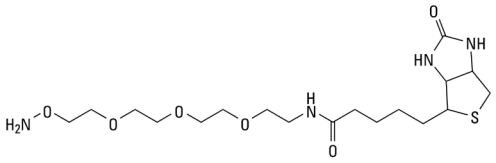
INSTRUCTIONS

EZ-Link™ Alkoxyamine-PEG-Biotin Reagents

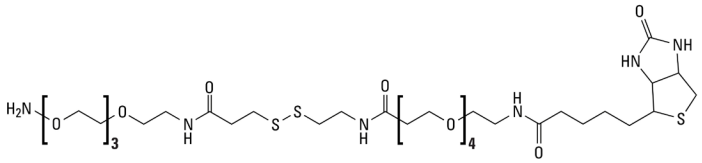
26137 26138 26139

2343.1

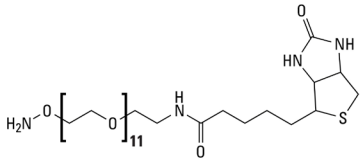
Number	Description
26137	**EZ-Link Alkoxyamine-PEG$_4$-Biotin,** 50mg Molecular Weight: 434.55 Spacer Arm Length: 27.0 Å Net Mass Added to Target: 416.53



26138	**EZ-Link Alkoxyamine-PEG$_4$-SS-PEG$_4$-Biotin,** 50mg Molecular Weight: 845.10 Spacer Arm Length: 56.6 Å Net Mass Added to Target: 827.08



26139	**EZ-Link Alkoxyamine-PEG$_{12}$-Biotin,** 50mg Molecular Weight: 786.97 Spacer Arm Length: 55.4 Å Net Mass Added to Target: 768.95



Storage: Upon receipt store product at -20°C protected from moisture. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific™ EZ-Link™ Alkoxyamine-PEG-Biotin Reagents are used for biotinylating macromolecules at carbohydrate groups that have been oxidized to form aldehydes. The alkoxyamine group reacts to carbonyls (aldehydes and ketones), resulting in an oxime linkage. Sialic acid is a common sugar component of protein polysaccharides and is easily oxidized with 1mM sodium *meta*-periodate ($NaIO_4$). Other sugars are effectively oxidized with 5-10mM sodium *meta*-periodate. Oxidation of sugar moieties enables directed labeling away from polypeptide domains that are important for protein function. Because most polyclonal antibodies are glycosylated in regions other than the antigen-binding sites, labeling is achieved without adversely affecting antibody function in immunoassays. Note that monoclonal antibodies may be deficient in glycosylation.

The EZ-Link Alkoxyamine-PEG-Biotin Reagents contain a multi-functional extended spacer arm that is a flexible, non-immunogenic hydrophilic polyethylene glycol (PEG), which imparts water solubility to labeled molecules. Consequently, antibodies labeled with biotin alkoxyamine reagents exhibit less aggregation when stored in solution compared to antibodies labeled with reagents having only hydrocarbon spacers. The EZ-Link Alkoxyamine-PEG$_4$-SS-PEG$_4$-Biotin has a disulfide bond in the spacer and two PEG chains. The disulfide bond enables cleavage of the biotin group from the labeled proteins, which is especially useful when affinity purifying biotinylated proteins using immobilized avidin, streptavidin or Thermo Scientific™ NeutrAvidin™ Protein.

Alkoxyamine 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 of the alkoxyamine, forming an amide linkage. Using EDC may result in polymerization of the peptide or protein if the molecule has both carboxyls and primary amines on its surface; however, decreasing the EDC amount or increasing the amount of the biotin reagent 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 alkoxyamines.
- For best results, dissolved the alkoxyamine reagent with dimethylsulfoxide (DMSO) at 50mM and then dilute into an aqueous reaction mixture.
- Alkoxyamines 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 (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 might be necessary to obtain the optimal alkoxyamine reaction.
- Aniline (Product No. 88944) is a catalyst that can be used to accelerate the coupling rate of hydrazide and alkoxyamine moieties with reactive aldehydes / ketones (carbonyls).[1]
- EDC-mediated reactions are generally performed in MES buffer at pH 4.5-5. Avoid buffers containing primary amines (e.g., Tris, glycine) or carboxyls (e.g., acetate, citrate) because they will quench the reaction. Phosphate buffers are suboptimal because they reduce conjugation efficiency, although this effect is overcome by adding more EDC.

Example Protocol for Labeling Glycoproteins with an Alkoxyamine-biotin Reagent

Note: The optimal alkoxyamine-biotin concentration and reaction conditions depend on the specific protein and downstream application. For best results, empirically optimize the molar ratio of reagent and glycoprotein.

A. Materials Required

- Alkoxyamine-biotin Solution: 50mM alkoxyamine-biotin reagent in DMSO. Prepare a volume sufficient to achieve the desired final concentration in step B.3.

  Note: Alkoxyamine biotin reagents are hygroscopic solids that are difficult to weigh and dispense. To facilitate handling, make a 250mM stock solution by dissolving the entire contents of the vial (50mg) in a dry (anhydrous, molecular sieve-treated) organic solvent such as DMSO. Store the stock solution at -20°C for up to 1 month; warm the vial to room temperature before opening to prevent moisture condensation.
- Oxidation Buffer: 0.1M sodium acetate, pH 5.5
- Sodium *meta*-periodate (Product No. 20504) at 20mM in Oxidation Buffer: Prepare solution immediately before use in an amber vial or other light-protecting vessel.
- Coupling Buffer: 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2 (PBS, Product No. 28372) or other neutral or slightly alkaline, non-amine buffer
- Glycoprotein at 2mg/mL 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 and mix well. Incubate mixture for 30 minutes on ice or at 4°C protected from light.

   Note: To oxidize only sialic acid groups, add 50µL of sodium *meta*-periodate instead of 1mL, which results in 1mM periodate final concentration rather than 10mM).[2]
2. Remove excess sodium *meta*-periodate and exchange the buffer by dialysis against coupling buffer or with a desalting column equilibrated with coupling buffer.
3. Add 1 part of Alkoxyamine-biotin Solution to 9 parts oxidized and buffer-exchanged protein (results in 5mM alkoxyamine biotin). Mix the reaction for 2 hours at room temperature.
4. Separate the biotinylated protein from non-reacted material by dialysis or desalting. Store the biotinylated protein using the same conditions as for the non-biotinylated sample.

Example Protocol for Labeling Carboxyl Groups with an Alkoxyamine-biotin Reagent

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

A. Materials Required

- Alkoxyamine-biotin Solution: 50mM alkoxyamine-biotin reagent in DMSO
- 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). Prepare EDC immediately before use in step B3.
- Dialysis cassette or desalting column (e.g., Thermo Scientific Slide-A-Lyzer Dialysis Cassette Kit, Product No. 66382 or Thermo Scientific 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 Alkoxyamine-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 for 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 desalting.

   Note: Store the biotinylated sample using the same conditions as for the non-biotinylated sample. A typical storage condition is 4°C for several weeks.

Additional Information from Our Website

- Tech Tip #43: Protein stability and storage

Related Thermo Scientific Products

See the website or catalog for a complete list of biotin-binding resins and beads.

20036	**Bioconjugate Techniques,** 2nd edition, by Greg T. Hermanson, 2008, Academic Press
21339	**EZ-Link Biotin Hydrazide,** 100mg
21340	**EZ-Link Biotin-LC-Hydrazide,** 50mg
20504	**Sodium *meta*-Periodate,** 25g
22980	**EDC,** 5g
88944	**GlycoLink™ Coupling Catalyst,** 100mL kit (includes aniline and buffer)
28005	**Pierce™ Biotin Quantitation Kit**
28372	**BupH™ Phosphate Buffered Saline Packs,** 40 packs, each pack makes 500mL
28390	**BupH MES Buffered Saline Packs,** 10 packs, each pack makes 500mL
66382	**Slide-A-Lyzer Dialysis Cassette Kit**, 3mL capacity, 10K MWCO
89891	**Zeba Spin Desalting Columns**, 7K MWCO, 5 columns for 500–2,000µL samples
20688	**Dimethylsulfoxide (DMSO), Sequanal grade,** 950mL

Cited References

1. Lempens, E., *et al.* (2009). Efficient and chemoselective surface immobilization of proteins by using aniline-catalyzed oxime chemistry. *Chembiochem* **10:**658-62.
2. Zeng, Y., *et al.* (2009). High efficiency labeling of glycoproteins on living cells. *Nat Methods* **6:**207-9.

General References

Mahal, L.K., *et al*. (1997). Engineering chemical reactivity on cell surfaces through oligosaccharide biosynthesis. *Science* **276:**1125-8.

Dirksen, A., *et al*. (2006). Nucleophilic catalysis of oxime ligation. *Angew Chem Int Ed* **45:**7581-4.

Dirksen, A. and Dawson, P. (2008). Rapid oxime and hydrazone ligations with aromatic aldehydes for biomolecular labeling. *Bioconjugate Chem* **19:**2543-8.

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

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