INSTRUCTIONS

EZ-Link Hydrazide-PEG$_4$-Desthiobiotin, No-Weigh Format

MAN0011871
Rev. B.0
Pub. Part No. 2162530

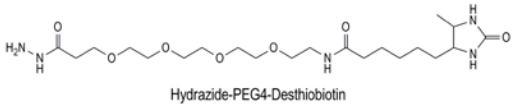
A39262

Number	Description
A39262	**EZ-Link Hydrazide-PEG$_4$-Desthiobiotin, No-Weigh Format,** 5 × 1mg

Molecular Weight: 475.58

Spacer Arm: 31.22Å

Net Mass Addition: 457.26


Hydrazide-PEG4-Desthiobiotin

Solubility: Soluble in DMSO, DMF and aqueous buffer.

Storage: Upon receipt store at 4°C. Product shipped with an ice pack.

Note: Product labels have been provided for your convenience. Please label the vials using one of the labels provided in the Al foil pouch to avoid any confusion as you work with this No-Weigh reagent.

Introduction

The Thermo Scientific™ EZ-Link™ Hydrazide-PEG$_4$-Desthiobiotin reagent is useful for desthiobiotinylating 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, which 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 EZ-Link Hydrazide-PEG$_4$-Desthiobiotin reagent without adversely affecting their function in immunoassays. Be aware that monoclonal antibodies may be deficient in glycosylation.

EZ-Link Hydrazide-PEG$_4$-Desthiobiotin contains a short polyethylene glycol (PEG) spacer arm, which transfers properties to the labeled molecule. For example, antibodies modified with EZ-Link Hydrazide-PEG$_4$-Desthiobiotin have decreased levels of aggregation when stored in solution over time.

Desthiobiotin is a non-sulfur-containing biotin analogue that binds to streptavidin with less affinity than biotin (K_d of 10^{-11}M versus a K_d of 10^{-16}M, respectively).[1-4] Unlike biotinylated proteins, desthiobiotinylated bait proteins and their interacting partners can be readily and specifically eluted under mild conditions when captured on streptavidin by using a biotin elution buffer. The soft release characteristics of desthiobiotin minimize the isolation of naturally biotinylated molecules that can interfere with results and also eliminate the use of harsh elution conditions that can disassociate complexes and/or damage the target protein or cell. This technique is ideal when using native or recombinant proteins that are not expressed with a fusion tag; when isolating captured proteins under native conditions; or when targeting intact cells or cell surface proteins.

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Important Product Information

- Avoid Tris or other primary amine-containing buffers in the oxidation and desthiobiotinylation steps because these buffers react with aldehydes and will quench the reaction with hydrazides.
- Hydrazide reagents can be dissolved in DMSO (Product No. 85190) or DMF (Product No. 20673) and then diluted into aqueous reaction mixtures.
- 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.

Example Protocol for Labeling Glycoproteins with EZ-Link Hydrazide-PEG_4-Desthiobiotin Reagent

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

A. Materials Required

- Water-miscible organic solvent such as dimethylsulfoxide (DMSO, Product No. 85190) or dimethylformamide (DMF, Product No. 20673)
- Oxidation buffer: 0.1M sodium acetate buffer, pH 5.5
- Sodium meta-periodate solution (Product No. 20504): 20mM sodium meta-periodate 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 NaCl, pH 7.2 (PBS, Product No. 28372) or other neutral or slightly alkaline, non-amine buffer
- Glycoprotein solution: 0.2-2mg/mL of glycoprotein in oxidation buffer
- Method for removal of non-reacted desthiobiotin (buffer exchange): gel filtration (e.g., Thermo Scientific™ Zeba™ Spin Desalting Columns, Product No. 89891 or 89894) or dialysis (e.g., Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cassettes, Product No. 66382).

B. Calculations

Note: The extent of desthiobiotin labeling depends on the size and distribution of the aldehyde groups on the protein and the amount of reagent used. This protocol provides instructions for protein labeling at 15X molar excess of label to protein. This is a recommended starting point as this typically produces labeling of 100% of the target protein molecules over a range of protein concentrations. Depending on your sample and application, a range of 5-25X can also be used. For example, compared to reactions involving concentrated proteins solutions, labeling reactions with dilute protein solutions may require a greater-fold molar excess of desthiobiotin reagent to achieve the same incorporation level. For concentrated samples with an abundance of carbonyl groups, a lower molar excess may be desired to prevent over-labeling. In addition, proteins of differing molecular weights will also require different amounts of labeling reagents. Adjust calculations and labeling reagent's starting concentration as appropriate.

Note: Perform all calculations before starting an experiment.

1. Calculate amount of EZ-Link Hydrazide-PEG$_4$-Desthiobiotin required for a labeling reaction:

Step 1: Determine mg of target protein in sample:	Equation: (sample volume in mL) × (sample concentration in mg/mL) = mg protein
Step 2: Convert mg of protein in sample to mmol:	Equation: (mg of protein) / (molecular weight of the protein) = mmol protein
Step 3: Determine number of mmol label needed for desired molar excess:	Equation: (mmol protein) × (desired molar excess) = mmol EZ-Link Hydrazide-PEG$_4$-Desthiobiotin needed
Step 4: Determine amount of label solution required and convert to µL:	Equation: (mmol label required) / [(concentration of label stock in mM) × 10^6µL/L] = µL of stock solution to add to sample

Table 1. Starting sample properties for example calculations.

Volume =	1mL
Concentration =	1mg/mL IgG
IgG molecular weight =	~150kDa
Hydrazide-PEG$_4$-Desthiobiotin stock solution =	10mM
Desired molar excess =	15X

Example:

[(1mL sample) × (1mg/mL IgG)] / [(150,000mg IgG/mmol) × (15-fold excess)] / [(10mmol/L EZ-Link Hydrazide-PEG$_4$-Desthiobiotin) × (10^6µL/L)] = **10µL of 10mM EZ-Link Hydrazide-PEG$_4$-Desthiobiotin required**

C. Prepare Desthiobiotin Solution

1. Remove one 1mg vial of EZ-Link Hydrazide-PEG$_4$-Desthiobiotin. Return the unused vials of reagent to provided pouch and store desiccated at 4°C.
2. Prepare a 10mM solution of EZ-Link Hydrazide-PEG$_4$-Desthiobiotin. Unscrew the cap to the EZ-Link Hydrazide-PEG$_4$-Desthiobiotin reagent vial and solubilize entire contents with the addition of 210µL of DMSO (Product No. 85190) or DMF (Product No. 20673) and mix by pipetting up and down. **The maximum useable volume of the vial is 800µL.**

   Note: If an alternative to a 10mM stock concentration is desired, use the following calculations to determine the volume needed to reconstitute the 1mg vial.

   Final volume (XµL) = (1mg/475.58mg/mmol) / [desired stock concentration (mM)] × 10^6µL/L

D. Desthiobiotin Labeling Reaction

1. Ensure sample to be labeled has a starting concentration of between 0.2mg/mL and 2mg/mL and is in an amine-free buffer at pH 4.7-5.5 or use Thermo Scientific™ Coupling Buffer (Product No. 88944).

   Note: If starting sample contains Tris or other amine-containing buffers, it must be exchanged into coupling buffer. Buffer exchange can be performed by desalting or dialysis [e.g., Zeba Spin Desalting Columns 7K, 5mL (Product No. 89891) or Slide-A-Lyzer MINI Dialysis Device, 10K MWCO, 2mL (Product No. 88404)].

   Note: When desalting solution, equilibrate columns in coupling buffer to remain at a pH 4.7-5.5. Also check solutions to ensure a pH of 4.7-5.5.
2. Make a 0.1M sodium meta-periodate and dissolve in a sodium acetate buffer.
3. Add 110µL of stock 0.1M sodium meta-periodate to the 1mL of protein solution (adjust as appropriate). Cover tube with aluminum foil (oxidation process is light sensitive) and incubate for 30 minutes at 4°C or room temperature. Do not exceed 30 minutes.
4. Remove sodium meta-periodate and excess reagent by using a 7K Zeba Spin Desalting Column.
5. Add the appropriate volume of EZ-Link Hydrazide-PEG$_4$-Desthiobiotin solution to the oxidized protein solution to achieve the desired molar excess of labeling reagent (see calculations in Section B).

   Note: When using low-molecular weight proteins, be sure to not exceed 0.1M of reagent in the labeling reaction, because this will result in excess unlabeled desthiobiotin reagent.

6. Incubate for 1 hour at room temperature or overnight at 4°C with mixing.
7. Desalt the solution by using a 7K Zeba Spin Desalting Column.
8. Dispose of any unused labeling reagent. Alternatively, any unused EZ-Link Hydrazide-PEG_4-Desthiobiotin solution can be stored at -20ºC for up to 2 months, but only if the reagent has been prepared in a high-quality anhydrous DMSO or DMF.

E. Buffer Exchange and Removal of Excess Desthiobiotin Reagent Using a Desalting Column

1. Prepare a Zeba Spin Desalting Column by breaking off the bottom plug and placing the column into a collection tube. Centrifuge the column at 1000 × *g* for 2 minutes; discard the storage buffer and return column to the same collection tube. Place a mark on the side of the column where the compacted resin is slanted upwards. Place the column in centrifuge with the mark facing outward in all subsequent centrifugation steps.
2. Equilibrate the column by adding the equivalent of 50% of resin volume in PBS to the top of the resin bed and centrifuge at 1000 × *g* for 2 minutes. Discard the flow-through and repeat this step 2-3 times. Place column into a new collection tube and apply protein sample directly onto the center of the resin bed. Allow the sample to absorb into the resin.
3. Centrifuge the column at 1000 × *g* for 2 minutes. Collected flow-through containing the purified and labeled protein sample is now ready for coupling and pull-down experiments. Store the protein solution at appropriate conditions. Dispose of desalting column after use.

General Procedure for Pull-down Interaction Assays

Note: See our full line of biotin-binding affinity resins and beads for a product suited for your application and needs. See our EZ-Link Desthiobiotinylation and Pull-Down Kit Instructions (Product No. 16138) for an example protocol of a pull-down interaction assay.

A. Procedure for Coupling Desthiobiotinylated Bait Protein to a Resin

1. Wash and equilibrate resin by adding a suitable wash buffer.
2. Add appropriate amounts of desalted desthiobiotinylated protein (typical range is 10-100µg) and incubate for 30 minutes.
3. Wash and equilibrate resin to remove unlabeled protein. Resin is now ready for a pull-down experiment.

B. Procedure for Pull-down and Protein Elution

1. Add lysate (or other sample containing suspected prey protein) to the resin bound to the labeled bait and incubate for 60 minutes.
2. Centrifuge and wash resin by adding a suitable wash buffer. Remove wash buffer and save for analysis if desired. Repeat as required.
3. Add elution buffer (4mM biotin, 20mM Tris and 50mM NaCl) and incubate at 37°C for 10 minutes or longer. Repeat as required.

Troubleshooting

Problem	Cause	Solution
High levels of desthiobiotinylation	Un-reacted desthiobiotin reagent was not removed	Desalt or dialyze sample before performing assay
Low desthiobiotinylation efficiency	Suboptimal reaction conditions	Optimize molar excess of desthiobiotin reagent
		Perform reactions at 37°C and increase incubation time
Protein is not desthiobiotinylated	There were no available free sugars (carbonyl groups)	Modify protein
		Choose alternative reactive chemistry
	Sugars were not fully oxidized	Optimize oxidation conditions (sodium meta-periodate)

Additional Information Available on Our Website

Refer to our website for a protocol for affinity purification of a desthiobiotinylated molecule from the EZ-Link Desthiobiotinylation and Pull-Down Kit (Product No. 16138).

Related Thermo Scientific Products

16138 **EZ-Link Desthiobiotinylation and Pull-Down Kit**

16129 **NHS-Desthiobiotin,** 50mg

A39263 **EZ-Link Amine-PEG$_4$-Desthiobiotin, No-Weigh™ Format,** 5 × 1mg vials

A39264 **EZ-Link Phosphine-PEG$_4$-Desthiobiotin, No-Weigh Format,** 5 × 1mg vials

A39265 **EZ-Link Sulfo-NHS-LC-Desthiobiotin, No-Weigh Format,** 5 × 1mg vials

28372 **BupH™ Phosphate Buffered Saline Packs,** 40 pack

89891 **Zeba Spin Desalting Columns, 7K MWCO, 5mL,** 5/pkg

89893 **Zeba Spin Desalting Columns, 7K MWCO, 10mL,** 5/pkg

20357 **High Capacity Streptavidin Agarose Resin,** 2mL

20673 **Dimethylformamide (DMF), Sequencing Grade,** 50mL

85190 **Dimethylsulfoxide (DMSO), Sequencing Grade,** 50mL

88816 **Pierce Magnetic Strepavidin Beads,** 1mL

References

1. Green, N.M. (1970) *Meth Enzymol* **18A**:418.
2. Hirsch, J., *et al.* (2002) Easily reversible desthiobiotin binding to streptavidin, avidin, and other biotin-binding proteins: uses for protein labeling, detection and isolation. *Anal Biochem* **308**:343-57.
3. Hofmann, K., *et al.* (1982) Avidin binding of carboxyl-substituted biotin and analogues. *Biochem* **21**:978-84.
4. Hofmann, K., *et al.* (1984) Syntheses of biotinylated and desthiobiotinylated insulins. *Biochem* **23:**2547-53.

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