Instructions for Biotin-$(PEG)_n$-PFP

Introduction

The BroadPharm Biotin-$(PEG)_n$-PFP is a biotin labeling reagent that reacts with primary and secondary amines. The pentafluorophenyl (PFP) ester-activated biotin is less subject to hydrolysis than NHS esters, resulting in more efficient reactions. Biotin-$(PEG)_n$-PFP must be first dissolved in a minimal amount of an organic solvent, such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF) and then added to the buffer containing the protein or other molecule. The reagent forms an emulsion that allows the reaction to proceed.

Important Product Information

• Biotin-$(PEG)_n$-PFP is moisture-sensitive. Store the vial of biotin reagent at -20°C with desiccant. To avoid moisture condensation onto the product, equilibrate vial to room temperature before opening.

• As directed in the procedure, dissolve the biotin reagent immediately before use. The PFP moiety hydrolyzes and becomes non-reactive; therefore, weigh and dissolve only a small amount of the reagent at a time, and do not prepare stock solutions for storage. Discard any unused reconstituted reagent.

• Avoid buffers containing primary amines (e.g., Tris or glycine) as these will compete with the reaction. If necessary, dialyze or desalt to exchange the protein sample into an amine-free buffer such as phosphate-buffered saline (PBS).

• After pegylation, unreacted Biotin-$(PEG)_n$-PFP is easily removed by size exclusion using either desalting columns or dialysis). A 10 mL desalting column is best suited for processing biotinylation reactions involving 1-10 mg of protein in approximately 0.5-2 mL. For smaller amounts of protein and/or smaller reaction volumes, both the biotinylation reaction and subsequent buffer exchange may be performed in a single Thermo Scientific Slide-A-Lyzer MINI Dialysis Unit.

General Procedure for BiotinylatingIgG and other Proteins

The following protocol typically results in approximately two biotin molecules per IgG. The degree of Biotin-$(PEG)_n$ incorporation can vary depending on the parameters of the biotinylation reaction, including protein concentration, PFP-$(PEG)_n$-Biotin concentration, pH and time. Commonly used reaction conditions include incubation at 4-37°C, pH values from 7 to 9, and incubation times from a few minutes to overnight.

1. Dissolve 2 mg of IgG in 1 mL of PBS (for example, 0.1M sodium phosphate 0.15M NaCl, pH 7.2).
2. Immediately before use, dissolve 1 mg of Biotin-(PEG)n-PFP in 75 µL of DMF or DMSO. Add 25 µL of the PFP-Biotin solution to the IgG solution.
3. Incubate the reaction on ice for two hours or at room temperature for 30 minutes.
4. Remove unreacted Biotin-$(PEG)_n$-PFP by dialysis or gel filtration.
5. Store the biotinylated protein at the specified conditions as unbiotinylated protein until ready for use.

A. Determination of Biotin Incorporation

Biotin incorporation can be estimated using the HABA (4´-hydroxyazobenzene-2-carboxylic acid) method. In solution, the HABA dye binds avidin, forming a complex with maximal absorption at 500nm. When biotin is added to the solution, its higher affinity for avidin displaces the HABA and the absorption at 500nm decreases proportionately. The absorbance of the HABA-avidin solution is measured before and after adding the biotin-containing sample. The change in absorbance relates to the amount of biotin in the sample.