Procedure for Biotinylating Proteins

The following procedure ordinarily will yield incorporation of 3-5 biotins per molecule of protein. Antibodies, which are large proteins, often will label with ~8-12 biotin molecules per molecule of IgG, especially when greater molar excesses of biotin reagent are used (see Calculations). The molar ratio of biotin reagent to protein may be adjusted to obtain the level of incorporation desired.

A. Calculations

The amount of biotin reagent to use for each reaction depends on the amount of protein to be labeled and its concentration. By using the appropriate molar ratio of biotin to protein, the extent of labeling can be controlled. When labeling more dilute protein solutions, a greater molar fold excess of biotin is necessary to achieve the same results. For best results, use ≥ 12-fold molar excess of biotin for a 10mg/mL protein solution or ≥ 20-fold molar excess of biotin for a 2mg/mL protein solution

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 = Recommended molar fold excess of biotin for 2 mg/ml protein sample

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. If the biotin reagent has been stored cold, remove the vial from storage and fully equilibrate it to room temperature before opening in step 3.

2. Dissolve 1-10mg protein in 0.5-2.0mL 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 10mM solution of the biotin reagent in an organic solvent such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF):

4. Add the appropriate volume (see Calculations in section A) of 10mM 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, the labeled protein may be purified for optimal performance and stability using desalting or dialysis. If the level of biotin incorporation will be determined using the Pierce Biotin Quantitation Kit, the protein first must be desalted or dialyzed to remove non-reacted biotin.

Notice

NHS-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.