

Biotinylation of Proteins for Immobilization Onto Streptavidin Biosensors



Technical Note

Scope

This Technical Note on Octet® Streptavidin (SA) Biosensors provides insight into the strategy of protein biotinylation to capture them on the biosensor surface.

Keywords or phrases:

Octet®, Bio-Layer Interferometry, BLI, protein-protein interaction, biotinylation, optimization, affinity analysis, Streptavidin

Executive Summary

The interaction between streptavidin and biotin is widely used as a system for the rapid, stable and irreversible non-covalent binding of biological molecules. The Octet® platform's Streptavidin Biosensors have been developed for the capture of biotinylated ligands for both quantitation and kinetic applications. The first protein captured onto the Streptavidin Biosensors must be biotinylated prior to an assaying on the Octet® system.

It provides guidelines for biotinylating a protein of interest utilizing Pierce-ThermoFisher (piercenet.com) biotinylation reagents for kinetic and quantitation assays with Streptavidin Biosensors on the Octet® platform.

Materials Required

- EZ-Link NHS-PEG₄-Biotin (Thermo, part no. 21329) prepared as a 1 mM solution in water
 - Can also use the NHS-PEG₁₂-Biotin (Thermo, part no. 21312) or Sulfo-NHS-LC-LC-Biotin (Thermo, part no. 21338)
- Distilled water
- 1 × PBS
- Zeba desalting spin columns for buffer exchange
 - For protein samples of 30 – 130 μL, use 0.5 mL columns (Thermo, part no. 89882)
 - For protein samples of 200 – 700 μL, use the 2 mL columns (Thermo, part no. 89889)
 - For protein samples of 600 – 2000 μL, use the 5 mL columns (Thermo, part no. 89891)
- Protein to be biotinylated (requires at least 50 μg total at a concentration of at least 100 μg/mL)
- (Optional) Slide-A-Lyzer (Thermo, part no. 66370)

Protein sample preparation

- Ensure that the protein is carrier-protein free
- Ensure that the protein to be biotinylated is not in a buffer containing primary amines (i.e., Tris or glycine). If protein is in a buffer containing primary amines, exchange into PBS either by dialysis or desalting spin columns. The protein concentration is recommended to be at least 1 mg/mL for this process.

Protein Sample Preparation by Dialysis

(Recommended for samples >0.5mL)

1. Dialyze each sample 1:1000 in 1 × PBS using a Slide-A-Lyzer.
2. Allow the protein and Slide-A-Lyzer to stir gently in PBS for a minimum of 3 hours before changing the PBS.
3. Change PBS at least 4 times before extracting the protein.

Protein Sample Preparation by Desalting Column

(Recommended for samples <0.5mL)

1. Follow the manufacturer's instructions for using the desalting columns for buffer exchange.

Biotin calculation and preparation

The amount of 1 mM biotin reagent required depends on the molar coupling ratio (MCR) of the biotin reagent to protein. In general, it is recommended to use a 1:1 ratio (1 biotin for every protein molecule). If the protein concentration is less than 500 μg/mL or if the extent of biotinylation at an MCR of 1:1 is found to be insufficient, a 3:1 or 5:1 ratio is recommended.

$$\text{Vol 1 mM biotin reagent } (\mu\text{L}) = \frac{\text{Protein Conc (mg/mL)}}{\text{MW Protein (kDa)}} \times \text{MCR} \times \text{volume Protein } (\mu\text{L})$$

Example 1:

1 mL solution of IgG (150 kDa)
at 1 mg/mL with an MCR of 1: μL
1 mM biotin reagent =
 $(1/150) \times 1 \times 1000 = 6.67 \mu\text{L}$

Example 2:

0.5 mL solution of IgG (150 kDa)
at 0.5 mg/mL with an MCR of 1: μL
1 mM biotin reagent =
 $(0.5/150) \times 1 \times 500 = 1.67 \mu\text{L}$

Example 3:

0.5 mL solution of IgG (150 kDa)
at 0.5 mg/mL with an MCR of 5: μL
1 mM biotin reagent =
 $(0.5/150) \times 5 \times 500 = 8.33 \mu\text{L}$

Figure 1: Equation for calculating the required volume of 1 mM biotin reagent.

1. Based on the selected MCR, calculate the volume of 1 mM biotin reagent needed. A sample calculation is shown in Figure 1.
2. Prepare a 1 mM biotin reagent solution.
 - a. If using NHS-PEG₄-Biotin, pierce the foil top and add 170 μL of distilled water to the tube to create a 20 mM stock solution. Add 50 μL of the 20 mM biotin stock solution to 950 μL of distilled water to create a 1 mM solution of NHS-PEG₄-Biotin.
 - b. For the NHS-PEG₁₂-Biotin, the product insert instructs on how to create a 250 mM stock solution; add 4 μL of this stock solution to 996 μL water to create a 1 mM solution.
 - c. For the sulfo-NHS-LC-LC-Biotin, the product insert instructs on how to create a 10 mM stock solution; add 100 μL of this stock solution to 900 μL distilled water to create a 1 mM solution.
3. To each sample, add the appropriate volume (μL) of biotin reagent as calculated in Step 1.
4. Mix immediately.
5. Incubate 30 minutes at room temperature.
6. Stop the reaction by removing the excess biotin reagent using the desalting column (follow manufacturer's product insert).

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