INSTRUCTIONS

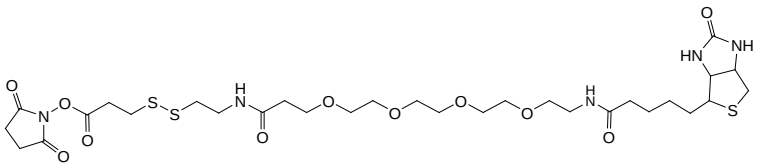
EZ-Link NHS-SS-PEG$_4$-Biotin

MAN0016366
Rev. A.0
Pub. Part No. 2161842.3

21442

Number	Description
21442	**EZ-Link NHS-SS-PEG$_4$-Biotin**

Package size: 50mg
Molecular Weight: 751.94
Spacer Arm: 37.9Å



Storage: Upon receipt store desiccated at -20°C. Product is shipped at ambient temperature.

Introduction

Thermo Scientific™ EZ-Link™ NHS-SS-PEG$_4$-Biotin enables simple and efficient biotin labeling of antibodies, proteins and other primary amine-containing molecules. This biotinylation reagent contains a multi-functional extended spacer arm that is a flexible, non-immunogenic hydrophilic polyethylene glycol (PEG), which imparts water solubility that is transferred to the labeled molecule. Consequently, antibodies labeled with NHS-SS-PEG$_4$-Biotin exhibit less aggregation when stored in solution compared to antibodies labeled with reagents having only hydrocarbon spacers. The spacer also contains a disulfide bond reducible with dithiothreitol (DTT) or other reducing agents, enabling labeled proteins to be cleaved from the biotin group. This feature is especially useful for affinity purifications when it is necessary to elute the biotinylated protein from its bound state to avidin or streptavidin.

N-Hydroxysuccinimide (NHS) esters are the most popular biotinylation reagents. In pH 7-9 buffers, NHS esters react efficiently with primary amino groups (-NH_2) 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 (K) residues and the N-terminus of each polypeptide.

Biotin is a vitamin that has a high affinity to avidin and avidin-like 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 biotin-avidin interaction is strong and unaffected by most extremes of pH, organic solvents and other denaturing agents. Labeled proteins can be purified using immobilized streptavidin, avidin or Thermo Scientific™ NeutrAvidin™ Protein affinity resins and detected in ELISA, dot blot and Western blot applications.

Important Product Information

- The NHS-ester moiety readily hydrolyzes and becomes non-reactive. 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. 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.
- During biotinylation, avoid buffers containing primary amines (e.g., Tris or glycine) and imidazole, as these will compete with the intended reaction. If necessary, dialyze or desalt to exchange the sample into an amine-free, well-buffered solution at pH 7.2 -8.0, such as phosphate-buffered saline (100mM phosphate, 150mM sodium chloride; Product No. 28372). For reaction volumes of 10-100µL, the buffer exchange and biotinylation may be conveniently performed in a single Thermo Scientific™ Slide-A-Lyzer™ MINI Dialysis Unit. For reaction volumes of 0.1-30mL, Slide-A-Lyzer Dialysis Cassettes may be used. Alternatively, Thermo Scientific™ Zeba™ Spin Desalting Columns enable faster buffer exchange.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | Thermofisher.com

Additional Materials Required

- Phosphate-buffered Saline (PBS): 100mM phosphate, 150mM sodium chloride; pH 7.2 (Product No. 28372) or other non-amine-containing buffer at pH 7.0-8.0
- Water-miscible organic solvent such as DMSO (Product No. 20688) or DMF (Product No. 20672)
- Method for removing non-reacted biotin and hydrolyzed NHS ester (buffer exchange): Slide-A-Lyzer MINI Dialysis Units for 10-100µL samples, Slide-A-Lyzer Dialysis Cassette Kit for 0.1-30.0mL samples, or Zeba Spin Desalting Columns for < 10µL to 4mL samples.
- Reducing Agent: DTT (Product No. 20290), 2-mercaptoethanol (Product No. 35602), TCEP (Product No. 20490) or other reducing agent for cleaving the disulfide bond

Procedure for Biotinylating Proteins in Solution

A. Calculations

The extent of biotin labeling depends on the distribution of amino groups on the protein, and the concentration and amount of protein to be labeled. By using the appropriate molar ratio of biotin to protein, the extent of labeling can be controlled. Labeling reactions with dilute protein solutions require a greater fold molar excess of biotin reagent to achieve the same incorporation level as reactions involving concentrated solutions. Generally, use ≥ 12-fold molar excess of biotin for proteins at 2-10mg/mL or ≥ 20-fold molar excess of biotin for proteins at ≤ 2mg/mL. Adjust the molar ratio of biotin reagent 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 for 2mg/mL protein sample

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

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

Example: For 1mL of a 2mg/mL IgG (150,000 MW) solution, ~27µL of 10mM biotin reagent will be added.

$$1\,\text{mL IgG} \times \frac{2\,\text{mg IgG}}{1\,\text{mL IgG}} \times \frac{1\,\text{mmol IgG}}{150{,}000\,\text{mg IgG}} \times \frac{20\,\text{mmol Biotin}}{1\,\text{mmol IgG}} = 0.000266\,\text{mmol Biotin}$$

$$0.000266\,\text{mmol Biotin} \times \frac{1{,}000{,}000\,\mu\text{L}}{\text{L}} \times \frac{\text{L}}{\text{10 mmol}} = 26.6\,\mu\text{L Biotin Reagent}$$

B. Biotin-labeling Reaction

1. Equilibrate the vial of NHS-SS-PEG_4-Biotin to room temperature before opening in Step 3.
2. Dissolve or transfer protein into an appropriate buffer. If protein is already dissolved in amine-free buffer at pH 7.2-8.0, it may be used without buffer exchange. Determine protein concentration if unknown.
3. Immediately before use, prepare 10mM of of NHS-SS-PEG_4-Biotin by dissolving 2mg in 265µL of DMF or DMSO.
4. Add the appropriate volume of 10mM NHS-SS-PEG_4-Biotin to the protein to achieve the desired molar excess (see Calculations section).
5. Incubate the reaction on ice for two hours or at room temperature for 30 minutes. Other than the possibility of ordinary protein degradation or microbial growth, there is no harm in reacting longer than the specified time.
6. (If needed) Remove non-reacted NHS-PEG_4-SS-Biotin and hydrolysis byproducts by dialysis or gel filtration. See Important Product Information and Related Pierce Product sections for more information.
7. Store the biotinylated protein using the same condition that is optimal for the non-biotinylated protein.
8. To cleave the disulfide bond in the spacer arm, incubate the labeled protein in 50mM DTT for 2 hours at room temperature or for 30 minutes at 50°C. Other reducing agents also may be used to cleave the disulfide bond.

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 or convert sulfhydryl to amine using Aminoethyl-8 (Product No. 23010)
	Buffer contains primary amines	Use a non-amine containing buffer
	Hydrolysis of the NHS ester	Allow reagent to equilibrate to room temperature before opening and use reagent immediately upon reconstitution
	Incomplete removal of primary amines	Dialyze or desalt into a buffer that is free of primary amines
Protein is not functional	Excessive biotinylation	Reduce molar excess of biotinylation reagent, or reduce time or temperature for biotinylation
		Choose a biotinylation reagent that targets different groups

Additional Information

A. Determination of Biotin Incorporation

Biotin incorporation can be estimated using the HABA (4´-hydroxyazobenzene-2-carboxylic acid) method. This method is based on the ability of the HABA dye to bind avidin forming a complex with maximal absorption at 500nm. Biotin is then added to the solution and because of its higher affinity for avidin, biotin displaces the HABA and the absorption at 500nm decreases proportionately to the dissociation of avidin from the HABA dye. 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. (See Related Thermo Scientific Products Section.)

B. Please visit our web site for additional information including the following:

- Tech Tip #14: Perform labeling and other reactions in Slide-A-Lyzer Dialysis Cassettes
- Tech Tip #43: Protein stability and storage
- HABA Calculator

Related Thermo Scientific Products

28005	**Pierce Biotin Quantitation Kit**
21441	**EZ-Link NHS-SS-Biotin,** 50mg
21331	**EZ-Link Sulfo-NHS-SS-Biotin,** 100mg
21330	**EZ-Link NHS-PEG$_4$-Biotin,** 25mg
20347	**Streptavidin Agarose Resin**, 2mL
29200	**NeutrAvidin Agarose Resin**, 5mL
20290	**Dithiothreitol (DTT),** 5g
20291	**No-Weigh™ DTT,** 48 × 7.7mg microtubes
77720	**Bond-Breaker™ TCEP Solution, Neutral pH,** 5mL
28372	**BupH™ Phosphate Buffered Saline Packs,** 40 packs
69576	**Slide-A-Lyzer MINI Dialysis Unit Kit**
66382, 66807	**Slide-A-Lyzer Dialysis Cassette Kits**
89891	**Zeba Spin Desalting Columns, 7K MWCO, 5mL**

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Pierce Biotechnology
3747 N. Meridian Road
PO Box 117
Rockford, IL 61105 USA
(815) 968-0747
(815) 968-7316 fax
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