Biotinylating Antibody in Stocks Containing Carrier Protein

Recommended Procedure for Use with ForteBio Streptavidin Biosensors

OVERVIEW

The interaction between streptavidin and biotin is widely used as a system for the rapid, stable, and irreversible non-covalent binding of proteins to ligands. The Streptavidin biosensors (Standard Binding Capacity (SBC), High Binding, and Super Streptavidin) for the Octet Q/QK and Octet RED Systems have been developed for kinetic analysis of protein-protein or protein-small molecule interactions between a biotinylated ligand and analytes in a crude sample matrix. The first protein immobilized onto the Streptavidin biosensors requires biotinylation prior to assaying on the Octet Systems.

Often suppliers of antibodies provide their reagents in the presence of carrier protein(s) to increase the long-term stability of their product. In order to efficiently biotinylate the antibody, it must be isolated from the carrier protein. This can be done conveniently using commercially available isolation kits. The resulting purified antibody can then be biotinylated and immobilized onto the Streptavidin biosensors for use in kinetic and custom quantitation applications.

OBJECTIVE

This technical note provides a detailed protocol for isolating antibodies from carrier protein and subsequently biotinylating the purified antibodies.

MATERIALS REQUIRED

- Antibody to be biotinylated (>200 µg antibody in the presence of carrier protein; lyophilyzed or in solution)
- NAb Protein A or G Purification Kit (Pierce part no. 45200 or 45201)
- EZ-Link NHS-LC-LC-Biotin (Pierce part no. 21343)
- Dimethylformamide (DMF)
- 1x PBS
- Slide-A-Lyzer, 10000 MWCO (Pierce part no. 66383)
- (Optional) De-salt Dextran Columns (Pierce part no. 43230)

WORKFLOW

A. Isolate antibody from carrier protein using spin purification kit

B. Biotinylate purified antibody

A. ANTIBODY ISOLATION

NOTE: For mIgG2a, mIgG2b or IgG3, use Protein A Spin Purification Kit. For mIgG1, use the Protein G Spin Purification Kit.

1. Cut a pipet tip to provide a wider opening for pipetting the resin. Mix resin gently to make a slurry; use cut pipet tip to transfer 200 µL of the slurry to a spin cup column placed in a collection tube.
2. Add 300 µL of the appropriate binding buffer from the Purification Kit to the resin. Mix gently. Centrifuge 1 minute at ~5000 g. Discard the solution from the collection tube.
3. Add 400 µL of binding buffer to resin. Mix gently. Centrifuge 1 minute at ~5000 g. Discard the solution from the collection tube.
4. Repeat wash one more time.
5. *If the antibody is lyophilized*, add 300 µL of binding buffer to it.

If the antibody is in solution, add an equal volume of binding buffer to it (e.g., 250 µL of antibody plus 250 µL binding buffer).

6. Add the antibody solution to the resin. Cap the cup and incubate 30 minutes with gentle shaking.
7. Uncap the cup and centrifuge it in a collection tube for 1 minute.
8. Move the cup to a new collection tube. Add 400 µL binding buffer and mix briefly. Centrifuge 1 minute.
9. Repeat the previous wash step 2 more times for a total of three washes.
10. Transfer the cup to a new collection tube. Add 400 µL elution buffer. Cap the cup and mix gently for 5 minutes.
11. Uncap the cup and centrifuge 1 minute. Transfer the cup to a new collection tube.
12. Repeat steps 10 and 11 two more times for a total of 3 elutions. Elutions should be neutralized with 40 µL 1M sodium phosphate (pH 8).
14. 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).

B. BIOTINYLATION

1. Using a MWCO 10000 Slide-A-Lyzer, dialyze the elution fractions containing the antibody against PBS at 4 °C (Elution 1 in both examples shown in Figure 1).

   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. Recover the dialyzed sample from the Slide-A-Lyzer and transfer to a new Eppendorf tube.
3. Prepare a 10 mM Biotin reagent solution: add 2.0 mg NHS-LC-LC-Biotin reagent in 350 µL of DMF. Mix to dissolve.

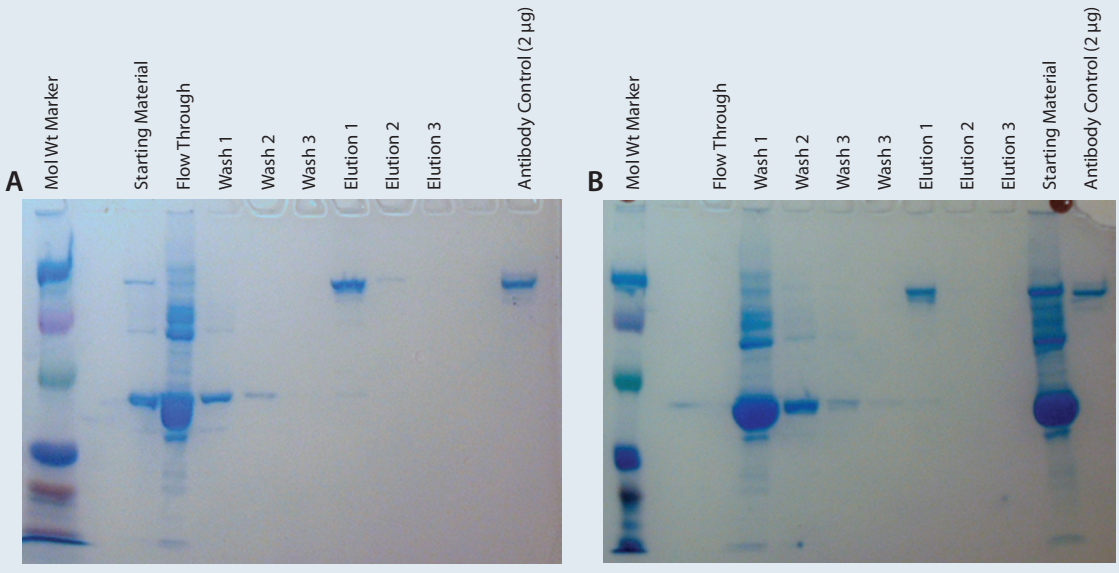

Figure 1: Denaturing, non-reducing gels of fractions from antibody isolation. (A) Fractionated using Protein G spin purification with mIgG1. (B) Fractionated using Protein A spin purification with mIgG2a.

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

Figure 2: Equation for calculating the required volume of 10 mM biotin reagent.

4. Calculate the volume of 10 mM biotin reagent needed based on the mass of antibody prior to antibody isolation (Figure 2, at left). For most antibodies, a 5:1 molar coupling ratio (moles NHS-LCLC-biotin: moles antibody at start of procedure) works well.
5. To each sample, add the appropriate volume (µL) of NHS-LC-LC-Biotin reagent as calculated. Mix immediately.
6. Incubate 30 minutes at room temperature.
7. 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.

   NOTE: All free biotin must be removed to most efficiently bind the biotinylated protein to the streptavidin biosensor surface.
8. Biotinylated antibodies in PBS can be stored at 4 °C. See Figure 3 for an example immobilization of biotinylated antibody onto Streptavidin SBC biosensors.

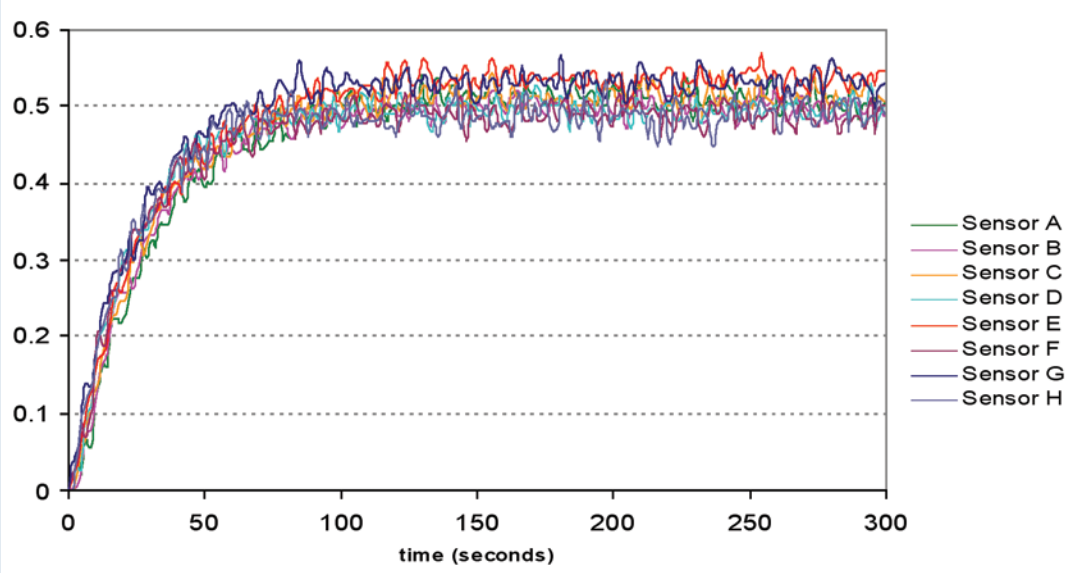

Figure 3: Sample data for the parallel immobilization of a biotinylated protein onto eight Streptavidin SBC biosensors.

RELATED TECHNICAL NOTES

Other related technical notes are available for download from ForteBio's web site (www.fortebo.com):

- Octet Technical Note 6: *Biotinylation of Protein for Immobilization onto Streptavidin Biosensors (TN-3006)*
- Octet Technical Note 10: *Batch Immobilization of Protein onto Streptavidin Biosensors (TN-3010)*
- Octet Technical Note 12: *Biotinylation of Protein for Immobilization onto Streptavidin Biosensors When Very Small Quantities of Protein are Available (TN-3012*)

DISCLAIMER

ForteBio reserves the right to change its products and services at any time to incorporate the latest technological developments. This technical note is subject to change without notice.

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