Protocol

TD-P Revision 2.0

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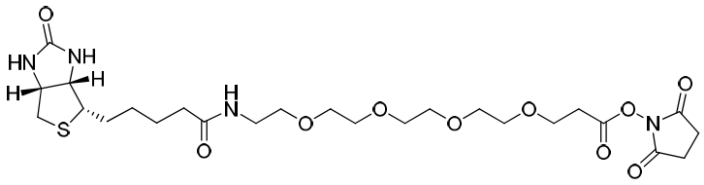
Biotin-PEG4-NHS Ester Protocol and Technical Information

Product No.:	B-720
Product Name:	Biotin-PEG4-NHS Ester
Chemical Structure:	
Chemical Composition:	$C_{25}H_{40}N_4O_{10}S$
Molecular Weight:	588.67 g/mol
Net Mass Addition:	473.22 g/mol
Spacer Arm:	29Å
Solubility:	10 mg/ml in water
Storage:	Store at -20°C. (Product shipped at ambient temperature).

Introduction

Biotin-PEG4-NHS Ester enables simple and efficient biotin labeling of antibodies, proteins and any other primary amine-containing macromolecule. The hydrophilic polyethylene glycol (PEG) spacer arm imparts water solubility that is transferred to the biotinylated molecule. Consequently, antibodies that have been labeled with Biotin-PEG4-NHS Ester exhibit less aggregation when stored in solution compared to antibodies labeled with reagents having only hydrocarbon spacers.

Biotin is a small naturally occurring vitamin that binds with high affinity to avidin and streptavidin proteins. Biotinylated proteins typically retain biological activity because the biotin group is relatively small. An antibody conjugated with several biotin molecules can amplify signal, thereby increasing the sensitivity of many assays. The bond formation between biotin and avidin is rapid and, once formed, is unaffected by most extremes of pH, organic solvents

and other denaturing agents. Labeled proteins can be purified and detected using avidin, streptavidin products.

N-Hydroxysuccinimide (NHS) esters are the most commonly used biotinylation reagents. In pH 7-9 buffers, NHS-biotin reagents react efficiently with primary amino groups (-NH2) by nucleophilic attack, forming an amide bond and releasing the NHS. Proteins typically have many sites for labeling, including the primary amine in the side chain of lysine residues and the N-terminus of each polypeptide.

Materials

Important Product Information

- NHS esters are moisture-sensitive. To avoid moisture condensation onto the product always let vial come to room temperature before opening; be careful to limit exposure to moisture and restore under an inert atmosphere. The NHS-ester moiety readily hydrolyzes and becomes non- reactive; therefore, prepare stock solutions immediately before use. Stock solutions in anhydrous solvents can be kept for several days (freeze when not in use).
- Hydrolysis of the NHS ester is a competing reaction. Conjugation with primary amines of proteins/peptides (i.e., acylation) is favored at near neutral pH (7-9) and with concentrated protein solutions. For conjugation, use non-amine-containing buffers at pH 7-9 such as PBS (20mM sodium phosphate, 150mM sodium chloride, pH 7.4); 20mM HEPES; 100mM carbonate/bicarbonate; or 50mM borate buffer.
- Do not use buffers that contain primary amines, (e.g., Tris, glycine).
- Dissolve Biotin-PEG4-NHS Ester in a dry water-miscible organic solvent such as DMSO or DMF before diluting in final reaction buffer.

Additional Materials Required

- Water-miscible organic solvent such as dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF).
- Reaction buffer: Phosphate-buffered saline (PBS) or other buffer at pH 7-9
- Spin Desalting Columns

Method

Example Procedure for IgG Biotinylation

The following protocol is an example application for this product. Specific applications require optimization. This protocol typically results in ~3-5 biotin molecules per molecule of IgG. Adjust the molar excess of Biotin-PEG4-NHS Ester to optimize for the level of labeling desired.

Protein Derivitization

1. Dissolve or buffer exchange the IgG into pH 7.5 PBS buffer.

2. Immediately before use, prepare 20mM of the Biotin-PEG4-NHS Ester reagent in DMSO or DMF.

3. Add the appropriate volume of the NHS reagent to the protein sample to achieve desired molar excess of the Biotin-PEG4-NHS Ester reagent.

4. Incubate the reaction at room temperature for 30 minutes or on ice for 2 hours.

5. Remove non-reactive reagent by dialysis or desalting.

6. Store the biotinylated protein using the same condition that is optimal for the non-biotinylated protein.

Determination of Biotin Incorporation (optional)
Biotin incorporation can be estimated using commercially available Streptavidin-binding assays such as HABA or FluoReporter™ (trademark of Thermo Scientific). Alternatively, use biotinylation reagents with built-in signal quantification for quick and accurate determination of the number of biotins incorporated into a protein by means of non-destructive UV-Vis spectroscopy.

Calculations

The amount of biotin reagent to use for each reaction depends on the amount and concentration of the protein to be labeled. To control the extent of labeling, adjust the molar excess of Biotin-PEG4-NHS Ester.

For dilute protein solutions (e.g., 1 mg/ml) use a greater molar-fold excess of biotin compared to a concentrated protein solution (e.g., 10 mg/ml).

For example, use ≥20-30-fold molar excess for IgG at 1mg/ml and ≥10-fold molar excess for IgG at 5 mg/ml.

Troubleshooting

Problem	Possible Cause	Solution
Lack of biotinylation	No amines available on molecule of interest	Use a biotinylation reagent that targets a different functional group
	NHS-ester hydrolyzed	Allow product to equilibrate to room temperature before opening
		Prepare new solutions in the indicated dry solvents
		Avoid buffers that contain primary amines such as Tris and glycine
Biotinylated protein does not function in downstream application	Excessive biotinylation	Reduce molar excess of biotinylation reagent
		Choose biotinylation reagent that targets different groups