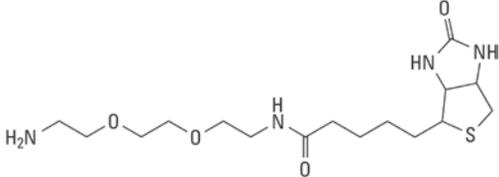
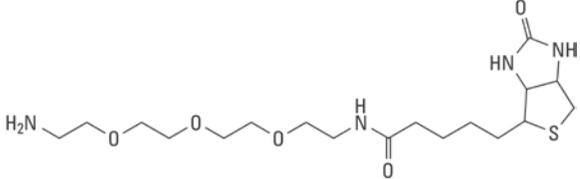
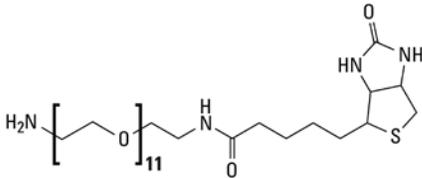


# EZ-Link<sup>®</sup> Amine-PEG<sub>n</sub>-Biotin

26136 21346 21347

0750.6

Number	Description
21346	<p><b>EZ-Link Amine-PEG<sub>2</sub>-Biotin</b>, (+)-biotinyl-3,6-dioxaoctanediamine, 50mg</p> <p>Molecular Weight: 374.50</p> <p>Spacer Arm: 20.4Å</p> <p>Maximum solubility: &gt; 25mg/mL in water or buffer</p> 
21347	<p><b>EZ-Link Amine-PEG<sub>3</sub>-Biotin</b>, (+)-biotinyl-3,6,9-trioxaundecanediamine, 50mg</p> <p>Molecular Weight: 418.55</p> <p>Spacer Arm: 22.9Å</p> <p>Maximum solubility: &gt; 25mg/mL in water or buffer</p> 
26136	<p><b>EZ-Link Amine-PEG<sub>11</sub>-Biotin</b>, (+)-biotinyl-undeca-oxapentatriacontanediamine, 100mg</p> <p>Molecular Weight: 770.97</p> <p>Spacer Arm: 53.2Å</p> <p>Maximum solubility: &gt; 25mg/mL in water or buffer</p> 

**Storage:** Upon receipt store amine-PEG<sub>2</sub>-biotin and amine-PEG<sub>3</sub>-biotin at 4°C; store amine-PEG<sub>11</sub>-biotin at -20°C. Product is shipped at ambient temperature.

## Introduction

The Thermo Scientific EZ-Link Amine-PEG<sub>n</sub>-Biotin reagents are water-soluble, polyethylene glycol (PEG)-containing reagents with terminal primary amines (-NH<sub>2</sub>). The PEG spacer arm is hydrophilic and confers greater solubility to labeled proteins compared to reagents having only hydrocarbon spacers. The amine group of these reagents can be reacted with carboxyl groups on carboxy termini, aspartate residues or glutamate residues using EDC (Product No. 22980), a water-soluble carbodiimide crosslinker. EDC activates carboxyl groups to bind to the -NH<sub>2</sub> group of the amine-biotin, forming an amide bond. For more information, consult the product instructions for EDC.

## Protein Biotinylation

The example procedure in these instructions uses amine-PEG<sub>n</sub>-biotin with EDC to label carboxyl groups on a protein. Reagent proportions must be optimized to achieve the desired extent of labeling and to control undesired polymerization. Because EDC causes conjugation of carboxyl groups to primary amino groups, protein (or peptide) polymerization might result if the protein has both functional groups on its surface. To minimize polymerization, use a large molar excess (e.g., 100-fold over the protein) of amine-PEG<sub>n</sub>-biotin and a limiting amount of EDC. Using a large molar excess of amine-PEG<sub>n</sub>-

biotin ensures that every EDC-activated carboxyl group on the protein is more likely to react with amine-PEG<sub>n</sub>-biotin than an amine on the protein. Using a limiting amount of EDC (e.g., 5- to 20-fold molar excess over the protein) ensures that carboxyl-to-amine conjugation will cease after only a few protein carboxyl groups have been modified. Not every carboxyl group that is activated by EDC will result in reaction to an amine; a significant proportion will hydrolyze before encountering an amine. Consequently, a five-fold molar excess of EDC will usually result in only 1-2 conjugations, depending on reactant concentrations.

## Example Procedure for Biotinylating BSA

**Note:** This example procedure uses a 1:100:10 molar ratio of BSA:amine-PEG<sub>n</sub>-biotin:EDC. Reagent proportions must be optimized to achieve the desired extent of labeling and to control undesired polymerization.

### A. Materials Required

- MES Buffer: 0.1M MES [(2-*N*-morpholino) ethanesulfonic acid], pH 4.7-5.5 (Thermo Scientific BupH MES Buffered Saline Packs, Product No. 28390). EDC reactions are generally performed using MES buffer at pH 5-6. Avoid buffers containing primary amines (Tris, glycine, etc.) or carboxyls (acetate, citrate, etc.) because they will quench the reaction. Phosphate buffers (pH 6.5-7.3) can be used but result in lower conjugation efficiency, often requiring more EDC to obtain the same results.
- Bovine serum albumin (BSA): 2mg (0.03μmol)
- EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride), Product No. 22980 or 22981
- Method for removal of non-reacted biotin (buffer exchange): Dialysis (e.g., Thermo Scientific Slide-A-Lyzer Dialysis Cassettes, Product No. 66382) or gel filtration (e.g., Thermo Scientific Zeba Spin Desalting Columns, Product No. 89891 or 89894)

### B. Procedure

1. Dissolve 2mg (0.03μmol) of BSA in 0.5-1mL of MES buffer.
2. Prepare 50mM solution of amine-PEG<sub>n</sub>-biotin in MES buffer. For example, dissolve the following amounts in 1mL of MES buffer:
  - 19mg of Amine-PEG<sub>2</sub>-Biotin
  - 21mg of Amine-PEG<sub>3</sub>-Biotin
  - 39mg of Amine-PEG<sub>11</sub>-Biotin
3. Add 60μL of amine-PEG<sub>n</sub>-biotin solution to the BSA solution and mix. This reaction results in 100-fold molar excess of biotinylation reagent over BSA (i.e., 3μmol of amine-PEG<sub>n</sub>-biotin).
4. Immediately before use, prepare 100mM solution of EDC in MES Buffer. For example, dissolve 19mg in 1mL buffer.
5. Add 3μL of the EDC to the solution from Step 3 and mix. This reaction results in a 10-fold molar excess of EDC over BSA (i.e., 0.3μmol of EDC)
6. Incubate for 2 hours at room temperature with stirring or mixing.
7. Centrifuge to remove any precipitate that formed during the reaction.
8. Remove the non-reacted biotinylation reagent and EDC by-products by desalting or dialysis.

## Additional Information

Visit the website for the following information.

- Tech Tip #30: Label and modify oligonucleotide 5'-phosphate groups

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## Related Thermo Scientific Products

20036	<b>Bioconjugate Techniques</b> , by Greg T. Hermanson, 2008, Academic Press, 1202 pages
28005	<b>Pierce® Biotin Quantitation Kit</b>
22980	<b>EDC</b> , 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, 5g
24510	<b>Sulfo-NHS</b> , 500mg
20227	<b>Monomeric Avidin Agarose Kit</b> , for reversible biotin immobilization
21126	<b>Streptavidin, Horseradish Peroxidase Conjugated</b> , 1mg

## Product Reference

Bronfman, F. C., *et al.* (2003). Ligand-induced internalization of the p75 neurotrophin receptor: A slow route to the signaling endosome. *J Neurosci* **23(8)**:3209-20.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at [www.thermoscientific.com/pierce](http://www.thermoscientific.com/pierce). For a faxed copy, call 800-874-3723 or contact your local distributor.

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