INSTRUCTIONS

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

MAN0011870
Rev. B.0
Pub. Part No. 2162529

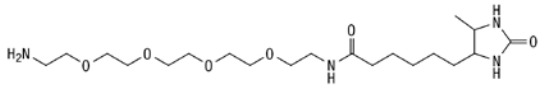
A39263

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

Molecular Weight: 432.55

Spacer Arm: 28.81Å

Net Mass Addition: 431.55



Amine-PEG$_4$-Desthiobiotin

Solubility: 10mg/mL in DMSO; also soluble in water and aqueous buffers

Storage: Upon receipt store at -20°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™ Amine-PEG$_4$-Desthiobiotin reagent is a water-soluble, polyethylene glycol (PEG)-containing reagent with a terminal primary amine (–NH_2). The PEG spacer arm is hydrophilic and confers greater solubility to labeled proteins compared to reagents having only hydrocarbon spacers. The amine group of this reagent can be reacted with carboxyl groups on carboxy termini, aspartate residues or glutamate residues using EDC (Product No. 22980), a water-soluble carbodiimide crosslinker. EDC activates carboxyl groups to bind to the –NH_2 group of the EZ-Link Amine-PEG$_4$-Desthiobiotin, forming an amide bond. For more information, consult the product instructions for EDC.

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.

Additional Materials Required

- MES buffer: 0.1M [(2-*N*-morpholino)ethanesulfonic acid], pH 4.7-5.5 (Thermo Scientific™ BupH™ MES Buffered Saline Packs, Product No. 28390)

  Note: EDC reactions are generally performed using MES buffer at pH 5-6. Avoid buffers containing primary amines (Tris, glycine, etc.) or carboxyls (acetate, citrate, etc.) because they will quench the reaction. Phosphate buffers (pH 6.5-7.3) may be used, but lower conjugation efficiency can result, requiring more EDC to obtain the same results.

- EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride) (Product No. 22980 or 22981)

- 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)

Procedure for Desthiobiotinylating Proteins

The example procedure in these instructions uses EZ-Link Amine-PEG$_4$-Desthiobiotin with EDC to label carboxyl groups on a protein. Reagent proportions must be optimized to achieve the desired extent of labeling and to control undesired polymerization. Because EDC causes conjugation of carboxyl groups to primary amino groups, protein (or peptide) polymerization may result if the protein has both functional groups on its surface. To minimize polymerization, use a large molar excess (e.g., 100-fold over the protein) of EZ-Link Amine-PEG$_4$-Desthiobiotin and a limiting amount of EDC. Using a large molar excess of EZ-Link Amine-PEG$_4$-Desthiobiotin ensures that every EDC-activated carboxyl group on the protein is more likely to react with EZ-Link Amine-PEG$_4$-Desthiobiotin than an amine on the protein. Using a limiting amount of EDC (e.g., five- to 20-fold molar excess over the protein) ensures that carboxyl-to-amine conjugation will cease after only a few protein carboxyl groups have been modified. Not every carboxyl group activated by EDC will result in reaction to an amine; a significant proportion will hydrolyze before encountering an amine. Consequently, a five-fold molar excess of EDC will usually result in one to two conjugations, depending on reactant concentrations.

A. Calculations

Note: Perform all calculations before starting an experiment.

1. Calculate amount of EZ-Link Amine-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 of label needed for desired molar excess:	Equation: (mmol protein) × (desired molar excess) = mmol EZ-Link Amine-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

2. Calculate amount of EDC required and convert to µL:

   Equation: (mmol EDC required) / (concentration of EDC in mM) × 10^6µL/L = µL of EDC needed

B. Example Protocol for Desthiobiotinylating BSA

Example calculations and protocol for a typical protein labeling are provided below (Table 1):

Table 1. Starting sample properties for example calculations.

Volume =	1mL
Concentration =	0.15mg/mL BSA
BSA molecular weight =	~66.5kDa
Amine-PEG$_4$-Desthiobiotin stock solution concentration =	50mM
Desired molar excess =	100X
EDC solution concentration =	10mM
Desired EDC molar excess =	10X

Note: This example procedure uses a 1:100:10 molar ratio of BSA:EZ-Link Amine-PEG$_4$-Desthiobiotin:EDC. Reagent proportions must be optimized to achieve the desired extent of labeling and to control undesired polymerization.

Example:

[(1mL sample) × (0.15mg/mL BSA)] / [(66,500mg BSA/mmol) × (100 fold excess)] / [(50mmol/L EZ-Link Amine-PEG$_4$-Desthiobiotin) × (10^6µL/L)] = **4.5µL of 50mM EZ-Link Amine-PEG$_4$-Desthiobiotin required**

[(1mL sample) × (0.15mg/mL BSA)] / [(66,500mg BSA/mmol) × (10-fold excess)] / [(10mmol/L EDC) × (10^6µL/L)] = **2.3µL of 10mM EDC required**

1. Ensure that the sample is in an amine-free and carboxyl-free buffer such as MES. Perform all necessary calculations for your sample to determine how much of each reagent is required in the following steps.
2. Remove one 1mg vial of EZ-Link Amine-PEG$_4$-Desthiobiotin and return the unused vials of reagent to provided pouch and store desiccated at 4°C.
3. Prepare a 50mM solution of EZ-Link Amine-PEG$_4$-Desthiobiotin in DMSO. For example, dissolve 1mg of EZ-Link Amine-PEG$_4$-Desthiobiotin in 46µL of DMSO (Product No. 85190). **The maximum useable volume of the vial is 800µL.**

   Note: If an alternative to a 50mM stock concentration is desired, use the following calculations to determine the volume needed to reconstitute the 1mg vial.

   Final volume (XµL) = (1mg/432.55mg/mmol) / (desired stock concentration mM) × 10^6µL/L
4. Add an appropriate volume of EZ-Link Amine-PEG$_4$-Desthiobiotin solution to the protein solution and mix.
5. Immediately before use, prepare a 10mM solution of EDC in MES Buffer (e.g., dissolve 1.9mg in 1000µL of buffer).
6. Add an appropriate volume of the EDC solution to the solution from Step 3 and mix.
7. Incubate for 2 hours at room temperature with stirring or mixing.
8. Centrifuge to remove any precipitate that formed during the reaction.
9. Remove the non-reacted desthiobiotinylation reagent and EDC by-products by desalting or dialysis (see below).

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

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 × *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 × *g* for 2 minutes. Discard the flow-through and repeat this step two to three 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 × *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 desired application and needs. See our EZ-Link Desthiobiotinylation and Pull-Down Kit instruction booklet (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 amount 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 37ºC 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 and/or perform reactions at 37°C and increase incubation time
Protein is not desthiobiotinylated	There were no available free carboxyl groups	Modify protein or choose alternative reactive chemistry
	Reagent hydrolyzed and became non-reactive	Do not store reagent in aqueous solutions or solvent that has been absorbed in water. Bring up reagents in organic solvents such as DMSO or DMF

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

16129	**EZ-Link NHS-Desthiobiotin,** 50mg
A39262	**EZ-Link Hydrazide-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
16138	**EZ-Link Desthiobiotinylation and Pull-Down Kit**
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, 5mL,** 5/pkg
20357	**High Capacity Streptavidin Agarose Resin,** 2mL
20673	**Dimethylformamide (DMF), Sequencing Grade,** 50mL
85190	**Dimethylsulfoxide (DMSO), Sequencing Grade,** 50mL
88816	**Pierce Streptavidin Magnetic 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 dethiobiotinylated insulins. *Biochem* **23:**2547-53.

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