Procedure for Biotinylating Proteins in Solution

A. Calculations

The extent of biotin labeling depends on the distribution of amino groups on the protein, protein concentration and the amount of reagent used. Compared to reactions involving concentrated protein solutions, labeling reactions with dilute solutions require a greater fold molar excess of biotin reagent to achieve the same incorporation level. Experiments performed at Pierce that used a 20-fold molar excess of biotin reagent to label 1-10 mg antibody (in 0.5-2 mL) resulted in 4-6 biotin groups per antibody molecule. Experiments that used a 50-fold molar excess of biotin reagent to label 50-200 µg of antibody (in 200-700 µL) resulted in 1-3 biotin groups per antibody molecule. Adjust the molar ratio of Sulfo-NHS-LC-Biotin to protein to obtain the level of incorporation desired.

1. Calculate millimoles of biotin reagent to add to the reaction for a 20-fold molar excess:

$$\text{ml protein} \times \frac{\text{mg protein}}{\text{ml protein}} \times \frac{\text{mmol protein}}{\text{mg protein}} \times \frac{\text{20 mmol Biotin}}{\text{mmol protein}} = \text{mmol Biotin}$$

* 20 = Molar fold excess of biotin

2. Calculate microliters of 10 mM biotin reagent solution (prepared in Step B.3) to add to the reaction:

$$\text{mmol Biotin} \times \frac{\text{1,000,000 µl}}{\text{L}} \times \frac{\text{L}}{\text{10 mmol}} = \text{µl Biotin}$$

B. Biotin Labeling Reaction

1. Remove vial of Sulfo-NHS-LC-Biotin from freezer and equilibrate it to room temperature before opening in Step 3.
2. Prepare protein in PBS according to the calculation made in Section A.
   Note: Protein that is already dissolved in amine-free buffer at pH 7.2-8.0 may be used without buffer exchange or dilution with PBS. Proteins in Tris or other amine-containing buffers must be exchanged into a suitable buffer.
3. Immediately before use, prepare a 10 mM solution of the biotin reagent:
4. Add the appropriate volume (see Calculations in Section A) of 10 mM biotin reagent solution to the protein solution.
5. Incubate reaction on ice for two hours or at room temperature for 30 minutes.
   Note: Other than the possibility of ordinary protein degradation or microbial growth, there is no harm in reacting longer than the specified time.
6. Protein labeling is complete at this point, and although excess non-reacted and hydrolyzed biotin reagent remains in the solution, it is often possible to perform preliminary tests of the labeled protein by ELISA or Western blot. Once proper function and labeling of the protein has been confirmed, for optimal performance and stability, purify the labeled protein using desalting or dialysis. If the Pierce Biotin Quantitation Kit will be performed to determine the level of biotin incorporation, the protein first must be desalted or dialyzed to remove non-reacted biotin.

Procedure for Biotinylating Cell Surface Proteins

Labeling may be performed on cells in suspension or on adherent cells in culture plates. In the latter situation, diffusion of the Sulfo-NHS-LC-Biotin to all surfaces of the cells will be limited, and labeling will occur predominately on the exposed surface. Culture media must be washed from cells; otherwise, amine-containing components will compete and quench the reaction to cell surface proteins. Using a more concentrated cell suspension is most effective because less biotin reagent is required in the reaction. Generally, a final concentration of 2-5 mM Biotin Reagent is effective. NHS reactions occur more rapidly at high pH; therefore, pH 8.0 is used in the following example so that labeling can be completed as quickly as possible.

1. Wash cells three times with ice-cold PBS (pH 8.0) to remove amine-containing media and proteins from the cells.
2. Suspend cells at a concentration of ~25 $\times 10^6$ cells/ml in PBS (pH 8.0).
3. Add 1.0 mg of Sulfo-NHS-LC-Biotin reagent per mL of cell suspension (results in ~2 mM biotin reagent). Alternatively, add 200 µL of the 10 mM biotin reagent solution (see step B.3 on previous page) per mL of cell suspension.
4. Incubate reaction mixture at room temperature for 30 minutes.
   Note: Performing this incubation at 4 ℃ may reduce active internalization of the biotin reagent.
5. Wash cells three times with PBS + 100 mM glycine to quench and remove excess biotin reagent and byproducts.

Notice

Sulfo-NHS-LC-Biotin is moisture-sensitive . If the vial of reagent has been stored cold, fully equilibrate vial to room temperature before opening to avoid moisture condensation inside the container. Do not prepare stock solutions for storage, and dissolve the biotin reagent immediately before use. Avoid buffers containing primary amines (e.g., Tris or glycine) as these will compete with the reaction.