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 (NaIO₄). 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-PEG₄-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-PEG₄-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 -NH₂ group from the biotinylating 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.
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-PEG4-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 (NaIO₄; 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-PEG₄-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-PEG₄-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

28020  EZ-Link Biocytin Hydrazide, 25 mg
28005  Pierce® Biotin Quantitation Kit

General References


Product References

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