Technical Note

Biotinylate antibody from stock which contains carrier protein (Recommended procedure for use with FortéBio streptavidin biosensors)

Materials required:

- Antibody to be biotinylated (>200µg antibody in the presence of carrier protein; lyophilyzed or in solution)
- NAb Protein A or G Purification Kit (Pierce cat # 45200 or 45201)
- EZ-Link NHS-LC-LC-Biotin (Pierce cat # 21343)
- Dimethylformamide (DMF)
- 1x PBS
- Slide-A-Lyzer, 10000 MWCO (Pierce cat# 66383)
- De-salt Dextran Columns (Pierce cat #43230)- optional

Time required:

Antibody isolation: 1.5 hour hands-on; 1 day + overnight total time
Biotinylation: 1 hour hands-on; 1 day + overnight total time

Protocol:

1. **Antibody isolation**
   - 1.1. **NOTE:** For mIgG2a, mIgG2b and IgG3 use Protein A Spin Purification Kit. For mIgG1 use the Protein G Spin Purification Kit.
   - 1.2. Mix resin gently to make slurry; use cut pipet tip to transfer 200 µL of the slurry to spin cup column placed in a collection tube. Tip should be cut to provide a wider opening for pipeting the resin.
   - 1.3. Add 300 µL of the appropriate Binding buffer from Purification Kit to resin. Mix gently. Centrifuge 1 min at ~5000 g. Discard solution from collection tube.
   - 1.4. Add 400 µL of Binding buffer to resin. Mix gently. Centrifuge 1 min at ~5000 g. Discard solution from collection tube.
   - 1.5. Repeat wash 1 more time.
   - 1.6. If the antibody is lyophilized, add 300 µL of the binding buffer.
   - 1.7. If the antibody is in solution, add an equal volume of binding buffer (eg 250 µL of antibody plus 250 µL binding buffer)
   - 1.8. Add antibody solution to resin. Cap cup and incubate 30 minute with gentle shaking.
   - 1.9. Uncap cup and centrifuge in collection tube 1 minute.

1.10. Move cup to a new collection tube. Add 400µL binding buffer and mix briefly. Centrifuge 1 minute.

1.11. Repeat previous wash step 2 more times.

1.12. Transfer to a new collection tube. Add 400 µL of Elution Buffer. Cap cup and mix gently for 5 minutes.

1.13. Uncap and centrifuge 1 minute. Transfer cup to a new collection tube.

1.14. Repeat steps 2 more times for a total of 3 elutions. Elutions should be neutralized with 40µL of 1M sodium phosphate (pH 8).

1.15. A denaturing, non-reducing gel should be run of the washes and elutions to determine efficiency of isolation and location of the antibody (**Figure 1**).

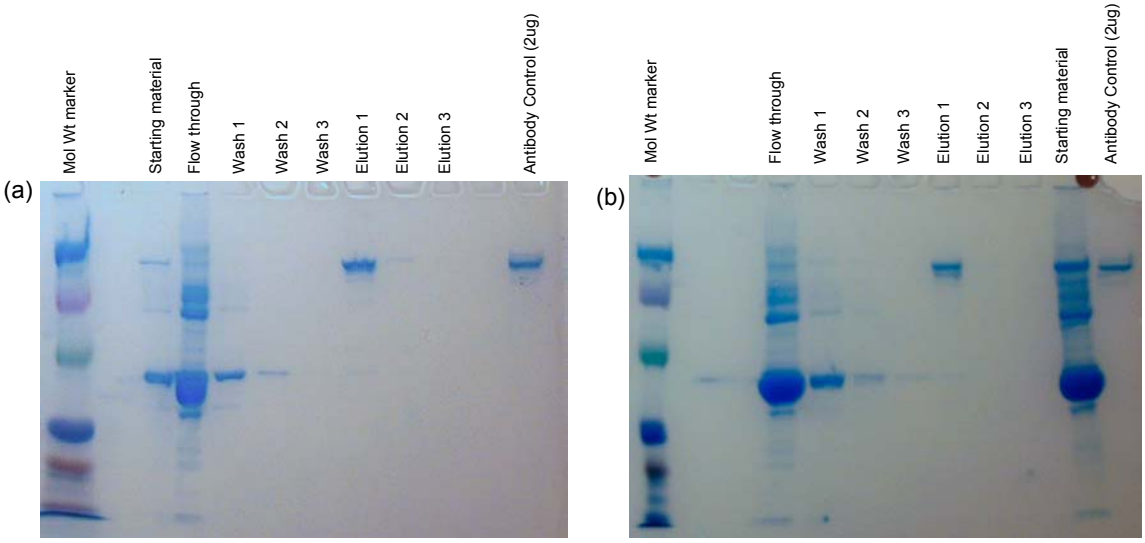

Figure 1: Denaturing, non-reducing gels of fractions from antibody isolation. **(a)** using Protein G spin purification with mIgG1. **(b)** using Protein A spin purification with mIgG2a

2. **Biotinylation**

2.1. Using a MWCO 10000 slidalyzer, dialyze the elution fractions containing the antibody against PBS at 4C (elution 1 in both examples shown in **Figure 1**).

2.2. **NOTE:** Since the elution buffer contains primary amines, dialysis must be extensive. Six changes at 1:1000 (> 3 hours between each change) are recommended. Use of a desalting column is not recommended for this step as the procedure is not efficient enough to remove all the reactive amines from the buffer.

2.3. Recover the dialyzed sample from the slidalyzer and transfer to a new eppendorf tube.

2.4. Prepare a 10mM Biotin reagent solution: add 2.0 mg NHS-LC-LC-Biotin reagent in 350 µL of DMF. Mix to dissolve.

2.5. Calculate the volume of 10 mM biotin reagent needed based on the mass of antibody started with prior to antibody isolation. For most antibodies, a

5:1 molar coupling ratio (moles NHS-LCLC-biotin: moles antibody at start of procedure).

$$\frac{\text{mg protein}}{\text{MW (mg/mmol)}} \times \frac{5\ \text{mmol biotin}}{1\ \text{mmol protein}} \times \frac{1000\text{mL}}{10\ \text{mmol biotin}} \times \frac{1000\ \mu\text{L}}{1\ \text{mL}} = \textbf{µL of 10mM solution of biotin reagent}$$

2.6. To each sample, add the appropriate **volume (µL)** of NHS-LC-LC-Biotin reagent as calculated. Mix immediately

2.7. Incubate 30 minutes at Room Temperature.

2.8. Stop the reaction by removing the excess biotin reagent by either dialysis (recommended) or desalting column. Dialysis should involve 4 changes of PBS at 1:1000.

2.9. **NOTE:** Take care to remove all free biotin in order to most efficiently bind the biotinylated protein to the streptavidin sensor surface.

2.10. Biotinylated antibodies in PBS can be stored at 4C. See **Figure 2** for example immobilization of biotinylated antibody onto streptavidin SBC biosensor.

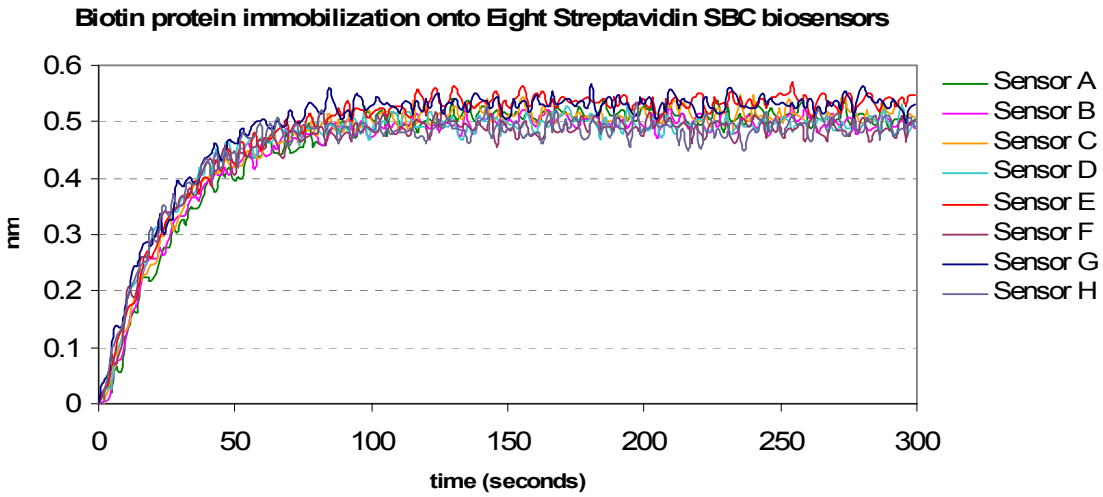

Figure 2: Example data for the parallel immobilization of a biotinylated protein onto 8 streptavidin SBC biosensors.