INSTRUCTIONS

Thermo SCIENTIFIC

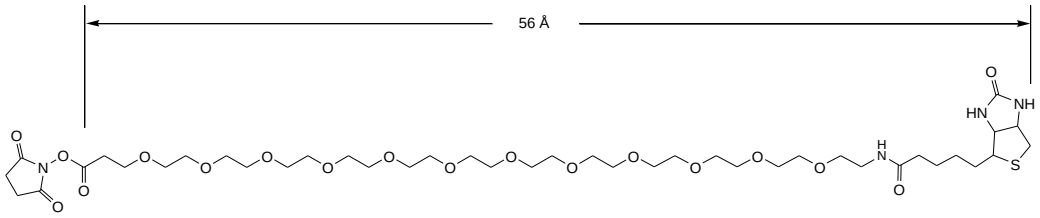
EZ-Link NHS-PEG_{12}-Biotin, No-Weigh Format

MAN0017096
Rev. A.0
Pub. Part No. 2162632.0

A35389

Number	Description
A35389	**EZ-Link NHS-PEG_{12}-Biotin, No-Weigh Format** 10 × 1mg

Molecular Weight: 941.09
Spacer Arm: 56Å
Net Mass Addition: 825.64



Storage: Upon receipt store desiccated at -20°C protected. Product is shipped on ice.

For Research Use Only. Not for use in diagnostic procedures.

Introduction

Thermo Scientific™ EZ-Link™ NHS-PEG_{12}-Biotin reagent 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. For example, antibodies labeled with NHS-PEG_4-Biotin exhibit less aggregation when stored in solution as compared to antibodies labeled with reagents having only hydrocarbon spacers. Specific labeling of cell surface proteins is another useful application for these water-soluble and membrane-impermeable reagents.

Thermo Scientific™ No-Weigh™ products are specialty reagents provided in a pre-aliquoted format. The pre-weighed packaging prevents the loss of reagent reactivity and contamination over time by eliminating the repetitive opening and closing of the vial. The format enables use of a fresh vial of reagent each time, eliminating the hassle of weighing small amounts of reagents and reducing concerns over reagent stability.

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 using immobilized streptavidin, avidin or Thermo Scientific™ NeutrAvidin™ Protein affinity resins and detected in ELISA, dot blot or Western blot applications.

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 (-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.

Cell surface biotinylation has emerged as an important tool for studying the expression and regulation of receptors and transporters, differentiation of plasma membrane proteins from those localized to organelle membranes, and distribution of membrane proteins in polarized epithelial cells. Because EZ-Link NHS-PEG_{12}-Biotin dissolves readily in polar solutions, it does not permeate the cell membrane. As long as the cell remains intact, only primary amines exposed on the surface will be biotinylated with this reagent.

Important Product Information

- The EZ-Link NHS-PEG$_{12}$-Biotin can be prepared by dissolving the reagent in dry (anhydrous, molecular-sieve treated) organic solvent, such as dimethylformamide (DMF, Product No. 20673) and dimethylsulfoxide (DMSO, Product No. 20688). Minimize reagent exposure to moisture because the NHS-ester reactive group is susceptible to hydrolysis. Equilibrate reagent vial to room temperature before opening to avoid moisture condensation inside the container.
- Avoid buffers containing primary amines (e.g., Tris or glycine) during conjugation because they compete with the intended reaction. If necessary, dialyze or desalt samples into a buffer such as phosphate-buffered saline (PBS).
- The reagent-to-protein molar ratio affects modification extent of available amine groups. Optimize this ratio to yield the extent of biotinylation that is best for the specific application.

Additional Materials Required

- Water-miscible organic solvent (molecular sieve-treated) such as dimethylsulfoxide (DMSO, Product No. 20688), acetonitrile (Product No. 51101) or dimethylformamide (DMF, Product No. 20673) for preparing reagent stock solution
- Phosphate-buffered saline (PBS) or other amine-free buffer at pH 7-8 for use as reaction buffer (see Important Product Information and Related Thermo Scientific Products)
- Desalting columns or dialysis units for buffer exchange and removal of excess reagent following modification (e.g., Thermo Scientific Zeba Spin Desalting Columns or Slide-A-Lyzer Dialysis Units)

Procedure for Protein Biotinylation using EZ-Link NHS-PEG$_{12}$-Biotin

The amount of reagent to use for a reaction depends on the amount of labeling desired, the amount of protein to be labeled, and its concentration. By regulating the molar ratio of reagent to target molecule, the extent of labeling can be controlled. As a starting point, consider using a 5- to 20-fold molar excess of EZ-Link NHS-PEG$_{12}$-Biotin for protein solutions > 2mg/mL. When labeling more dilute solutions, a greater relative molar excess of EZ-Link NHS-PEG$_{12}$-Biotin may be necessary to achieve the same labeling results. Example calculations for a typical antibody modification are provided for convenience.

A. Calculate the Amount of Reagent Needed

1. Calculate the quantity in millimoles of EZ-Link NHS-PEG$_{12}$-Biotin to add to the reaction for a 20-fold molar excess:

$$\text{ml protein} \times \frac{\text{mg protein}}{\text{ml protein}} \times \frac{\text{mmol protein}}{\text{mg protein}} \times \frac{\text{20 mmol Biotin Reagent}}{\text{mmol protein}} = \text{mmol Biotin Reagent}$$

Note: The value 20 in this equation corresponds to the suggested reagent molar fold excess for a 2mg/mL protein sample.

2. Calculate microliters of 10mM NHS-PEG$_{12}$-Biotin stock solution (prepared in Step B.1) to add to the reaction:

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

Example Calculation:

For 1mL of a 2mg/mL IgG (150,000 MW) solution, ~26µL of 10mM NHS-PEG$_{12}$-Biotin reagent will be added:

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

$$0.000266\,\text{mmol Biotin Reagent} \times \frac{1{,}000{,}000\,\mu\text{l}}{\text{L}} \times \frac{\text{L}}{\text{10 mmol}} = 26.6\,\mu\text{l of 10 mM Biotin Reagent stock solution}$$

B. Prepare 10mM Reagent Stock Solution

1. Read the Important Product Information (previous section) before preparing and storing this solution.
2. Remove vial of reagent from -20°C storage and fully equilibrate it to room temperature before opening.
3. Prepare a 10mM Biotin Reagent Stock Solution by dissolving 1 vial of EZ-Link NHS-PEG_{12}-Biotin in ~106µL of dry water-miscible solvent (e.g., DMF, acetonitrile or DMSO). The maximum useable volume of each tube is 800µL.
4. Cap, store and handle reagent stock solution as directed in the Important Product Information section.

C. Labeling Reaction

1. Dissolve 1-10mg protein to be modified in PBS according to the calculations 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.
2. Remove vial of Biotin Reagent Stock Solution from storage and fully equilibrate it to room temperature before use.
3. Using a pipette, remove an appropriate volume (see Calculations in section A) of 10mM Biotin Reagent Stock Solution, dispense it into the protein solution and mix well.
4. 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.
5. Labeling is complete at this point and, although excess nonreacted and hydrolyzed Biotin Reagent remains in the solution, it is often possible to perform preliminary tests of the labeled protein. Once proper function and labeling has been confirmed, the labeled protein may be purified from nonreacted Biotin Reagent using desalting or dialysis.

D. Determination of Biotin Incorporation (optional)

Biotin incorporation can be estimated using the HABA (4′-hydroxyazobenzene-2-carboxylic acid) method (e.g., Thermo Scientific™ Pierce™ Biotin Quantitation Kit, Product No. 28005). This method is based on the ability of the HABA dye to bind avidin, thereby 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. 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.

Troubleshooting

Problem	Possible Cause	Solution
Lack of biotinylation	No amines were available on molecule of interest	Use a biotinylation reagent that targets a different functional group
	Buffer contained primary amines	Use a non-amine containing buffer
		Extensively dialyze or desalt sample into a buffer free of primary amines
	Exposure of reagent to moisture caused hydrolysis of the NHS ester, inactivating the reagent	Ensure that reagent stock solution is not exposed to moisture
		Purchase new reagent
Biotinylated protein does not function in downstream application	Sites (primary amines) of biotinylation were active sites of molecule	Choose biotinylation reagent that targets different groups
	Excessive biotinylation	Reduce molar excess of biotinylation reagent, or reduce time or temperature for biotinylation

Related Thermo Scientific Products

28005	**Pierce™ Biotin Quantitation Kit**
21330	**EZ-Link NHS-PEG$_4$-Biotin,** 25mg
21329	**EZ-Link NHS-PEG$_4$-Biotin, No-Weigh™ Format, 8 X 2mg**
21334	**EZ-Link Iodoacetyl-PEG$_2$-Biotin,** 50mg, sulfhydryl-reactive biotinylation reagent
21911	**EZ-Link Maleimide-PEG$_{11}$-Biotin,** 25mg, sulfhydryl-reactive biotinylation reagent
21126	**Streptavidin, Horseradish Peroxidase Conjugated,** 1mg
20347	**Streptavidin Agarose Resin**, 2mL
20227	**Pierce Monomeric Avidin Kit**
46610	**Fluorescence Biotin Quantitation Kit**
88816	**Pierce™ Streptavidin Magnetic Beads,** 1mL

General References

Altin, J.G., *et al*. (1995). A one-step procedure for biotinylation and chemical cross-linking of lymphocyte surface and intracellular membrane-associated molecules. *Anal Biochem* **224:**382-9.

Gretch, D.R., Suter, M. and Stinski, M.F. (1987). The use of biotinylated monoclonal antibodies and streptavidin affinity chromatography to isolate herpes virus hydrophobic proteins or glycoproteins. *Anal Biochem* **163:**270-7.

Manning, J., *et al*. (1977). A method for gene enrichment based on the avidin-biotin interaction. Application to the Drosophila ribosomal RNA genes. *Biochemistry* **16:**1364-70.

Updyke, T.V. and Nicolson, G.L. (1984). Immunoaffinity isolation of membrane antigens with biotinylated monoclonal antibodies and immobilized streptavidin matrices. *J Immunol Meth* **73:**83-95.

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