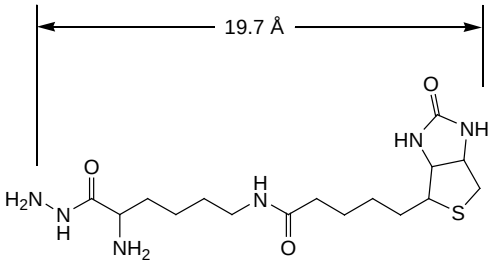
INSTRUCTIONS

EZ-Link Hydrazide Biocytin

MAN0016370
Rev. A.0
Pub. Part No. 2160451.5

28020

Number	Description
28020	**EZ-Link Hydrazide Biocytin,** 25mg Molecular Weight: 386.51 Spacer Arm: 19.7Å Net Mass Addition: 368.20



Storage: Upon receipt store product at room temperature. Product shipped at ambient temperature.

Introduction

Hydrazide-biotin reagents such as Thermo Scientific™ EZ-Link™ Hydrazide Biocytin are useful for biotinylating macromolecules at carbohydrate groups that have been oxidized to form aldehydes. The hydrazide group reacts with carbonyls (aldehydes and ketones), resulting in a hydrazone linkage (Figure 1).

Sialic acid is a common sugar component of protein polysaccharides, and the group is easily oxidized with 1mM sodium *meta*-periodate ($NaIO_4$). Other sugar groups can be oxidized effectively with 5-10mM sodium *meta*-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.

Hydrazide reagents also can be reacted with carboxyl groups using the carbodiimide EDC (Product No. 22980). EDC activates carboxyl groups to bind to the reagent -NH_2 group, 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.

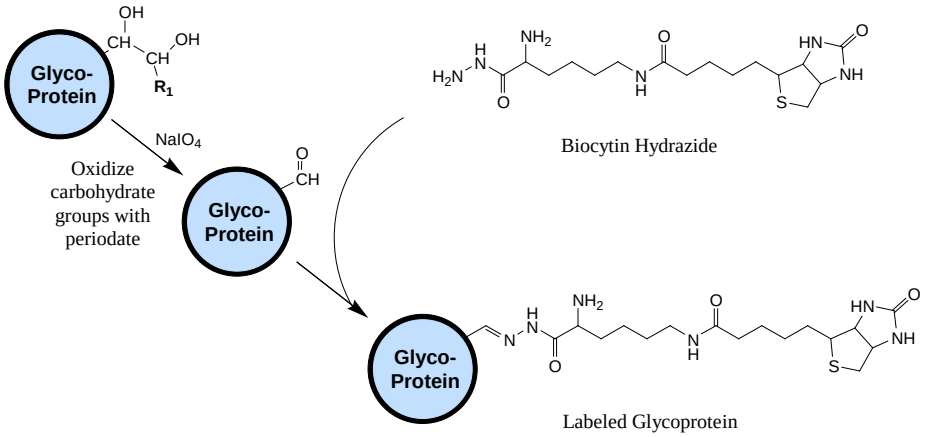


Figure 1. Biotinylation of oxidized carbohydrates with EZ-Link Hydrazide Biocytin.

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

Important Product Information

- Avoid Tris or other primary amine-containing buffers in the oxidation and biotinylation steps as these buffers react with aldehydes and quench the reaction with hydrazides.
- Hydrazide biocytin can be dissolved at 50mM in dimethylsulfoxide (DMSO) then diluted into aqueous reaction mixtures. Alternatively, the reagent can be dissolved directly in aqueous buffers to ~10mM.
- Hydrazides react with carbonyls most efficiently in amine-free, near-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 efficient reaction to the hydrazide group.
- 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 quench the reaction. Phosphate buffers reduce conjugation efficiency, although this effect can be overcome by adding more EDC.

Example Protocol for Labeling Glycoproteins with Hydrazide Biocytin

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

A. Materials Required

- Hydrazide-Biocytin Solution: 50mM hydrazide biocytin in dimethylsulfoxide (DMSO, Product No. 20688) or 5mM hydrazide biocytin in Coupling Buffer. Prepare a volume sufficient to achieve the desired final concentration in step B.4. (usually 1-5mM). Excess (unused) stock solution is stable for approximately 2 months at 4°C.
- 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 (Phosphate-buffered saline, PBS, Product No. 28372) or other neutral or slightly alkaline, non-amine buffer
- Glycoprotein Solution: 2mg/mL of glycoprotein in Oxidation Buffer
- Dialysis Cassette (e.g., Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cassette Kit, 10K MWCO, 0.5-3mL, Product No. 66382) or Desalting Column (e.g., Thermo Scientific™ Zeba™ Spin Desalting Columns, for desalting 0.5-2mL samples, 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-Biocytin Solution to 9 parts oxidized and buffer-exchanged sample (results in 5mM Hydrazide Biocytin); mix for 2 hours at room temperature.

   Note: Optimal Hydrazide-Biocytin 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).

   Note: Biotinylated samples may be stored using the same conditions as for the non-biotinylated sample.

Example Protocol for Labeling Carboxyl Groups with Hydrazide Biocytin

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

A. Materials Required

- Hydrazide-Biocytin Solution: 50mM hydrazide-biocytin reagent in dimethylsulfoxide (DMSO, Product No. 20688)
- 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 (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 (e.g., Slide-A-Lyzer Dialysis Cassette Kit, 10K MWCO, 0.5-3mL, Product No. 66382) or Desalting Column (e.g., Zeba Spin Desalting Columns, for desalting 0.5-2mL samples, Product No. 89891)

B. Procedure

1. Dissolve protein (carboxyl-containing molecule) in MES Buffer at 5-10mg/mL.
2. Add 25µL of Hydrazide-Biocytin 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: Store the biotinylated samples using the same conditions as for the non-biotinylated sample. A typical storage condition is 4°C for several weeks.

Related Thermo Scientific Products

21339	**EZ-Link Hydrazide Biotin,** 100mg
21340	**EZ-Link Hydrazide-LC-Biotin,** 50mg
21360	**EZ-Link Hydrazide-PEG$_4$-Biotin,** 50mg
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.

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 β-nerve growth factor. *J Neurochem* **46:**641-48.

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

Zsembery, Á., *et al.* (2003). Sustained calcium entry through P2X nucleotide receptor channels in human airway epithelial cells. *J Biol Chem* **278:** 13398-408.

Peng, Y., *et al.* (2001). ETB receptor activation causes exocytic insertion of NHE3 in OKP cells. *Am J Physiol Renal Physiol* **280:** 34-42.

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").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

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 thermofisher.com. For a faxed copy, call 800-874-3723 or contact your local distributor.

© 2016 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.

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