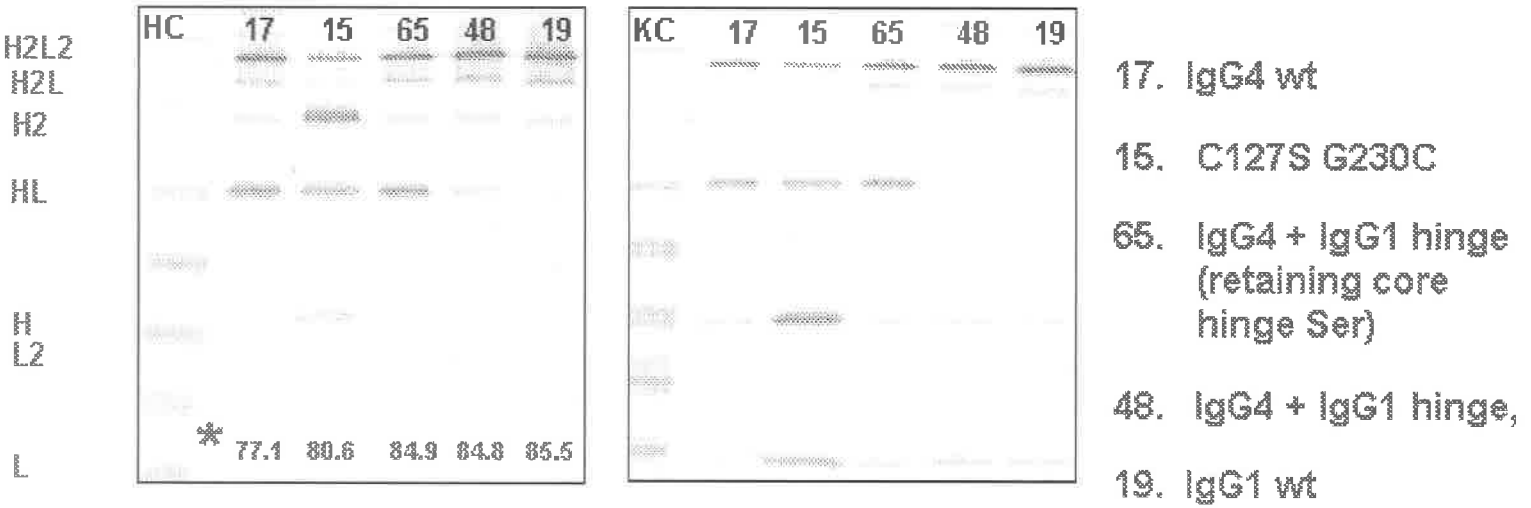
57. C127S G230C ^GGG

55. C127S G230C ^THT

19. IgG1 wt

* Fab stability ◦C

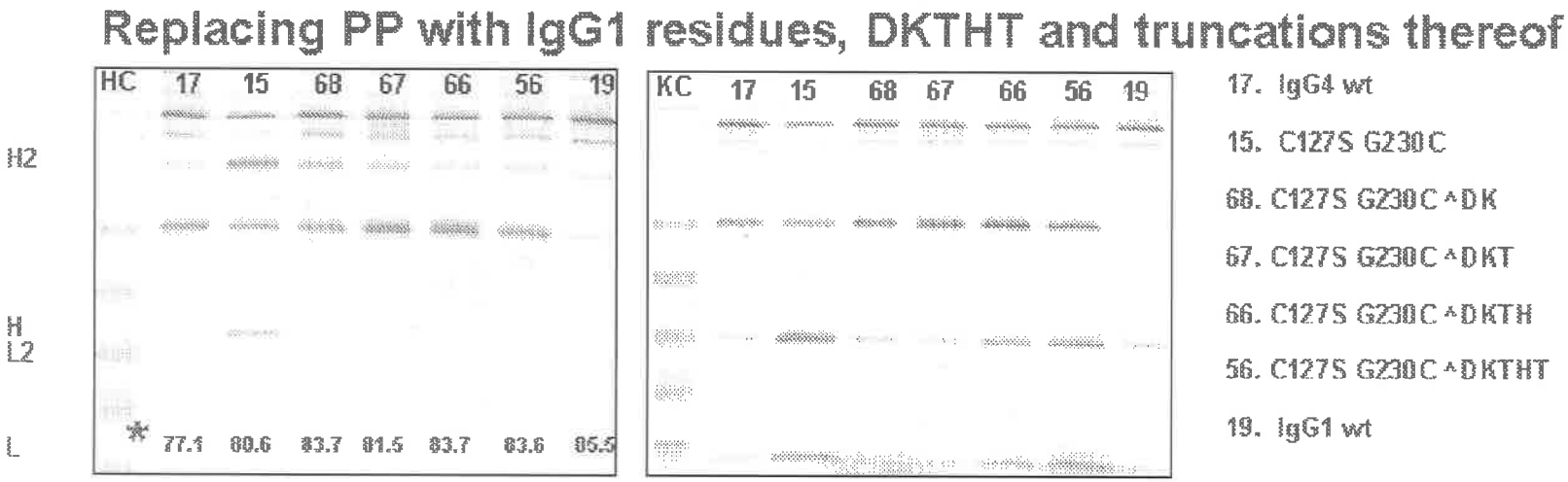
Figure 20



Re-introducing the Ser to the core hinge (mut 65) results in HL observed on SDS PAGE as in IgG4 wt, whilst retaining the Fab stability seen for mutant 48.

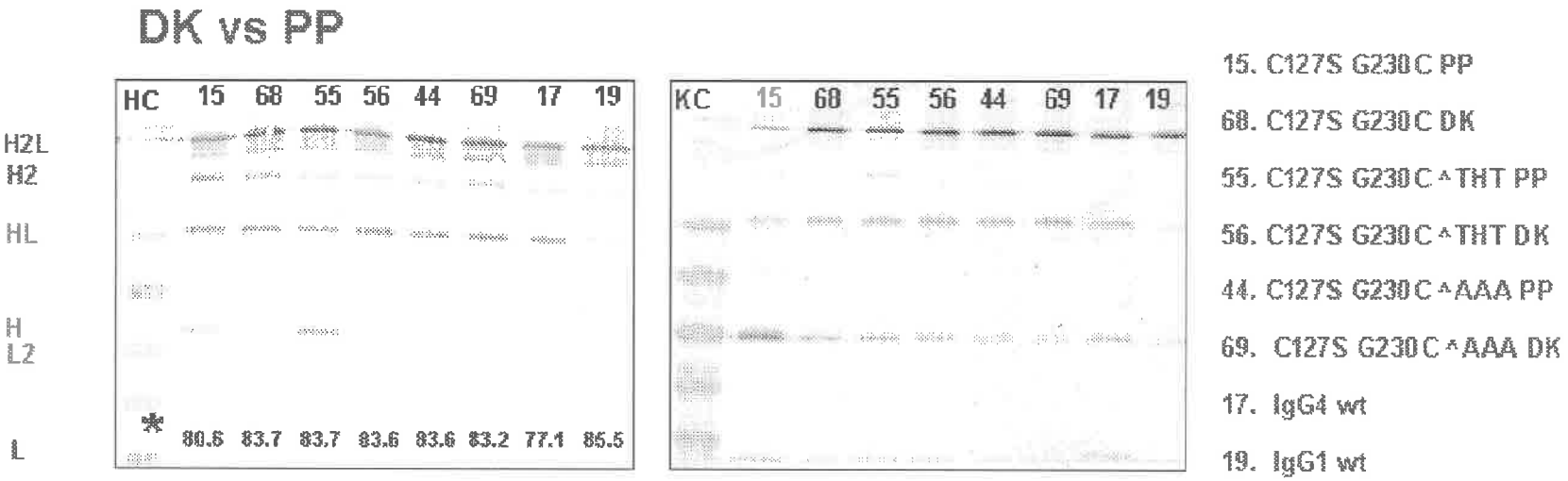
* Fab stability ◦C

Figure 21



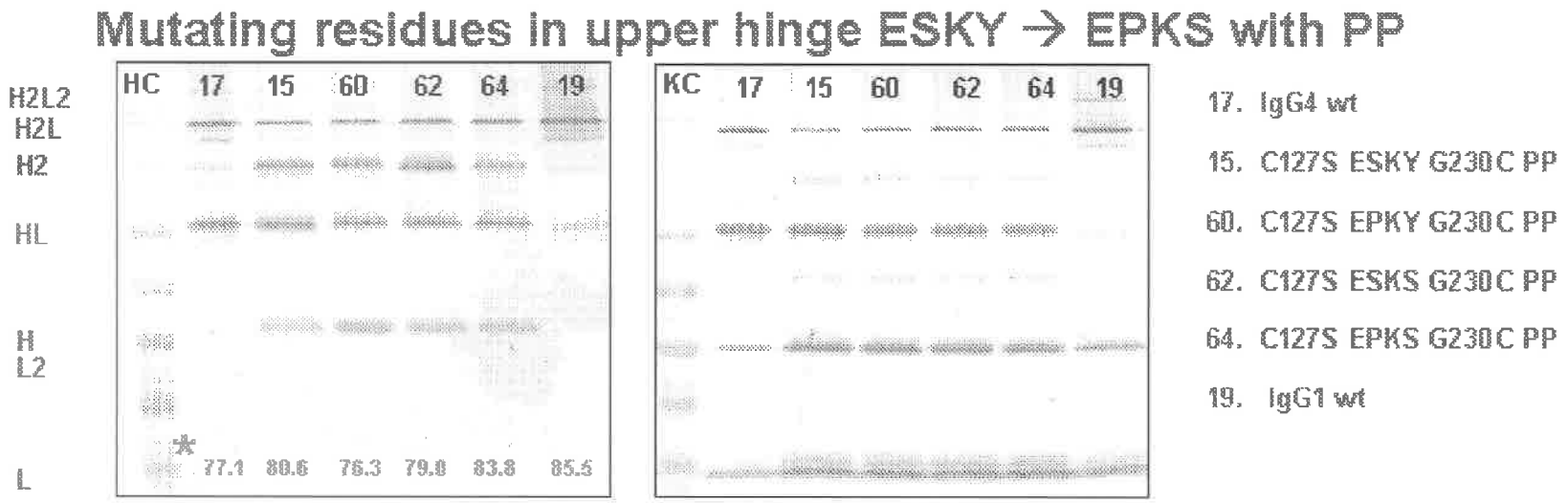
* Fab stability ◦C

Figure 22



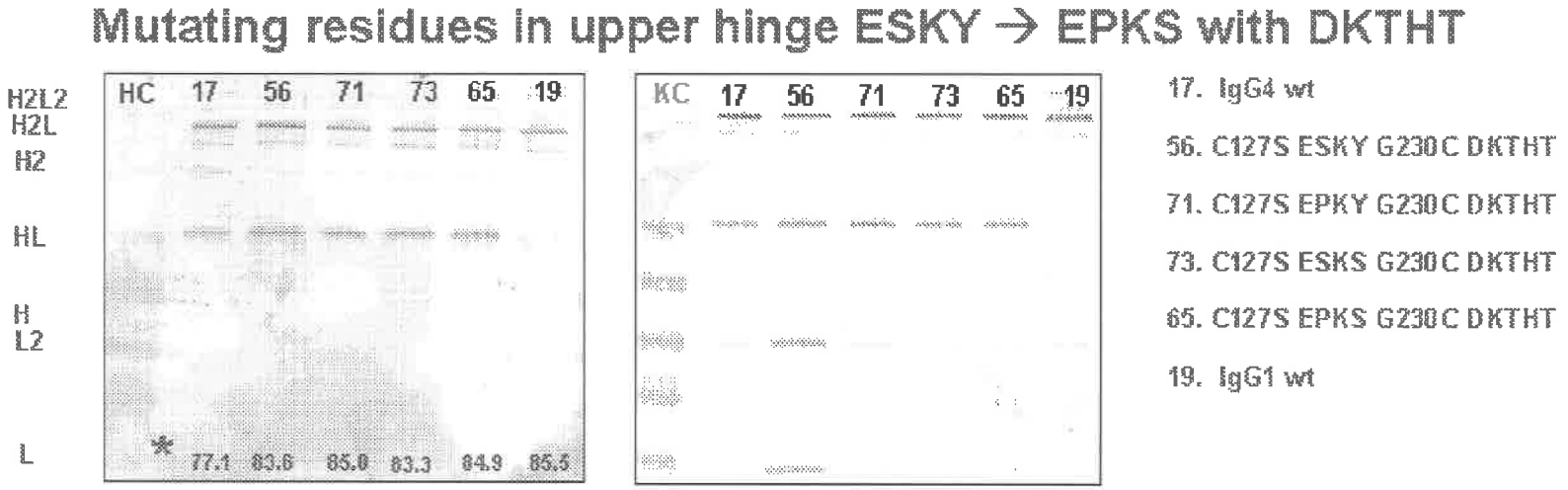
* Fab stability ◦C

Figure 23



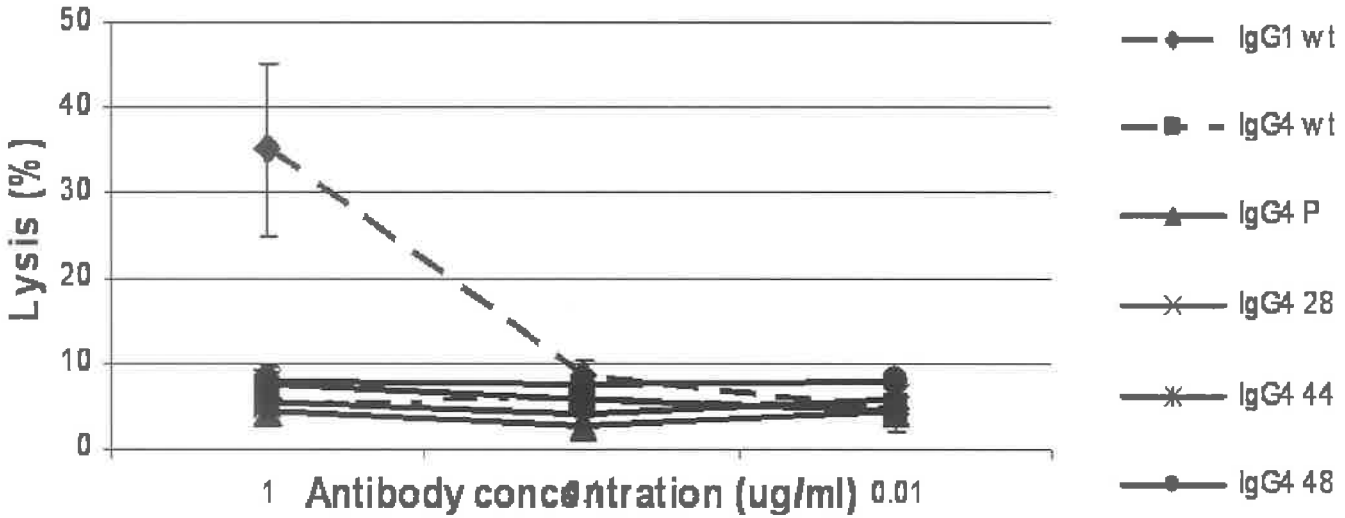
* Fab stability ◦C

Figure 24



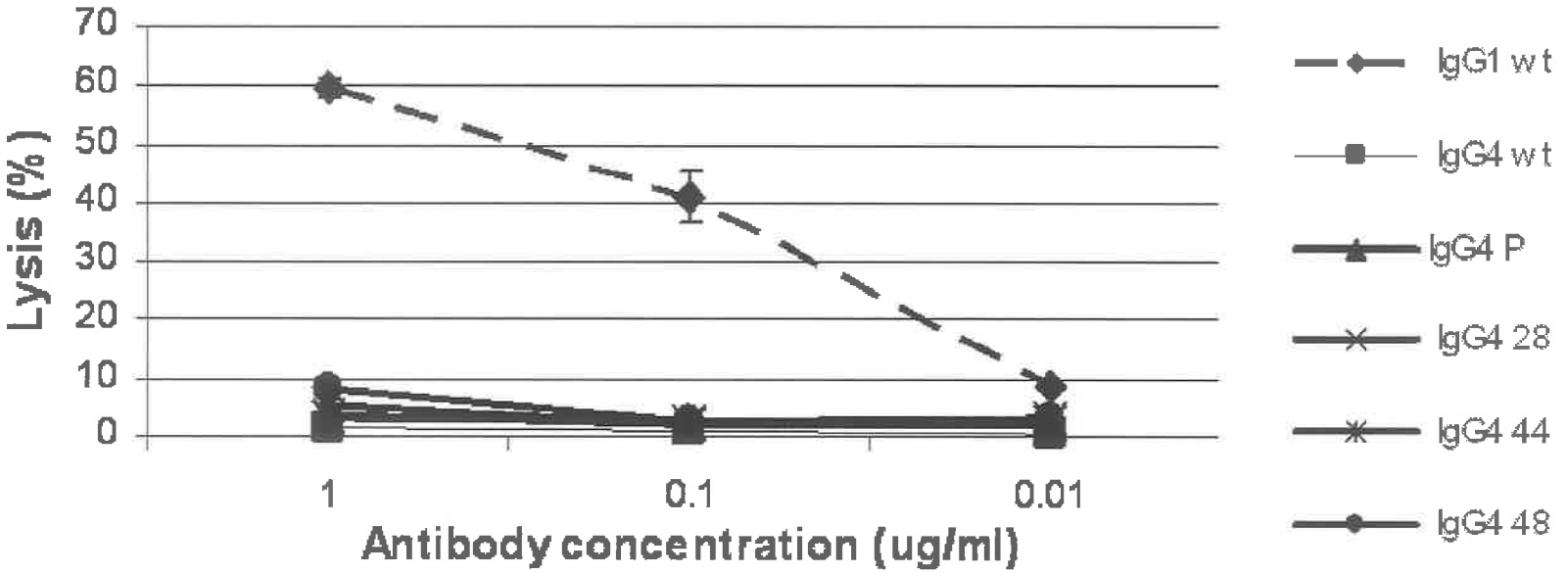
* Fab stability ◦C

Figure 25 MCF7



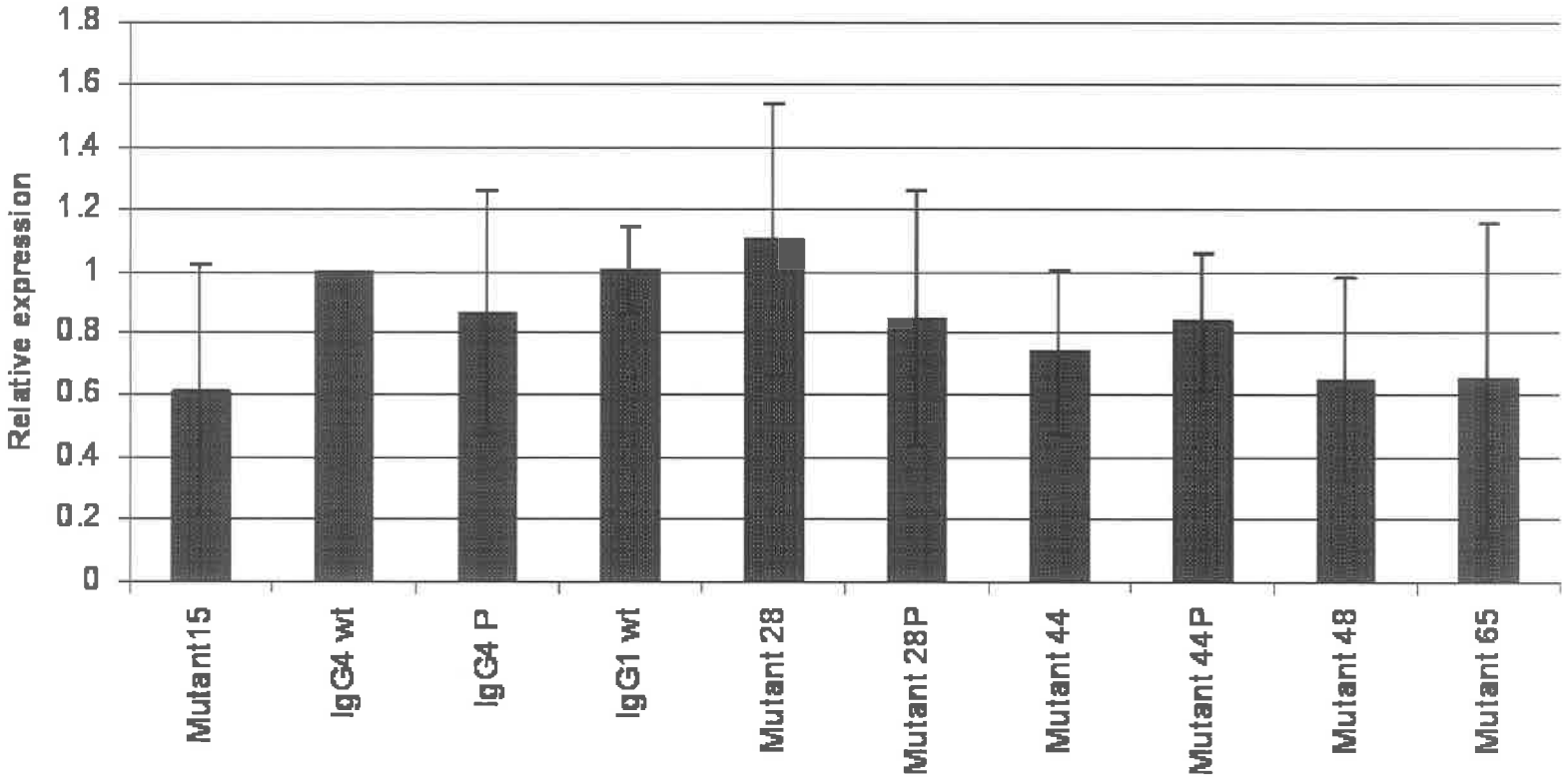
No increase in percentage lysis observed for IgG4 mutants compared to IgG4 wt in the McF7 cell line

Figure 26 Skbr3



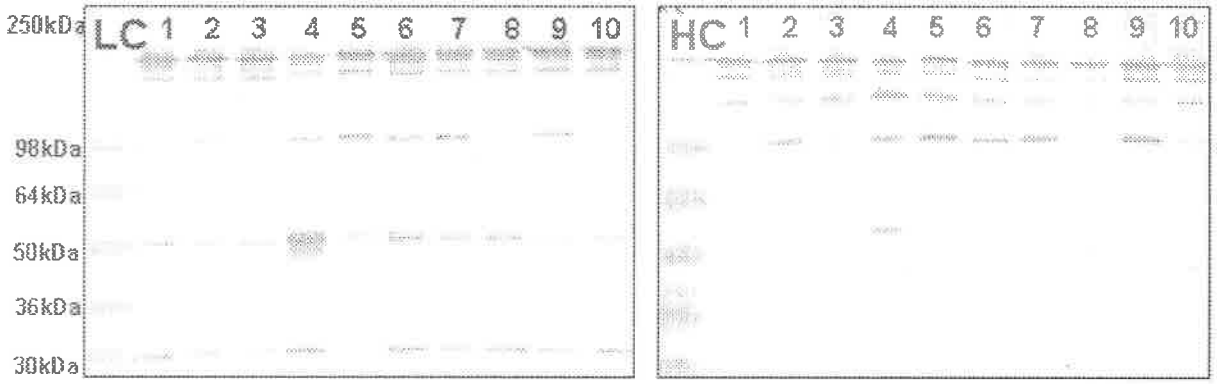
No increase in percentage lysis observed for IgG4 mutants compared to IgG4 wt in the Skbr3 cell line

Figure 27 Expression relative to wild type IgG4



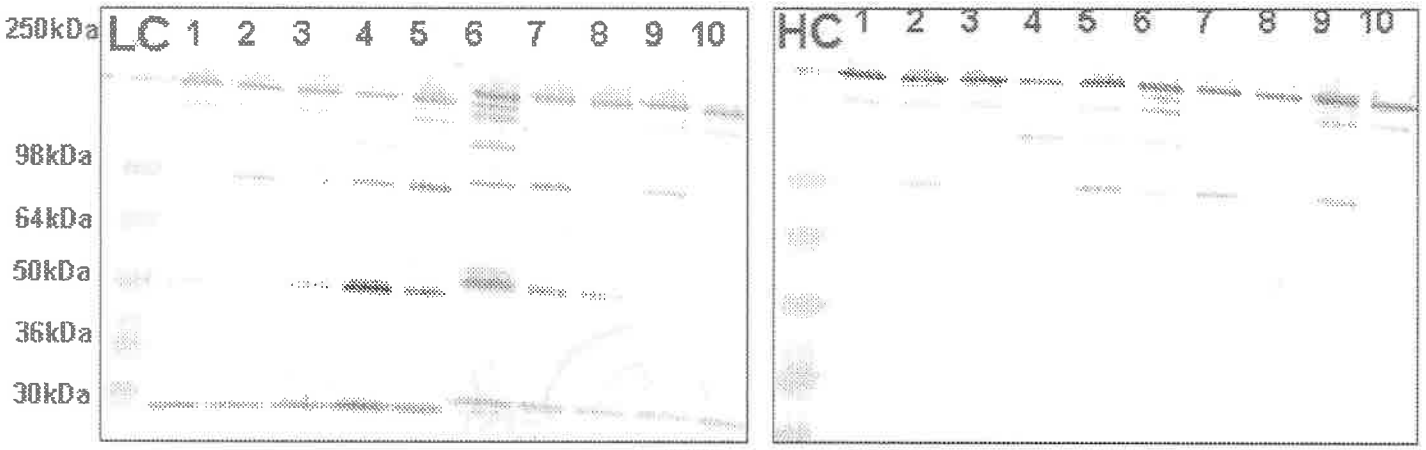
This is averages of 4 different antibodies' expression relative to IgG4 wt

Figure 28 Expression



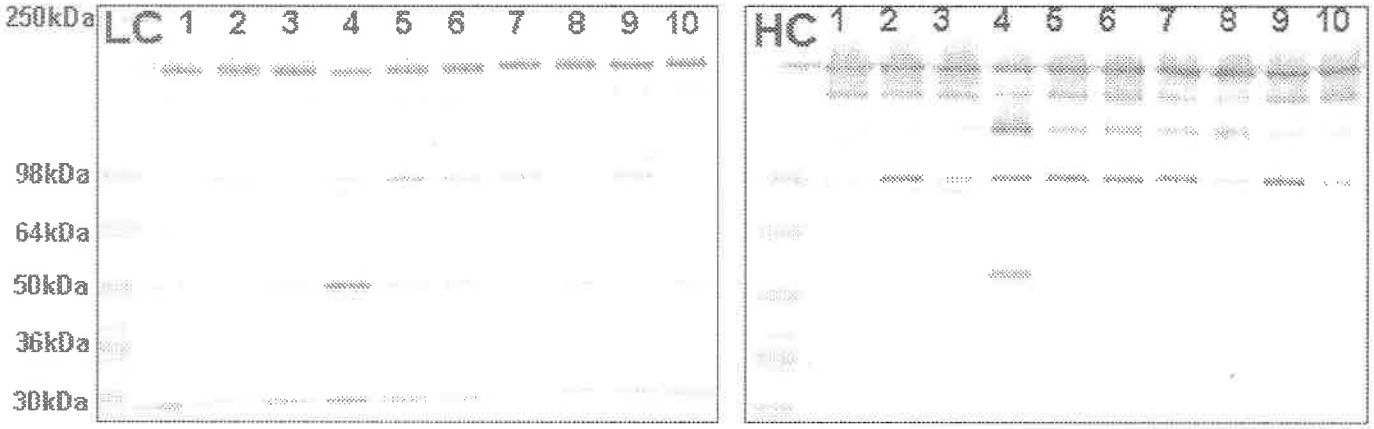
1. IgG1 wt
2. IgG4 wt
3. IgG4 P
4. IgG4 mutant 15
5. IgG4 mutant 28
6. IgG4 mutant 28P
7. IgG4 mutant 44
8. IgG4 mutant 44P
9. IgG4 mutant 48
10. IgG4 mutant 651

Figure 29 Expression



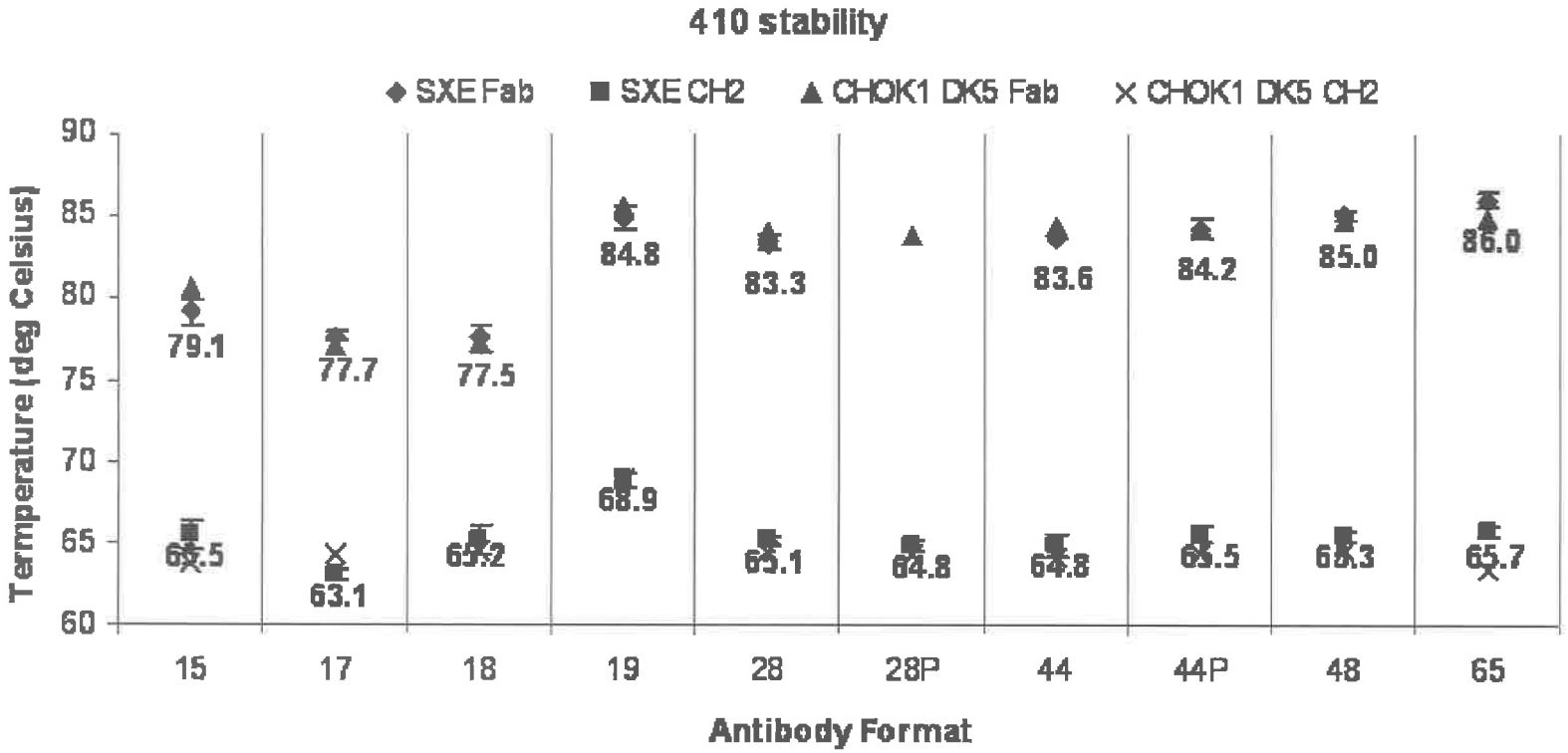
1. IgG1 wt
2. IgG4 wt
3. IgG4 P
4. IgG4 mutant 15
5. IgG4 mutant 28
6. IgG4 mutant 28P
7. IgG4 mutant 44
8. IgG4 mutant 44P
9. IgG4 mutant 65
10. IgG4 mutant 48

Figure 30 Expression



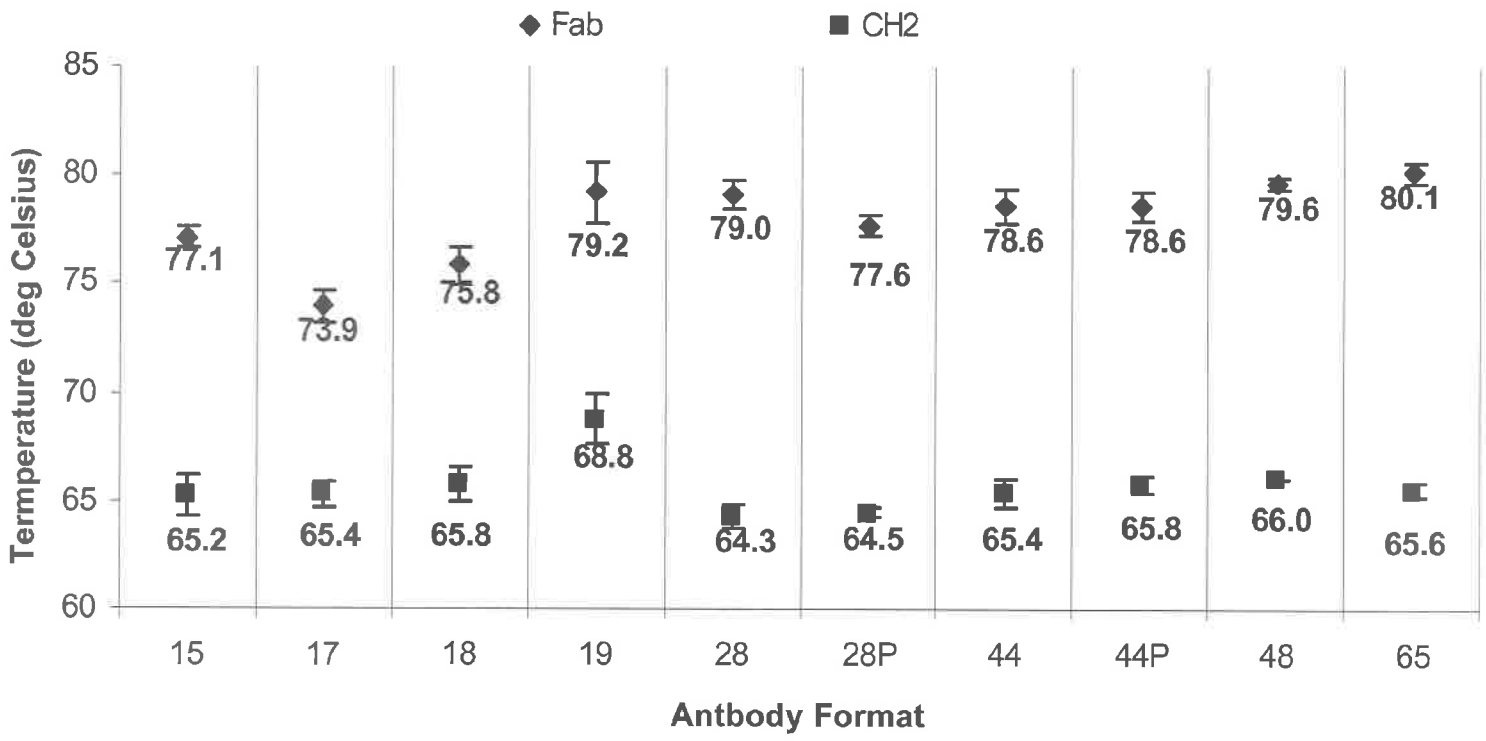
1. IgG1 wt
2. IgG4 wt
3. IgG4 P
4. IgG4 mutant 15
5. IgG4 mutant 28
6. IgG4 mutant 28P
7. IgG4 mutant 44
8. IgG4 mutant 44P
9. IgG4 mutant 65
10. IgG4 mutant 48

Figure 31



Comparison between material expressed from CHOSXE and CHOK1 DK5 shows no difference

Figure 32


◆ Fab
■ CH2
Termperature (deg Celsius)
85
80
75
70
65
60
77.1
73.9
75.8
79.2
79.0
77.6
78.6
78.6
79.6
80.1
65.2
65.4
65.8
68.8
64.3
64.5
65.4
65.8
66.0
65.6
15
17
18
19
28
28P
44
44P
48
65
Antbody Format

G0138-WO01

Figure 33

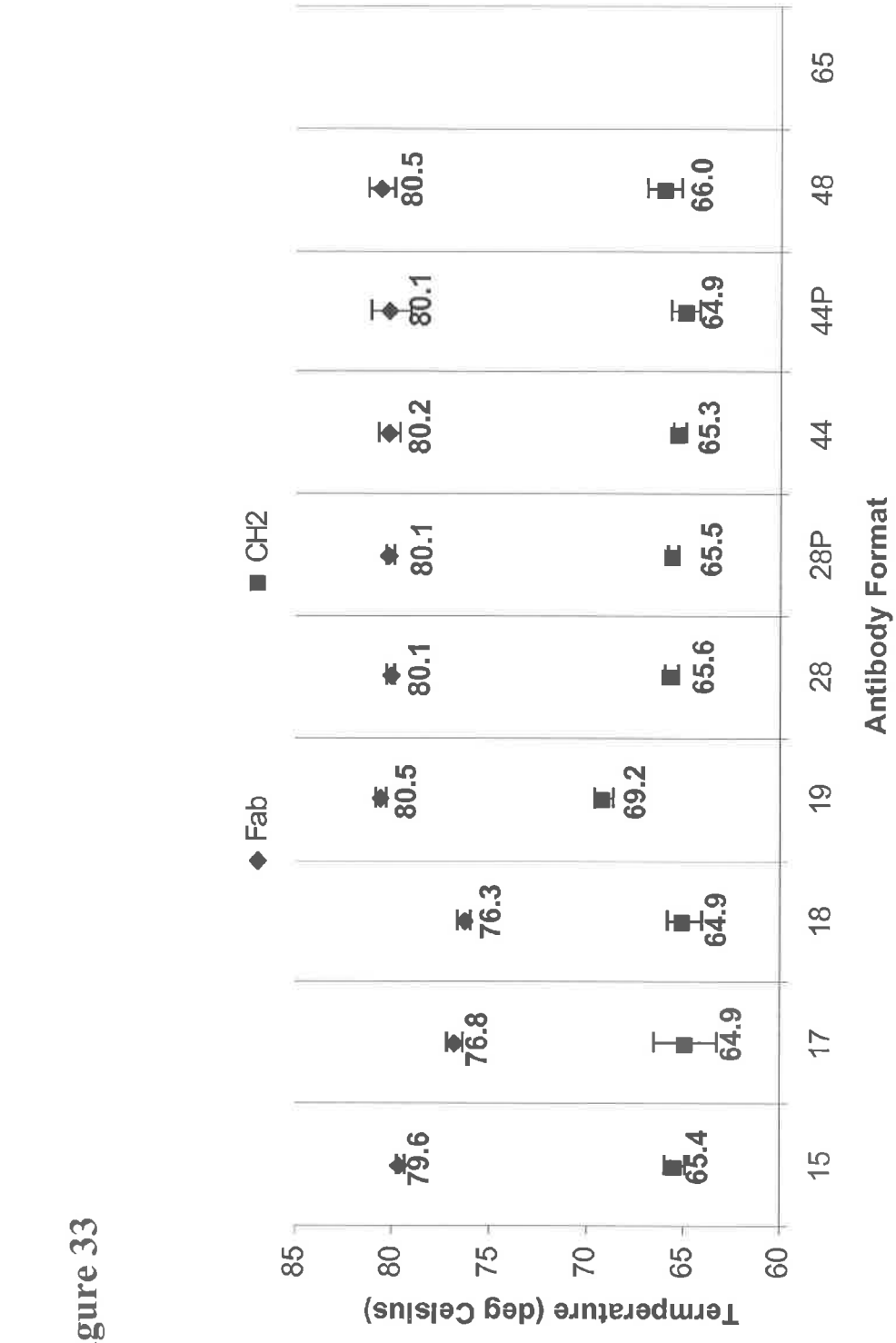
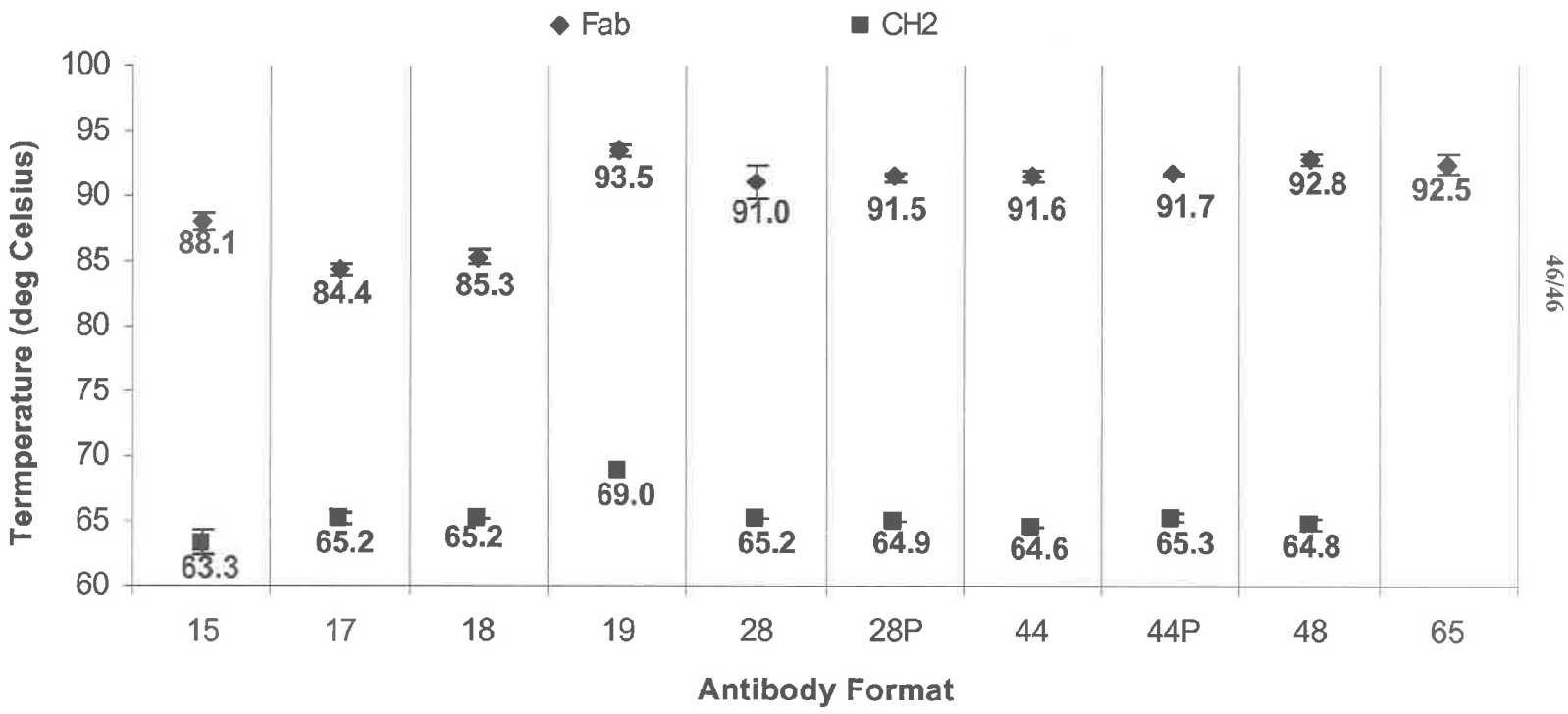


Figure 34


◆ Fab
■ CH2
Termperature (deg Celsius)
100
95
90
85
80
75
70
65
60
88.1
84.4
85.3
93.5
91.0
91.5
91.6
91.7
92.8
92.5
63.3
65.2
65.2
69.0
65.2
64.9
64.6
65.3
64.8
15
17
18
19
28
28P
44
44P
48
65
Antibody Format