Fab Production from IgG_{2b}

Day 1 - Purify IgG_{2b} from ascites

a. Wash Protein G column with 20 mM $NaPO_4$, pH 7.0, 0.05% NaN_3, at 0.5 ml/min.

b. Clarify aliquots of ascites by pelleting (5,000 rpm, 5 min, RT).

c. Dilute the clarified ascites 1:10 with 20 mM $NaPO_4$, pH 7.0, 0.05% NaN_3. Filter the diluted ascites through a 0.45 μm syringe filter or Steriflip.

d. Adjust pH of the clarified, filtered ascites to 7.0 with 1 M $NaPO_4$, pH 7.0.

e. Load the ascites onto the pre-equilibrated Protein G column at 0.5 ml/min. Wash the column with 20 mM $NaPO_4$, pH 7.0, 0.05% NaN_3 until the baseline returns to the starting level.

f. Prepare 10, 1.5 ml microcentrifuge tubes by sequentially numbering them and adding 400 μl 1 M $NaPO_4$, pH 7.4 to each.

g. Prepare 2L 100 mM NaOAc pH 5.5, 1 mM EDTA for dialysis, by combining:
   1700 ml 0.1M NaOAc
   4 ml 0.5M EDTA, pH 8.0
   ~300 ml 0.1M acetic acid (use to pH)

h. Elute the bound antibody by washing the Protein G column with 0.1 M citric acid, pH 3.0, 0.05% NaN_3. Collect 1 ml (final volume) fractions into the above prepared tubes. Invert to mix and check the pH with strip indicators to assure antibody is neutralized.

i. Pool the antibody-containing fractions and dialyze against 100 mM NaOAc, pH 5.5, 1 mM EDTA, O/N, 4°C.

j. Wash the Protein G column with 5 ml of 0.1M citric acid, pH 3.0, 0.05% NaN_3, followed by 5 column volumes of 20 mM $NaPO_4$, pH 7.0, 0.05% NaN_3. Store column at 4°C.

Fab Production (cont.)

Day 2 – Digest IgG_{2b} and purify Fab

a. Harvest IgG_{2b} from dialysis and measure A_{280} (blank with 100 mM NaOAc, pH 5.5 1 mM EDTA, ε_{IgG2b} (mg/ml) = 1.38)

b. Concentrate the IgG_{2b} using Amicon spin cell concentrator and Millipore YM10 membrane (pre-soaked in Millipore water, 30 min.). Concentrate the IgG_{2b} to ~2 mg/ml.

c. Wash the Superdex 75 column with 100 mM NaOAc, pH 5.5, 150 mM NaCl.

d. Harvest the concentrated IgG_{2b} and take post concentration A_{280} reading (1:20 with 100 mM NaOAc, pH 5.5 1 mM EDTA, ε_{IgG2b} (mg/ml) = 1.38)

e. Make 1 M cysteine (Sigma, C-7880)
   105.4 mg cysteine
   600 µl 100 mM NaOAc, pH 5.5, 1 mM EDTA

f. Dilute papain (Sigma, P-3125) in 100 mM NaOAc, pH 5.5, 1 mM EDTA
   1 µl 28 µg/µl stock papain + 9 µl diluent = 2.8 µg/µl
   2 µl 2.8 µg/µl + 198 µl diluent = 0.028 µg/µl

g. For IgG_{2b}, digests work best when done in 1 mg antibody per 1 ml reaction volume. Also, in my experience optimized yields are obtained when digesting and further purifying Fab from **no more than 5 mg** of IgG_{2b}. Prior to starting the digest, combine all components ***except*** the antibody in the 1.5 ml microcentrifuge tubes and pre-incubate at RT for 15 min to activate the papain. Reaction conditions:
   0.1 M NaOAc, pH 5.5, 1 mM EDTA to bring final reaction volume to 1 ml (don't forget to account for the volume of antibody to be added, see below)
   1 mM final EDTA
   50 mM final cysteine
   333 ng papain (1:3000 ratio of papain:MW1)

h. After the 15 min activation incubation, add 1 mg antibody to each tube and incubate at 35C for 30 min, on rotator.

i. Make 0.5 M iodoacetamide (Sigma, I-6125)
   92.5 mg iodoacetamide
   1 ml 100 mM NaOAc, pH 5.5, 1 mM EDTA
   Wrap in foil (light sensitive) and store at RT until needed.

Fab Production (cont.)

j. Begin washing the Protein A column with 0.1 M $NaPO_4$, pH 7.4, 0.05% NaN_3, at 0.5 ml/min (sensitivity set to 0.5 if purifying from 5 mg digest).

k. Terminate the digests by adding iodoacetamide to 70 mM and incubating the tubes wrapped in Al foil on the nutator (30 min, RT).

l. Combine all terminated digests into a 15 ml Falcon tube and neutralize by adding 1M Tris-HCl, pH 8.8 until pH is ~7.0.

m. Combine 15 μl of the digest with 15 μl reducing sample buffer for SDS-PAGE analysis. Load 20 μl.

n. Immediately add the terminated, neutralized digest to the pre-equilibrated Protein A column at 0.5 ml/min.

o. Collect the Fab-containing flow through.

p. Combine 15 μl of the Fab with 15 μl reducing sample buffer for SDS-PAGE analysis. Load 20 μl.

q. To the Fab-containing flow through, add NaCl to 200 mM and concentrate using an Ultrafree-4 spin concentrator (pre-rinse with 4 ml Millipore water.), to 500 μl.

r. Filter the concentrated Fab through a pre-rinsed (with water) 0.2 μm spin column.

s. Apply the Fab to the pre-equilibrated Superdex 75 column and elute using 0.1 M NaOAc, pH 5.5, 150 mM NaCl.

t. Take A_{280} (ε_{Fab} (mg/ml) = 1.6) readings of Fab-containing fractions (blank with 0.1 M NaOAc, pH 5.5, 150 mM NaCl.).

u. Elute the uncut IgG_{2b}, Fc and Fab/c fragments from the Protein A column with 0.1 M citric acid, pH 3.0, 0.05% NaN_3, and immediately neutralize with 1M Tris-HCl, pH 8.8. Store at 4C.

v. Combine 15 μl of the uncut IgG_{2b}, Fc and Fab/c fraction with 15 μl reducing sample buffer for SDS-PAGE analysis. Load 20 μl.

w. Wash the Protein A column with 5 ml 0.1 M citric acid, pH 3.0, 0.05% NaN_3, followed by 15 ml 0.1 M $NaPO_4$, pH 7.4, 0.0% NaN_3. Store column at 4C.