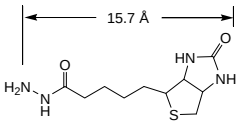
INSTRUCTIONS

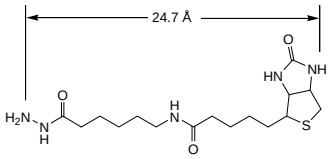
EZ-Link® Biotin Hydrazides

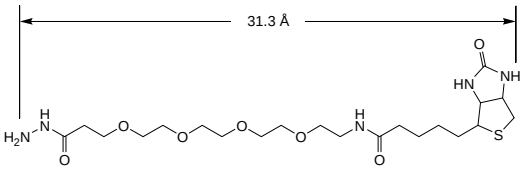
21339 21340 21360

0124.6

Number	Description
21339	**EZ-Link Biotin Hydrazide,** 100 mg Molecular Weight: 258.34 Spacer Arm: 15.7 Å Net Mass Added to Target: 240.11
21340	**EZ-Link Biotin-LC-Hydrazide,** 50 mg Molecular Weight: 371.50 Spacer Arm: 24.7 Å Net Mass Added to Target: 353.19
21360	**EZ-Link Biotin-PEG$_4$-Hydrazide,** 50 mg Molecular Weight: 505.26 Spacer Arm: 31.3 Å Net Mass Added to Target: 487.25







Storage: Upon receipt store product at 4°C. Product is shipped at ambient temperature.

Introduction

Thermo Scientific EZ-Link Biotin Hydrazide Reagents are useful for biotinylating macromolecules at carbohydrate groups that have been oxidized to form aldehydes. The hydrazide group reacts to carbonyls (aldehydes and ketones), resulting in a hydrazone linkage. Sialic acid is a common sugar component of protein polysaccharides, and the group is easily oxidized with 1 mM sodium periodate ($NaIO_4$). Other sugar groups can be oxidized effectively with 5-10 mM sodium periodate. For glycoproteins, oxidation of sugar moieties generates aldehyde groups that enable labeling to be directed away from polypeptide domains that are important for protein function. For example, most polyclonal antibodies are glycosylated in regions other than the antigen-binding sites, enabling them to be labeled with biotin-hydrazide reagents without adversely affecting their function in immunoassays. Be aware that monoclonal antibodies may be deficient in glycosylation.

The three biotin hydrazide reagents differ in spacer arm length and solubility, enabling the user to choose what is most appropriate for the application. Biotin Hydrazide is the simplest reagent, having the shortest possible spacer arm. Biotin-LC-Hydrazide contains a long-chain, albeit simple hydrocarbon, spacer arm that may reduce steric hindrance in biotin-binding assays. Biotin-PEG$_4$-Hydrazide contains a long-chain, water-soluble (hydrophilic), polyethylene glycol (PEG) spacer arm, whose properties are transferred to the labeled molecule. For example, antibodies modified with Biotin-PEG$_4$-Hydrazide have decreased levels of aggregation when stored in solution over time compared to proteins labeled with the other two biotin-hydrazide reagents.

Biotin hydrazide reagents also can be reacted with carboxyl groups using the carbodiimide EDC (Product No. 22980). EDC activates carboxyl groups to bind to the -NH_2 group from the biotinylation reagent, forming an amide linkage. Using EDC may result in some polymerization of the peptide or protein if the molecule has both carboxyls and primary amines on its surface. Decreasing the amount of EDC and/or increasing the amount of the biotin reagent used in the reaction can minimize polymerization.

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Important Product Information

- Avoid Tris or other primary amine-containing buffers in the oxidation and biotinylation steps as these buffers react with aldehydes and will quench the reaction with hydrazides.
- All three biotin hydrazide reagents can be dissolved at 50 mM in dimethylsulfoxide (DMSO) then diluted into aqueous reaction mixtures. (Do not use dimethylformamide, DMF, in which reagent solubility is poor.) Biotin Hydrazide and Biotin-LC-Hydrazide are soluble directly in aqueous buffers to ~5 mM; Biotin-PEG_4-Hydrazide is soluble in aqueous buffers to at least 20 mM.
- Hydrazides react with carbonyls most efficiently in amine-free, neutral conditions (pH 6.5-7.5). Carbonyls may exist at the reducing end of polysaccharides. To create additional carbonyls, oxidize sugar groups using either a specific oxidase, such as galactose oxidase, or 1-10 mM sodium *meta*-periodate ($NaIO_4$; Product No. 20504). Oxidation with periodate is most efficient in acidic conditions (e.g., 0.1 M sodium acetate, pH 5.5), although neutral buffers such as phosphate-buffered saline can be used. If oxidation is performed in acidic conditions, buffer exchange by dialysis or gel filtration into neutral buffer may be necessary to obtain optimal hydrazide reaction.
- EDC-mediated reactions are generally performed in an MES buffer at pH 4.5-5. Avoid buffers containing primary amines (Tris, glycine, etc.) or carboxyls (acetate, citrate, etc.) because they will quench the reaction. Phosphate buffers are suboptimal because they reduce conjugation efficiency, although this effect can be overcome by adding more EDC.

Example Protocol for Labeling Glycoproteins with Biotin Hydrazide Reagents

Note: For best results, optimize the molar ratio of reagent and glycoprotein by empirical testing.

A. Materials Required

- Biotin Hydrazide Solution: 50 mM biotin hydrazide reagent in dimethylsulfoxide (DMSO, Product No. 20684). Prepare a volume sufficient to achieve the desired final concentration in step B.4. Excess (unused) dissolved reagent may be stable, but this has not been verified. **Note:** Biotin-PEG_4-Hydrazide is a hygroscopic solid that is difficult to weigh and dispense. To facilitate handling, make a stock solution immediately before first use by dissolving the entire contents of the vial (50 mg) in 396 µl of dry (anhydrous, molecular sieve-treated) organic solvent such as DMSO. Store the resulting 250 mM stock solution of Biotin-PEG_4-Hydrazide at -20°C, and warm the vial fully to room temperature before opening for use to prevent moisture condensation, which may decrease its shelf-life.
- Oxidation Buffer: 0.1 M sodium acetate buffer, pH 5.5
- Sodium *meta*-periodate (Product No. 20504) solution: 20 mM sodium *meta*-periodate in Oxidation Buffer. Prepare solution immediately before use in amber vial or other light-protecting vessel.
- Coupling Buffer: 0.1 M sodium phosphate, 0.15 M NaCl, pH 7.2 (PBS, Product No. 28372) or other neutral or slightly alkaline, non-amine buffer
- Glycoprotein Solution: 2 mg/ml of glycoprotein in Oxidation Buffer
- Dialysis cassette or desalting column (e.g., Thermo Scientific Slide-A-Lyzer Dialysis Cassette Kit, Product No. 66382 or Zeba Spin Desalting Columns, Product No. 89891)

B. Procedure

1. Add 1 ml of cold sodium *meta*-periodate solution to 1 ml of cold glycoprotein solution; mix well and then protect reaction vessel from light and incubate mixture for 30 minutes on ice or at 4°C.

   Note: To oxidize only sialic acid groups, add 50 µl of sodium *meta*-periodate instead of 1 ml (results in 1 mM periodate final concentration rather than 10 mM).

2. Remove excess periodate and exchange the sample buffer by dialysis against coupling buffer or gel filtration through a desalting column that has been equilibrated with coupling buffer.

3. Add 1 part prepared 50 mM Biotin Hydrazide Solution to 9 parts oxidized and buffer-exchanged sample (results in 5 mM Biotin Hydrazide); mix for 2 hours at room temperature.

   Note: Optimal biotin-hydrazide concentration and reaction conditions depend on target protein and downstream application and must be determined empirically.

4. Separate the biotinylated molecule from non-reacted material by dialysis or gel filtration (desalting column). Biotinylated samples may be stored using the same conditions as for the non-biotinylated sample.

Example Protocol for Labeling Carboxyl Groups with Biotin Hydrazide Reagents

Note: For best results, optimize the molar ratio of reagents and carboxylate molecule by empirical testing.

A. Materials Required

- Biotin Hydrazide Solution: 50 mM biotin hydrazide reagent in dimethylsulfoxide (DMSO, Product No. 20684)
- MES Buffer: 0.1 M MES [(2-*N*-morpholino) ethanesulfonic acid], pH 4.7-5.5 (Product No. 28390)
- EDC (1-Ethyl-3-[3-Dimethylaminopropyl]carbodiimide Hydrochloride) solution: 100 mg/ml EDC (Product No. 22980 or 22981) in MES Buffer (results in ~0.5 M EDC solution). Prepare EDC immediately before use in step B3.
- Dialysis cassette or desalting column (e.g., Slide-A-Lyzer® Dialysis Cassette Kit, Product No. 66382 or Zeba™ Spin Desalting Columns, Product No. 89891)

B. Procedure

1. Dissolve protein (carboxyl-containing molecule) in MES Buffer at 5-10 mg/ml.
2. Add 25 µl of Biotin Hydrazide Solution per 1 ml of the protein solution and mix (results in 1.25 mM reagent).
3. Add 12.5 µl of the EDC solution per 1 ml of the protein solution and mix (results in ~6.5 mM EDC).
4. Incubate at 2 hours to overnight at room temperature with mixing.
5. Remove any precipitate that forms during the reaction by centrifugation. Separate the biotinylated molecule from non-reacted material by dialysis or gel filtration (desalting column).

   Note: Biotinylated samples may be stored using the same conditions as for the non-biotinylated sample. A typical storage condition is 4°C for several weeks.

Related Thermo Scientific Products

20036	**Bioconjugate Techniques,** 2nd edition, by Greg T. Hermanson, 2008, Academic Press
28020	**EZ-Link Biocytin Hydrazide,** 25 mg
28005	**Pierce® Biotin Quantitation Kit**

General References

Bayer, E.A., *et al.* (1988). Biocytin hydrazide-a selective label for sialic acids, galactose, and other sugars in glycoconjugates using avidin-biotin technology. *Anal. Biochem.* **170:**271-81.

O'Shannessy, D.J. and Quarles, R.H. (1987). Labeling of the oligosaccharide moieties of immunoglobulins. *J. Immunol. Meth.* **99:**153-61.

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Reisfield, A., *et al.* (1987). Nonradioactive hybridization probes prepared by the reaction of biotin hydrazide with DNA. *Biochem. Biophys. Res. Com.* **142:**519-26.

Rosenberg, M.B., *et al.* (1986). Receptor binding activities of biotinylated derivatives of ß-nerve growth factor. *J. Neurochem.* **46:**641-8.

Wade, D.P., *et al.* (1985). Detection of the low density-lipoprotein receptor with biotin-low density lipoprotein. *Biochem. J.* **229:**785-90.

Product References

Ayyagari, M.S., *et al.* (1995). Controlled free-radical polymerization of phenol derivatives by enzyme catalyzed reactions in organic solvents. Macromolecules. **28:** 5192-7.

Deepa, S.S., *et al.* (2002). Specific molecular interactions of oversulfated chrondroitin sulfate E with various heparin-binding growth factors. Implications as a physiological binding partner in the brain and other tissues. *J. Biol. Chem.* **277:** 43707-16.

DeSilva, N., Ofek, I. and Crouch, E.C. (2003). Interactions of surfactant protein D with fatty acids. *Amer. J. Respir. Cell and Molec. Biol.* **29:** 757-70.

Husi, H. (2001). Isolation of 2000-kDa complexes of N-methyl-D-aspartate receptor and postsynaptic density 95 from mouse brain. *J. Neurochem.* **77:** 281-91.

Visintin, A., *et al.* (2003). Lysines 128 and 132 enable lipopolysaccharide binding to MD-2, leading to toll-like receptor-4 aggregation and signal. *J. Biol. Chem.* **278:** 48313-20.

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 versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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