INSTRUCTIONS

EZ-Link® Hydrazide Biotins

21339 21340 21360

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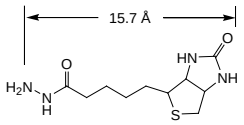
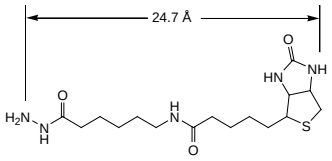
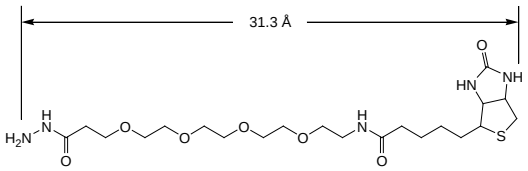
Number	Description	
21339	**EZ-Link Hydrazide Biotin,** 100mg Molecular Weight: 258.34 Spacer Arm: 15.7Å Net Mass Added to Target: 240.11	
21340	**EZ-Link Hydrazide-LC-Biotin,** 50mg Molecular Weight: 371.50 Spacer Arm: 24.7Å Net Mass Added to Target: 353.19	
21360	**EZ-Link Hydrazide-PEG$_4$-Biotin,** 50mg Molecular Weight: 505.26 Spacer Arm: 31.3Å 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 Hydrazide-Biotin 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 1mM sodium periodate ($NaIO_4$). Other sugar groups can be oxidized effectively with 5-10mM 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 hydrazide-biotin reagents without adversely affecting their function in immunoassays. Be aware that monoclonal antibodies may be deficient in glycosylation.

The three hydrazide-biotin reagents differ in spacer arm length and solubility, enabling the user to choose what is most appropriate for the application. Hydrazide Biotin is the simplest reagent, having the shortest possible spacer arm. Hydrazide-LC-Biotin contains a long-chain, albeit simple hydrocarbon, spacer arm that may reduce steric hindrance in biotin-binding assays. Hydrazide-PEG_4-Biotin 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 Hydrazide-PEG_4-Biotin have decreased levels of aggregation when stored in solution over time compared to proteins labeled with the other two hydrazide-biotin reagents.

Hydrazide-biotin 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 hydrazide-biotin reagents can be dissolved at 50mM in dimethylsulfoxide (DMSO) and then diluted into aqueous reaction mixtures. (Do not use dimethylformamide, DMF, in which reagent solubility is poor.) Hydrazide Biotin and Hydrazide-LC-Biotin are soluble directly in aqueous buffers to ~5mM; Hydrazide-PEG_4-Biotin is soluble in aqueous buffers to at least 20mM.
- 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 1-10mM sodium *meta*-periodate ($NaIO_4$; Product No. 20504). Oxidation with periodate is most efficient in acidic conditions (e.g., 0.1M 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 Hydrazide-Biotin Reagents

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

A. Materials Required

- Hydrazide-Biotin Solution: 50mM hydrazide-biotin reagent in dimethylsulfoxide (DMSO, Product No. 20684).
  - For a 50mM solution of Hydrazide Biotin (Product No. 21339), dissolve 12.9mg/mL DMSO.
  - For a 50mM solution of Hydrazide-LC-Biotin (Product No. 21340), dissolve 18.55mg/mL DMSO. Prepare a volume sufficient to achieve the desired final concentration in Step B3.
  - Hydrazide-PEG_4-Biotin (Product No. 21360) is a hygroscopic solid, which is difficult to weigh and dispense. To facilitate handling, make a stock solution immediately before the first use by dissolving the entire contents of the vial (50mg) in 396µL of dry (anhydrous, molecular sieve-treated) organic solvent, such as DMSO. Store the resulting 250mM stock solution of Hydrazide-PEG_4-Biotin at -20°C. Before opening, warm the vial to room temperature to prevent moisture condensation, which may decrease shelf-life.
- Oxidation Buffer: 0.1M sodium acetate buffer, pH 5.5
- Sodium *meta*-periodate (Product No. 20504) solution: 20mM sodium *meta*-periodate in Oxidation Buffer. Prepare solution immediately before use in amber vial or other light-protecting vessel.
- Coupling Buffer: 0.1M sodium phosphate, 0.15M NaCl, pH 7.2 (PBS, Product No. 28372) or other neutral or slightly alkaline, non-amine buffer
- Glycoprotein Solution: 2mg/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 1mL of cold sodium *meta*-periodate solution to 1mL 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 50µL of sodium *meta*-periodate instead of 1mL (results in 1mM periodate final concentration rather than 10mM).

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

   Note: Optimal hydrazide-biotin 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 Hydrazide-Biotin Reagents

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

A. Materials Required

- Hydrazide-Biotin Solution: 50mM hydrazide-biotin reagent in dimethylsulfoxide (DMSO, Product No. 20684)
- MES Buffer: 0.1M MES [(2-*N*-morpholino) ethanesulfonic acid], pH 4.7-5.5 (Product No. 28390)
- EDC (1-Ethyl-3-[3-Dimethylaminopropyl]carbodiimide Hydrochloride) solution: 100mg/mL EDC (Product No. 22980 or 22981) in MES Buffer (results in ~0.5M 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 5-10mg/mL.
2. Add 25µL of Hydrazide-Biotin Solution per 1mL of the protein solution and mix (results in 1.25mM reagent).
3. Add 12.5µL of the EDC solution per 1mL of the protein solution and mix (results in ~6.5mM 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

46610	**Fluorescence Biotin Quantitation Kit**
20036	**Bioconjugate Techniques,** 2nd edition, by Greg T. Hermanson, 2008, Academic Press
28020	**EZ-Link Hydrazide Biocytin,** 25mg
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.

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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

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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.

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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.

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