Instructions for the use of Biotin-(PEG)$_n$-NHS

Introduction

The BroadPharm Biotin-(PEG)$_n$-NHS is an D-(+)biotin labeling reagent with an extended spacer arm. This reagent is soluble in organic solvents such as DMSO or DMF. Once dissolved in an organic solvent, the reagent is further diluted in a non-amine containing aqueous buffer. The *N*-Hydroxysuccinimide (NHS) ester-activated PEG linker is an amine-reactive reagent. NHS esters react efficiently with primary amino groups (-NH_2) in pH 7-9 buffers to form stable amide bonds. Because antibodies and other proteins generally contain multiple lysine (K) residues in addition to the N-terminus of each polypeptide, they have multiple primary amines available as targets for labeling with NHS-activated PEG reagents.

Product Information

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

• Dissolve the Biotin-(PEG)$_n$-NHS linker immediately before use. The NHS-ester moiety readily 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 compete with the intended reaction. If necessary, dialyze or otherwise desalt to exchange the protein sample into an amine-free buffer such as phosphate buffered saline.

Additional Materials Required

• Phosphate-buffered Saline (PBS): 0.1M phosphate, 0.15M sodium chloride; pH 7.2 or other non-amine containing buffer at pH 7.0-8.0

• Quenching Buffer: Tris-buffered saline (TBS; 25mM Tris, 0.15M sodium chloride; pH 7.2; glycine or other amine-containing buffer)

• Water-miscible organic solvent such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF)

• 10-100 µL sample volumes; Slide-A-Lyzer® Dialysis Cassette Kit for 0.1-30 mL sample volumes; or Zeba Spin Desalting Columns for sample volumes ranging from >10 µL to 4 mL

Procedure for labeling IgG with Biotin-(PEG)$_n$-NHS

A. Calculations

The extent of biotin labeling depends on the size and distribution of amino groups on the protein and the amount of reagent used. Compared to reactions involving concentrated protein solutions, labeling reactions with dilute protein solutions require a greater fold molar excess of biotin reagent to achieve the same incorporation level. Typically using a 20-fold molar excess of biotin reagent to label 1-10 mg/mL

antibody (IgG) results in 4-6 biotin groups per antibody molecule. Adjust the molar ratio of Biotin-$(PEG)_n$-NHS to protein to obtain the desired level of incorporation.

1. Calculate millimoles of Biotin-$(PEG)_n$-NHS to add to the reaction for a 20-fold molar excess.

2. Calculate microliters of 10mM Biotin-$(PEG)_n$-NHS preparation for adding to the reaction.

B. Biotin-$(PEG)_n$-NHS Labeling Reaction

For reaction volumes from 10 µL to 100 µL, the buffer exchange may be conveniently performed in a single Slide-A-Lyzer MINI Dialysis Unit. For reaction volumes from 0.1 mL to 30 mL, Slide-A-Lyzer Dialysis Cassettes may be used. Alternatively, Zeba Spin Desalting Columns can be used for a faster buffer exchange.

1. Equilibrate the vial of Biotin-$(PEG)_n$-NHS to room temperature before opening in Step 3.
2. Dissolve 1-10 mg protein in 0.5-2 mL of PBS according to the calculation made.
3. Immediately before use, prepare a 10mM solution of Biotin-$(PEG)_n$-NHS by adding about 5 mg to 1 mL of DMSO or DMF.
4. Add the appropriate volume of the Biotin-$(PEG)_n$-NHS solution (a 20-fold molar excess) to the protein solution, making sure that the volume of organic solvent does not exceed 10% of the final reaction volume.
5. Incubate reaction on ice for two hours or at room temperature for 30-60 minutes.
6. Remove the unreacted Biotin-$(PEG)_n$-NHS by dialysis or gel filtration. See instructions provided with the preferred buffer exchange product.
7. Store the biotinylated protein using the same condition that is optimal for the non-biotinylated protein.

Additional Information

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.