

## Fab Production from IgG<sub>2b</sub>

### Day 1 - Purify IgG<sub>2b</sub> from ascites

- a. Wash Protein G column with 20 mM NaPO<sub>4</sub>, pH 7.0, 0.05% NaN<sub>3</sub>, 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<sub>4</sub>, pH 7.0, 0.05% NaN<sub>3</sub>. Filter the diluted ascites through a 0.45  $\mu$ m syringe filter or Steriflip.
- d. Adjust pH of the clarified, filtered ascites to 7.0 with 1 M NaPO<sub>4</sub>, 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<sub>4</sub>, pH 7.0, 0.05% NaN<sub>3</sub> until the baseline returns to the starting level.
- f. Prepare 10, 1.5 ml microcentrifuge tubes by sequentially numbering them and adding 400  $\mu$ l 1 M NaPO<sub>4</sub>, 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<sub>3</sub>. 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<sub>3</sub>, followed by 5 column volumes of 20 mM NaPO<sub>4</sub>, pH 7.0, 0.05% NaN<sub>3</sub>. Store column at 4°C.

### Fab Production (cont.)

#### Day 2 – Digest IgG<sub>2b</sub> and purify Fab

- a. Harvest IgG<sub>2b</sub> from dialysis and measure A<sub>280</sub> (blank with 100 mM NaOAc, pH 5.5 1 mM EDTA, [IgG<sub>2b</sub>] (mg/ml) = 1.38)
- b. Concentrate the IgG<sub>2b</sub> using Amicon spin cell concentrator and Millipore YM10 membrane (pre-soaked in Millipore water, 30 min.). Concentrate the IgG<sub>2b</sub> 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<sub>2b</sub> and take post concentration A<sub>280</sub> reading (1:20 with 100 mM NaOAc, pH 5.5 1 mM EDTA, [IgG<sub>2b</sub>] (mg/ml) = 1.38)
- e. Make 1 M cysteine (Sigma, C-7880)
  - 105.4 mg cysteine
  - 600  $\mu$ l 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  $\mu$ l 28  $\mu$ g/ $\mu$ l stock papain + 9  $\mu$ l diluent = 2.8  $\mu$ g/ $\mu$ l
  - 2  $\mu$ l 2.8  $\mu$ g/ $\mu$ l + 198  $\mu$ l diluent = 0.028  $\mu$ g/ $\mu$ l
- g. For IgG<sub>2b</sub>, 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<sub>2b</sub>. 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 mM 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<sub>4</sub>, pH 7.4, 0.05% NaN<sub>3</sub>, 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  $\mu$ l of the digest with 15  $\mu$ l reducing sample buffer for SDS-PAGE analysis. Load 20  $\mu$ l.
- 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  $\mu$ l of the Fab with 15  $\mu$ l reducing sample buffer for SDS-PAGE analysis. Load 20  $\mu$ l.
- 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  $\mu$ l.
- r. Filter the concentrated Fab through a pre-rinsed (with water) 0.2  $\mu$ m 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<sub>280</sub> ( $\mu$ 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<sub>2b</sub>, Fc and Fab/c fragments from the Protein A column with 0.1 M citric acid, pH 3.0, 0.05% NaN<sub>3</sub>, and immediately neutralize with 1M Tris-HCl, pH 8.8. Store at 4C.
- v. Combine 15  $\mu$ l of the uncut IgG<sub>2b</sub>, Fc and Fab/c fraction with 15  $\mu$ l reducing sample buffer for SDS-PAGE analysis. Load 20  $\mu$ l.
- w. Wash the Protein A column with 5 ml 0.1 M citric acid, pH 3.0, 0.05% NaN<sub>3</sub>, followed by 15 ml 0.1 M NaPO<sub>4</sub>, pH 7.4, 0.0% NaN<sub>3</sub>. Store column at 4C.