

Anti HA IgG2b purification

Column used : Hitrap Protein G HP (old stock from 2010) CV = 1mL

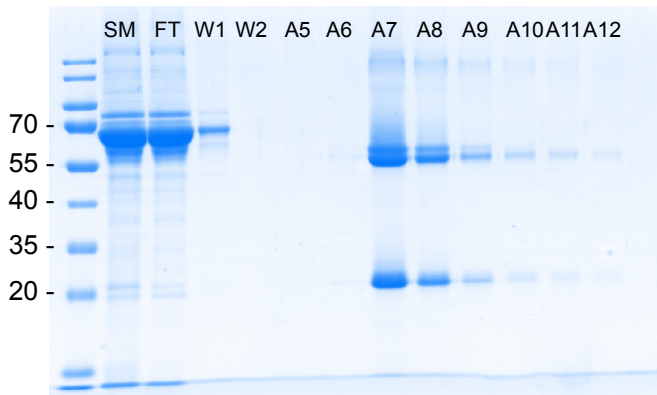
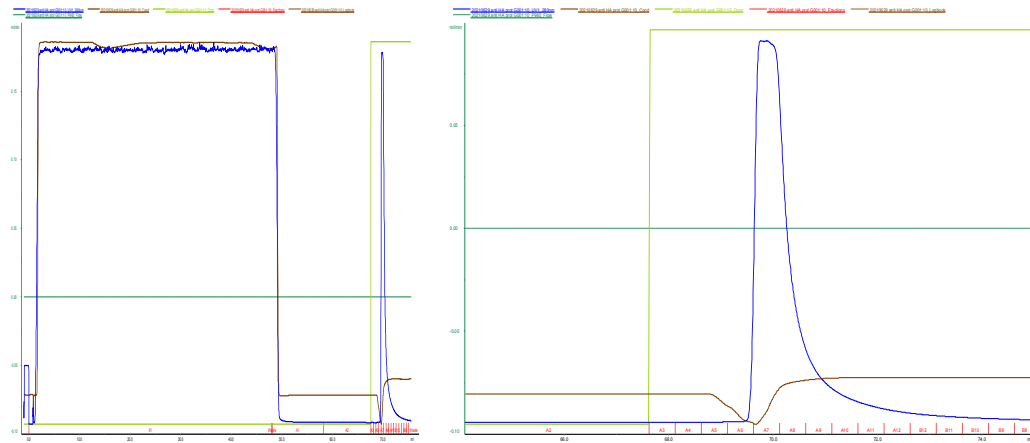
Sample : Anti HA (IgG2b) antibody in RPMI + 10%FCS (49 mL)

Buffer A = 20 mM NaPi pH7.0 (0.45 µm filtered)

Buffer B = 100 mM glycine pH2.7 (0.45 µm filtered)

Program on Akta purifier 2 :

Buffer	volume	Flow	Collection
mQ water	10 CV	1mL/min	Waste
Buffer A	10 CV	1mL/min	Waste
Sample	49 mL	1mL/min	Flow through
Buffer A	20 CV	1 mL/min	10 mL fractions A1 and A2
Buffer B	10 CV	1mL/min	0,5mL fractions with 100µL 1M TRIS pH9 (A3 to A12)
Buffer A	10 CV	1mL/min	Waste
mQ water	10 CV	1mL/min	Waste
20% Ethanol	10 CV	1mL/min	Waste



A7 and A8 are pooled and dialysed against PBS 3 times (250mL – once ON and 2 times 4h)

Antibody concentration : 2 mg/mL

Dilution de l'anticorps à 50% glycerol et stockage à -20°C en aliquots de 200 µL with a final concentration at 1 mg/mL