

EZ-Link Phosphine-PEG₄-Desthiobiotin, No-Weigh Format

16133

2533.0

Number

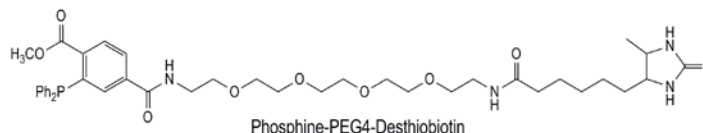
16133

Description**EZ-Link Phosphine-PEG₄-Desthiobiotin, No-Weigh Format, 5 x 1mg**

Molecular Weight: 778.87

Spacer Arm: 30.6Å

Net Mass Addition: 747.87

Solubility: Soluble in DMSO,
DMF and aqueous buffers**Storage:** Upon receipt store at 4°C. Product shipped with an ice pack.**Introduction**

The Thermo Scientific™ EZ-Link™ Phosphine-PEG₄-Desthiobiotin is a versatile desthiobiotinylation reagent for labeling azide-containing molecules. The phosphine group reacts with an azide to produce an aza-ylide intermediate, which is trapped to form a stable, covalent amide bond, also referred to as the Staudinger reaction. Because phosphines and azides are absent from biological systems, there is minimal background labeling of cells or lysates. Labeled proteins can be purified using immobilized streptavidin, avidin or Thermo Scientific™ Streptavidin Protein Affinity Resins.

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, which 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.

Important Product Information

- Reactions with phosphines and azides are more efficient at high concentrations and temperatures (i.e., 23-37°C). Typical reaction times are less than 4 hours; however, incubating for longer times can improve efficiency.

Note: There is no harm in reacting longer than the specified time other than the possibility of ordinary protein degradation or microbial growth. Although excess non-reacted and hydrolyzed desthiobiotin reagent remains in the solution, it is often possible to perform preliminary tests of the labeled protein by ELISA or Western blot. Once function has been confirmed, buffer exchange and label protein for optimal performance and stability using the procedure in Section C.

- Dissolve EZ-Link Phosphine-PEG₄-Desthiobiotin in a dry, water-miscible organic solvent such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF) before diluting in final reaction buffer. This solution will remain stable at -20°C for 2 months when stored with a desiccant in a foil pouch.

Note: If reconstituting the reagent in a non-organic solvent such as PBS, use the reagent immediately. If possible, avoid reducing agents in reaction buffers, which can interfere with azide stability.

Additional Materials Required

- Water-miscible organic solvent such as DMSO (Product No. 85190) or DMF (Product No. 20673)
- Reaction Buffer: Thermo Scientific™ BupH™ Phosphate Buffered Saline (Product No. 28372) or other sulfhydryl-free buffer having pH 6.5-7.5 to use as a reaction buffer (see Important Product Information and Related Thermo Scientific Products Sections)
- Desalting columns or dialysis units for buffer exchange and removal of excess reagent following modification [e.g., Thermo Scientific™ Zeba™ Spin Desalting Columns (Product No. 89891 or 89893) or Thermo Scientific™ Slide-A-Lyzer™ Dialysis Units (Product No. 66382 or 66807)]
- Elution buffer (4mM biotin, 20mM Tris and 50mM NaCl)

Procedure for Desthiobiotinylation of Proteins with EZ-Link Phosphine-PEG₄-Desthiobiotin

A. Calculations

The extent of desthiobiotin labeling depends on the size and distribution of azide 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 because 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 azide residues, a lower molar excess may be desired to prevent over-labeling. Adjust calculations and labeling reagent's starting concentration as appropriate. **Perform all calculations before starting an experiment.**

1. Calculate amount of EZ-Link Phosphine-PEG₄-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 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 Phosphine-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 ⁶ µL/L = µL stock solution to add to sample

Example calculations for a typical antibody labeling are provided below as a combined equation. Starting sample: Volume: 1ml, Concentration: 1mg/ml IgG, Approximate IgG molecular weight: 150,000, Concentration of EZ-Link Phosphine-PEG₄-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 Phosphine-PEG₄-Desthiobiotin) × 10⁶ µL/L = **10µL of 10mM EZ-Link Phosphine-PEG₄-Desthiobiotin required**

B. Prepare Desthiobiotin Solution

Note: See the Important Product Information Section above for instructions on proper handling and storage.

1. Prepare an azide-containing protein sample in reaction buffer.
2. Remove one 1mg vial of EZ-Link Phosphine-PEG₄-Desthiobiotin. Return the unused vials of reagent to provided pouch and store desiccated at 4°C.
3. Prepare a 10mM solution of EZ-Link Phosphine-PEG₄-Desthiobiotin. Unscrew the cap to the EZ-Link Phosphine-PEG₄-Desthiobiotin reagent vial and solubilize entire contents with the addition of 128µL of DMSO or DMF and mix by pipetting up and down.

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.

$$\text{Final volume (XµL)} = [(1\text{mg} / 778.87\text{mg/mmol}) / (\text{desired stock concentration mM})] \times 10^6 \mu\text{L/L}$$

C. 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 such as PBS at pH 7.2-8.

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

2. Add the appropriate volume of EZ-Link Phosphine-PEG₄-Desthiobiotin solution to the protein solution to achieve the desired molar excess of labeling reagent (see calculations in Section A).

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 excessive labeling and may make it difficult to remove the excess unlabeled desthiobiotin reagent in Section D.

3. Immediately dispose of any unused labeling reagent. Alternatively, any unused EZ-Link Phosphine-PEG₄-Desthiobiotin solution can be stored at -20°C for up to 2 months only if the reagent has been prepared in a high-quality anhydrous DMSO or DMF.

4. Incubate the reaction at 37°C for 2-4 hours or at room temperature for 16-24 hours.

Note: There is no harm in reacting longer than the specified time other than the possibility of ordinary protein degradation or microbial growth.

5. Remove excess non-reacted EZ-Link Phosphine-PEG₄-Desthiobiotin using a desalting column or a dialysis cassette. Refer to Section D for desalting procedure.

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

Note: See our full product line of Zeba Spin Desalting Columns for a format suited to your desired sample size. Because of the larger size of desthiobiotinylation reagents and the high molar excess used for labeling, use 30% less sample volume than the maximum recommended for any appropriate volume of desalting column to ensure removal of unreacted tag.

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 $1000 \times g$ 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 the 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 $1000 \times g$ for 2 minutes. Discard the flow-through and repeat this step 2-3 times.
3. 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.
4. Centrifuge the column at $1000 \times g$ for 2 minutes. Collected flow-through which contains 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 desired 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.
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	Possible Cause	Solution
Low desthiobiotinylation efficiency	Suboptimal reaction conditions	Optimize conjugation conditions by altering molar excess of desthiobiotin reagent to azide
		Perform conjugation reactions at 37°C and increase incubation time

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	EZ-Link NHS-Desthiobiotin, 50mg
16130	EZ-Link Hydrazide-PEG₄-Desthiobiotin, No-Weigh™ Format, 5 x 1mg vials
16131	EZ-Link Amine-PEG₄-Desthiobiotin, No-Weigh Format, 5 x 1mg vials
16136	EZ-Link Sulfo-NHS-LC-Desthiobiotin, No-Weigh Format, 5 x 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 Sequencing Grade (DMF), 50mL
85190	Dimethyl sulfoxide Sequencing Grade (DMSO), 50mL
88903	GlcNAz, 5mg
88904	ManNAz, 5mg
88905	GalNAz, 5mg
88816	Pierce Magnetic Streptavidin 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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