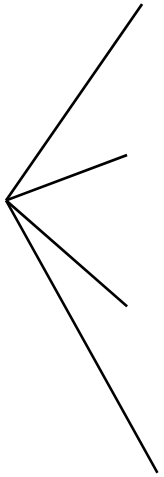
Affinity purification of SCA-2 antisera

Marion Schiffmann

06-21-2007

Purification of SCA-2 antibodies A,B,C,D

Purification of rabbit antisera - immunized with peptides:



A **AKVNGEHKEKDLE**
aa 359-371 (human)

B **EQVRKSTLNPNAKEFN**
aa 904-921

C **LGRGRNSNKG**
aa 241-250

D **ILSNTEHKRGPEV**
aa 867-879

Immunization of rabbits

day	week	
		Conjugation of peptide to KLH
0		pre-immune bleed
1		Primary injection
28	4	Boost 1
42	6	Boost 2
56	8	test bleed and Elisa for Titer
60	8,5	Boost 3
74	10,5	Production bleed
78	11	Boost 4
88	12,5	Production bleed
102	14,5	Final bleed

Preparation of sera / bleeds for affinity purification:

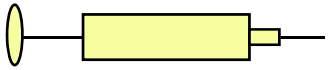
Ammonium sulfate preciptiation: Slowly addition of saturated ammonium sulfate solution to the antiserum. IgG is “salted out“ and can be centrifuged and collected as pellet. IgG concentration is measured with OD280: A280 of 1.35 = 1 mg/ml

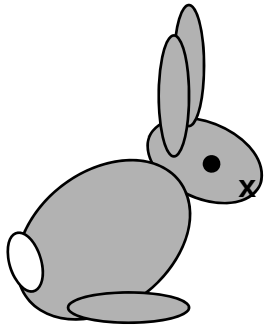
KLH: (Keyhole limpet hemocyanin) M.W.: $4.5 \times 10^{6} - 1.3 \times 10^{7}$ Da

Small molecules like peptides are not usually immunogenic. To generate an immune response it is necessary to conjugate them to a larger carrier protein. Hemocyanin is a high molecular weight respiratory protein found in mollusks and arthropods. Its large size makes it very immunogenic and the large number of lysine residues available for conjugation make it very useful as a carrier.

Coupling antibody via KLH or peptide

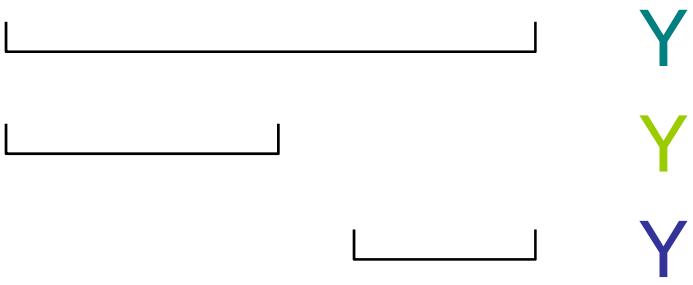
KLH peptide



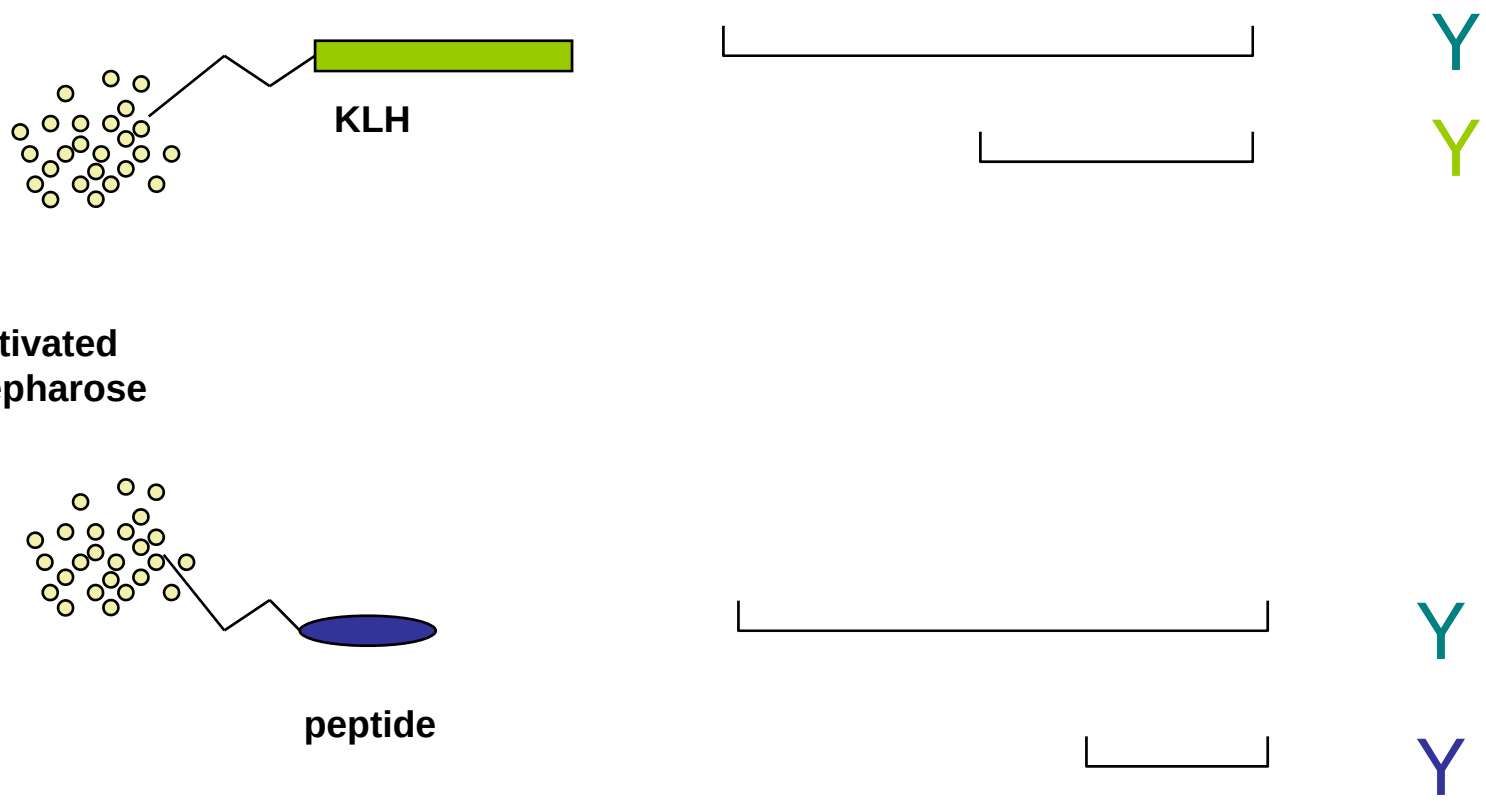


Generation of antibodies against antigen

KLH peptide



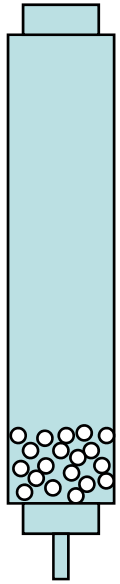
Affinity-purification of antibody via KLH or peptide



Protocol ligand-coupling to column

1) Ligand: KLH

2) Ligand: SCA2-peptide



Activated Sepharose

Sepharose is suspended and swollen to resin in cold 1 mM HCL, than added to column

Wash-steps with cold HCL

Wash-step with 0.1M NaHCO3 pH 6.5

Antigen is suspended in 0.1 M NaHCO3 pH 6.5

OD measurement of ligand-solution

1-2 hours incubation of ligand-solution to sepharose

Collection of ligand

OD measurement of collected ligand-solution

1-2 hours blocking-step with 0.1M TrisHCL, pH 8.0

Wash-step with cold NaHCO3 (unbound ligand)

Wash-step with 0.05 M Tris-HCl ,0.5M NaCl pH 8.0

Wash-step with 0.05 M NaAcetate,0.5M NaCl, pH 4.0

Wash-step with a neutral buffer (0.1M PBS pH 7.0)

Incubation of antibody to ligand at 4°C (ON)

Protocol affinity purification

Collection of antibody-solution

Resin is washed with 0.1M PBS pH 7,5 until absorbance OD280 is lower than 0.01.

2 wash cycles of

a) **20mM Tris HCl, 2M NaCl, pH 7.5**
b) **0.1 M Na borate pH 9.1**
c) **0.1 M PBS pH 4.5**

Elution of antibody fractions with 20mM glycine-HCL ,0.2M NaCl,

pH 2.4

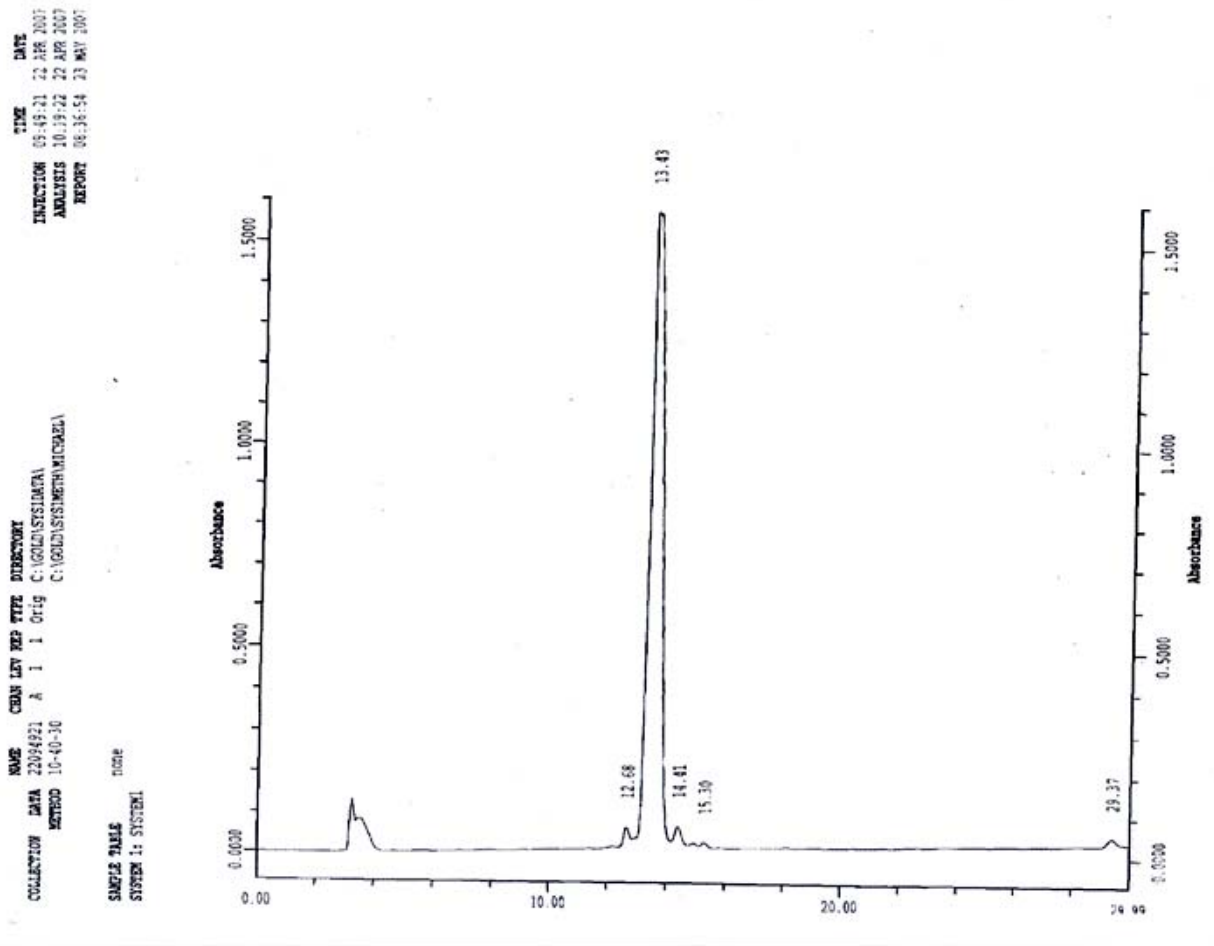
800ul-fractions are collected into 400 ul of 0.1 M Tris.HCl pH 8.8

pH of antibody fractions has to be controled.

Measurement of IgG concentration with OD280: 1 Abs. = 1.35 mg/ml IgG

Trouble shooting

Was purity of peptide not hight enough ? Did peptide degrade ?



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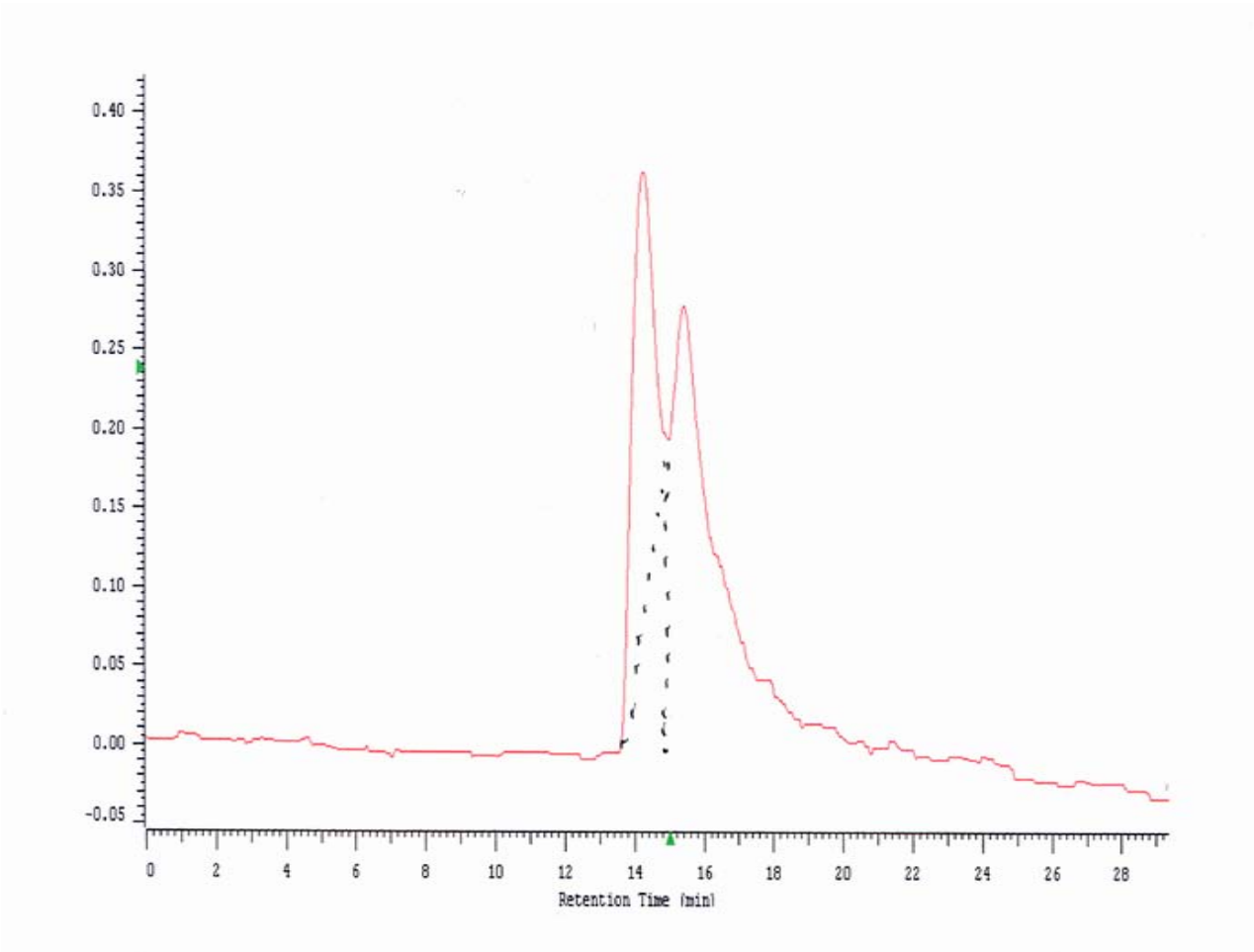
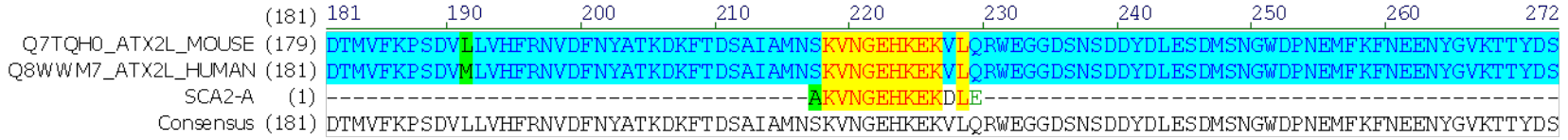
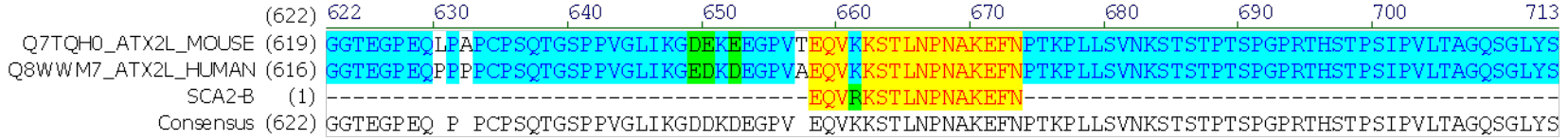


A2RP with SCA2 peptides A-D:

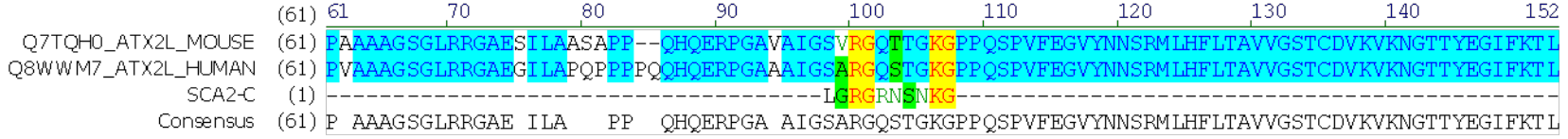
A



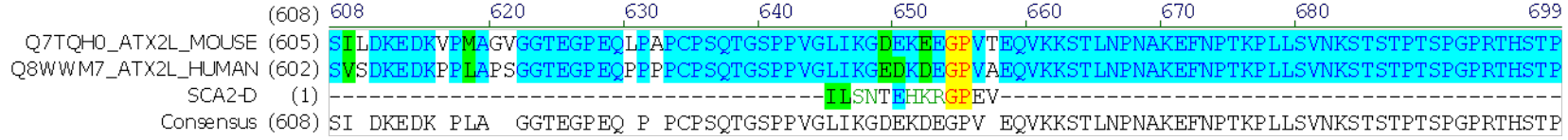
B



C



D



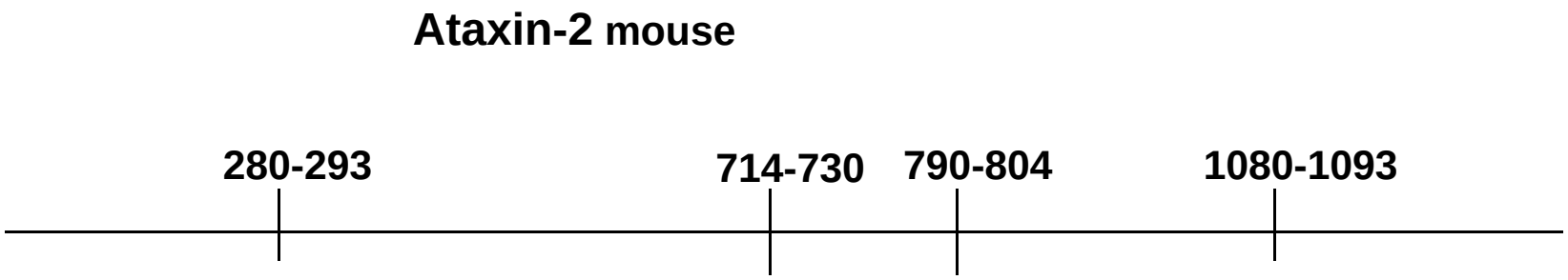
Criteria of new SCA-2 peptides:

No crossreactivity with other proteins especially with A2RP(Blast)

Immunogenicity (Average of Jameson and Wolf method)

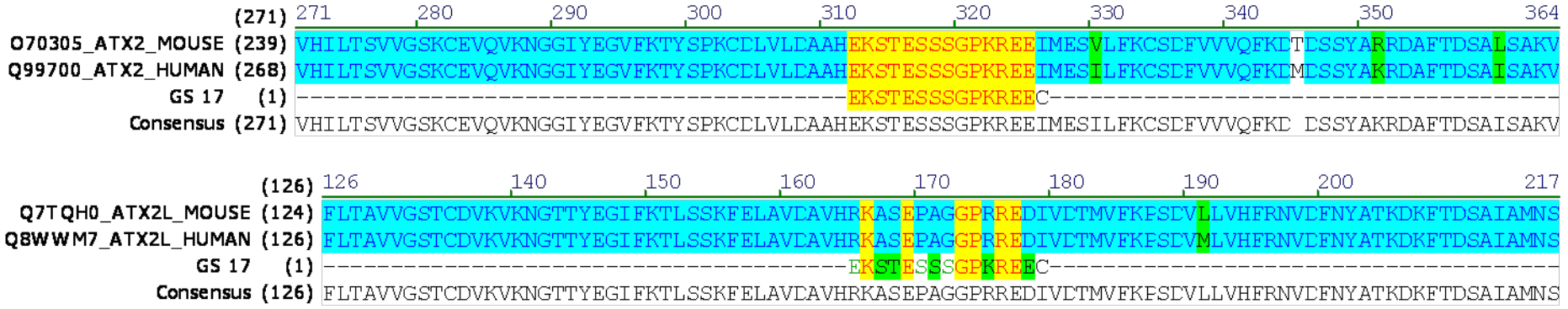
Distribution of peptides on mouse ataxin 2-protein sequence

(N-terminal, C-terminal and `middle` position)



mouse 280-293 / human 309-322

SCA2 280

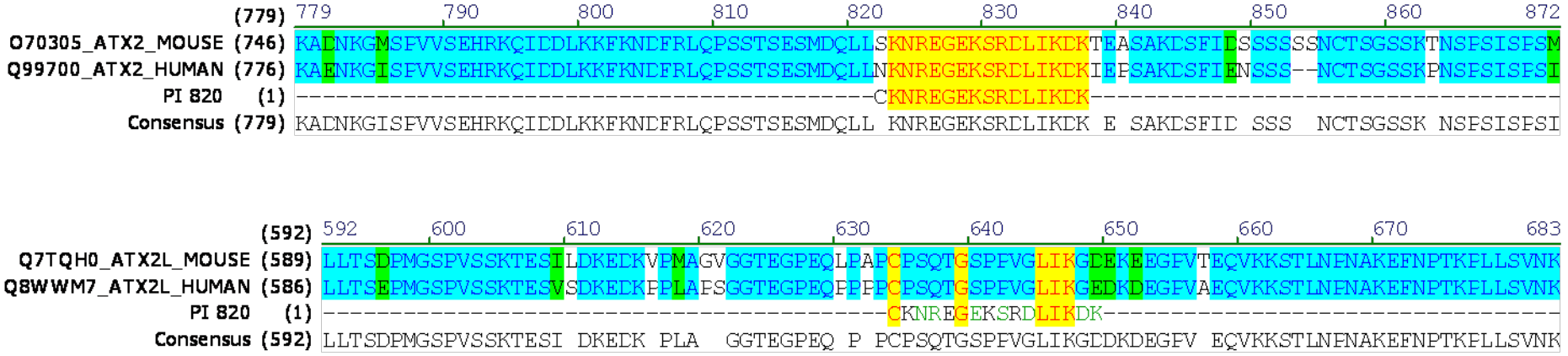


mouse 714-730 /human 748-764

SCA2 714

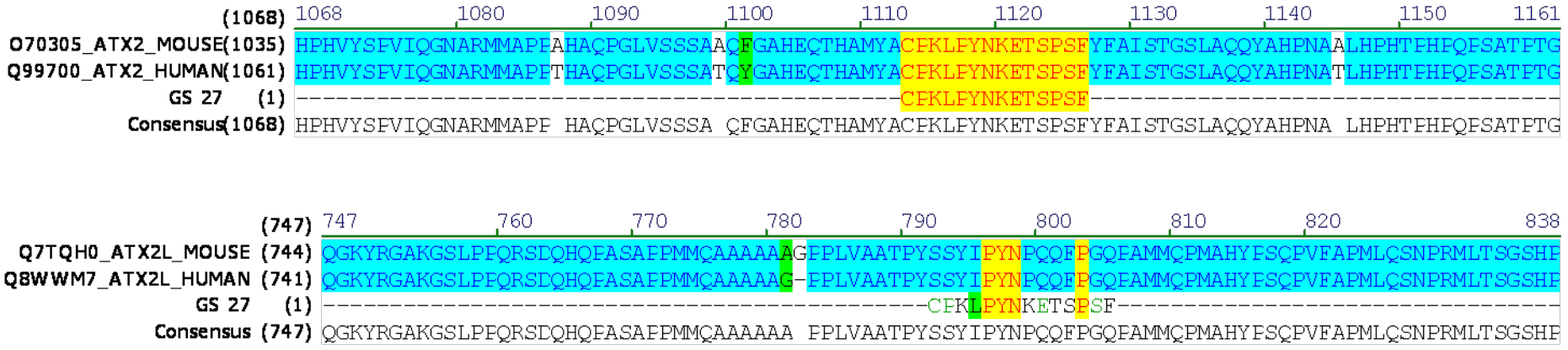
mouse 790-804 / human 820-834

SCA2 790



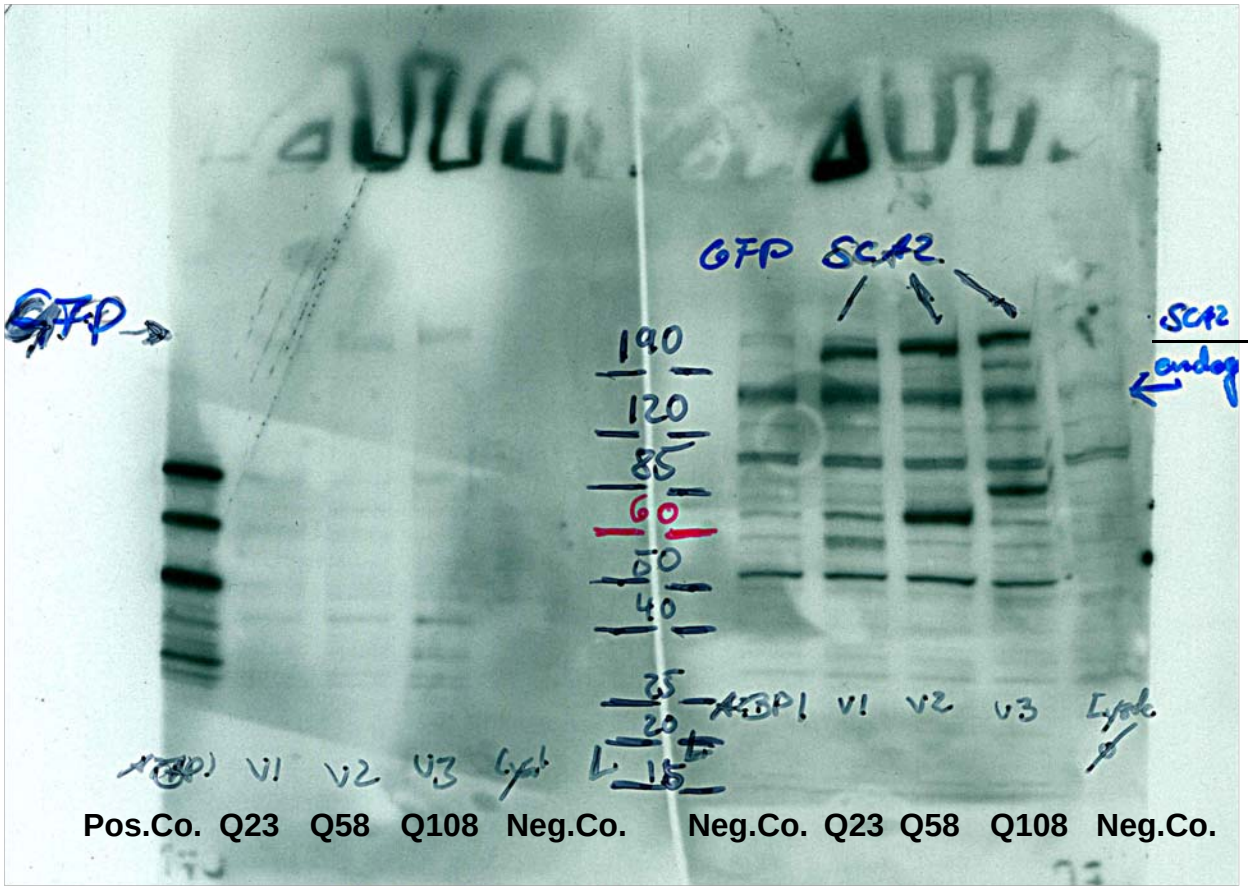
mouse 1080-1093 / human 1106-1119

SCA2 1080



Westernblot with antibodies SCA2-C and - D

SCA2-GFP- Lysates



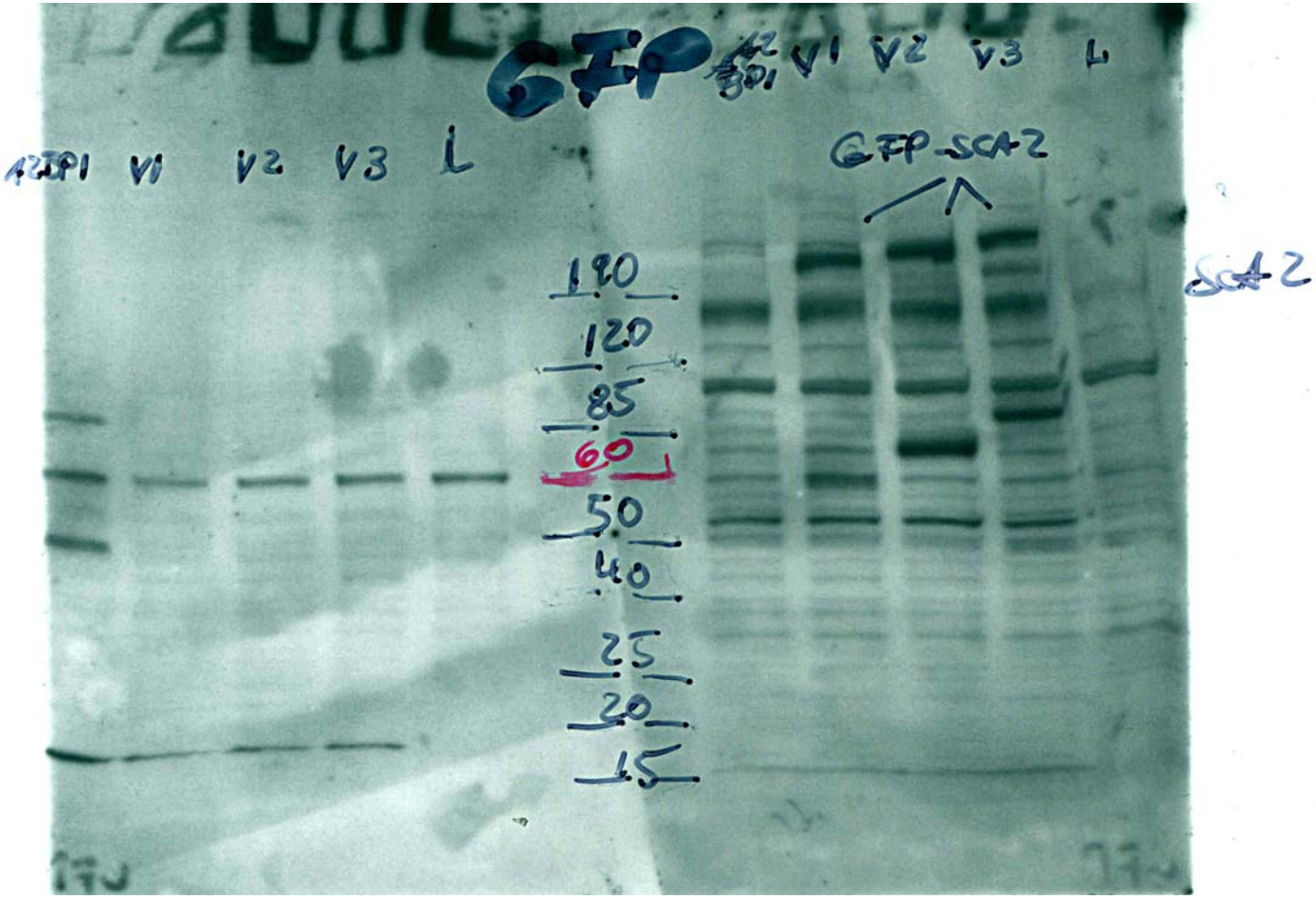
exp.Size 173 kDa

exp.Size 145 kDa

α-GFP

α-SCA2-D

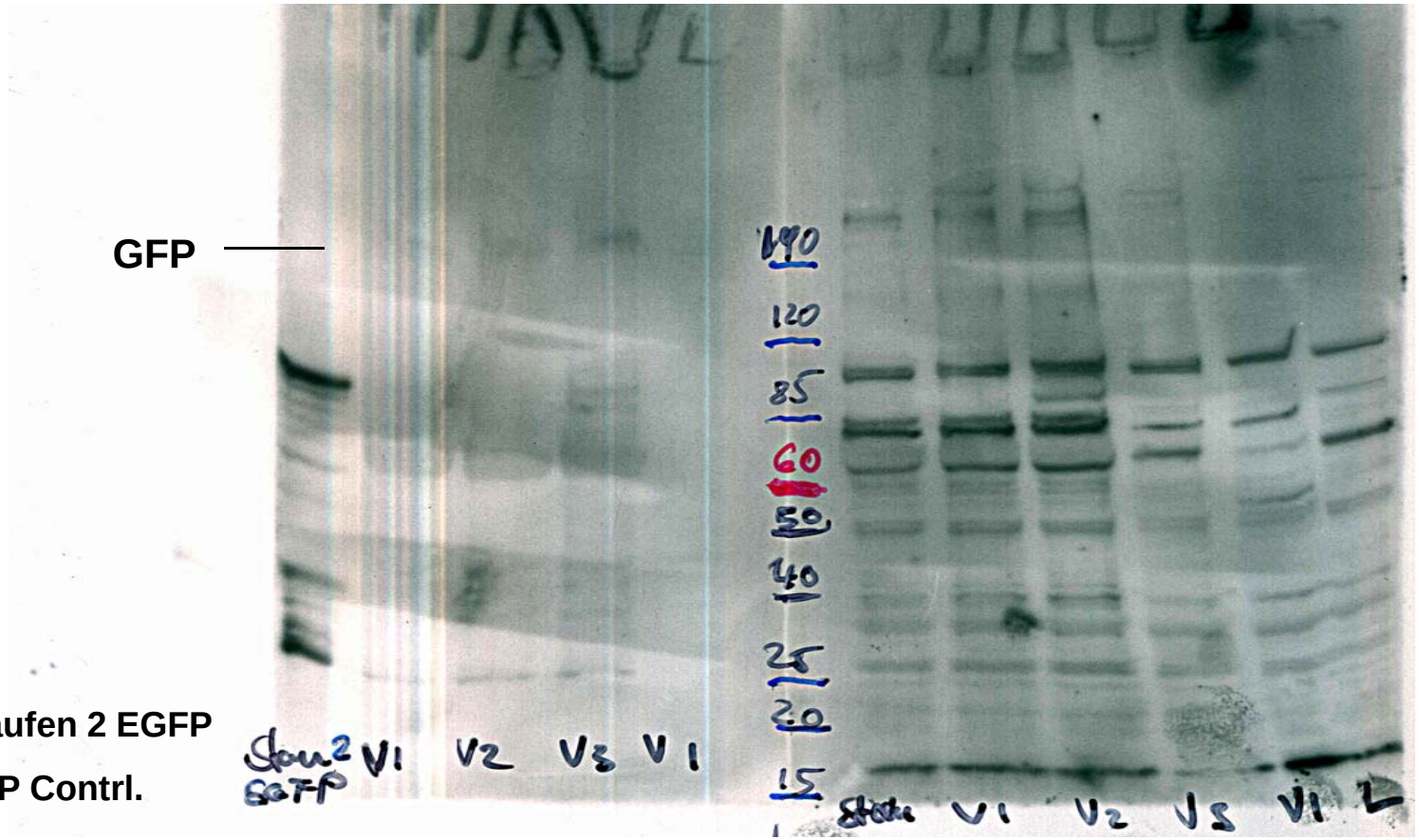
SCA2-GFP- Lysates



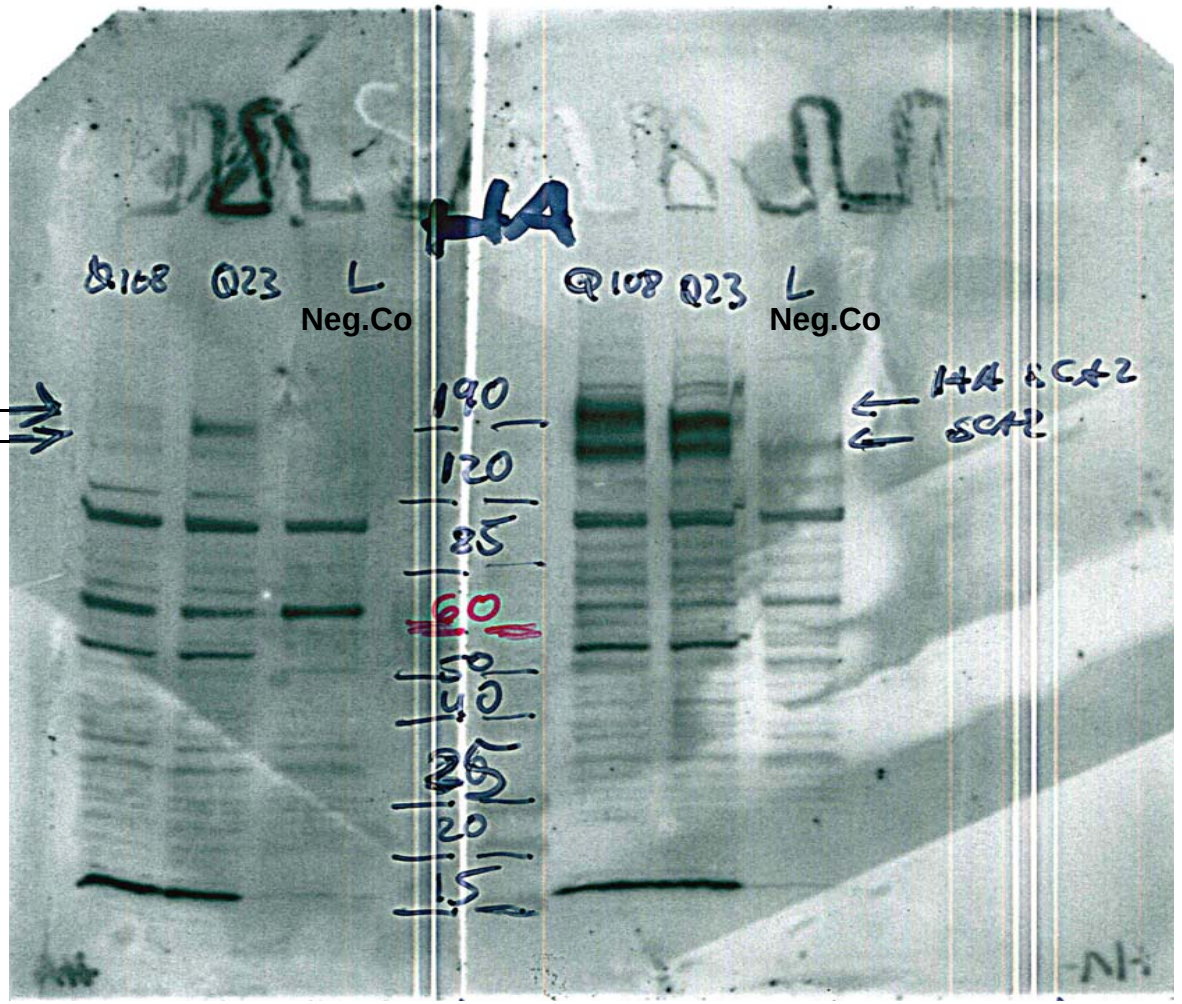
α-SCA2-C

α-SCA2-D

SCA2-GFP-lysates



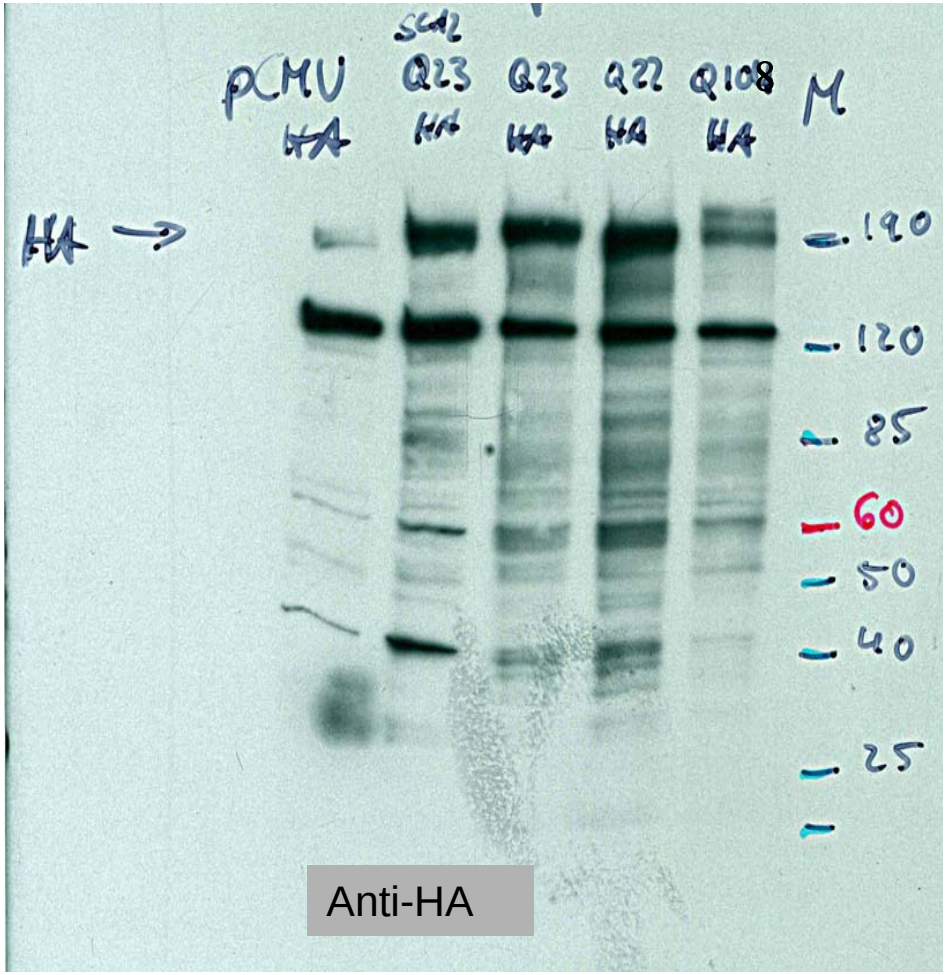
SCA2-HA-lysates

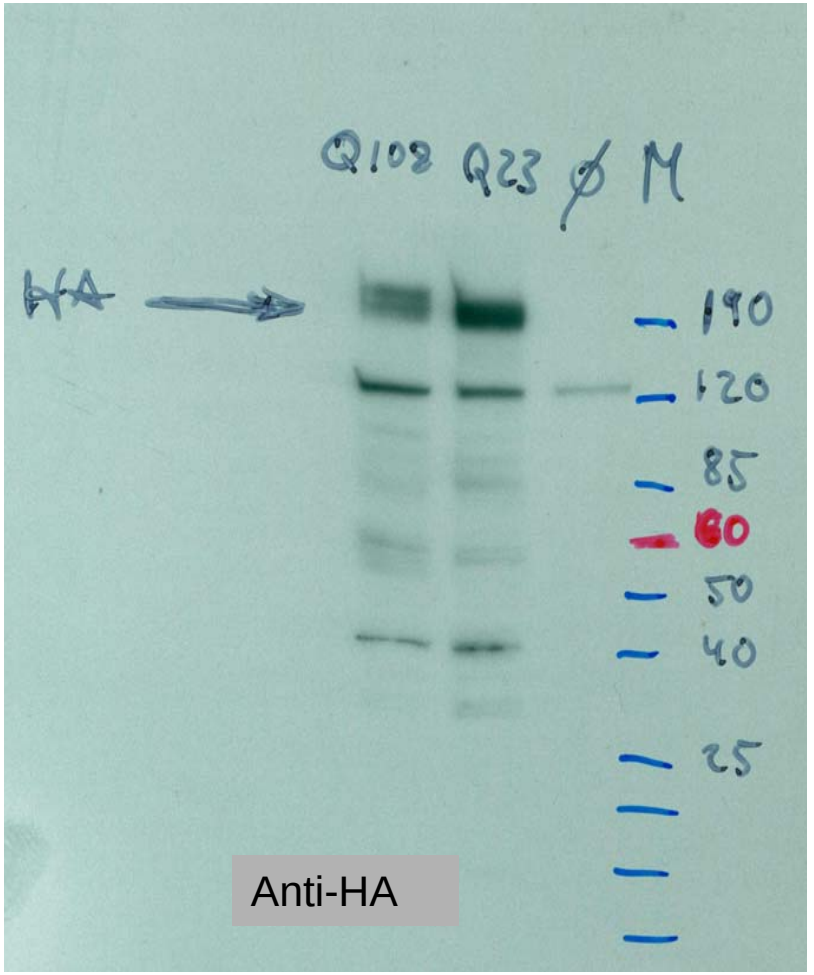


α SCA2-C

α SCA2-D

SCA- HA – lysates





Testing SCA2 C+D Serum on HA-SCA2 Westernblotstripes