INSTRUCTIONS

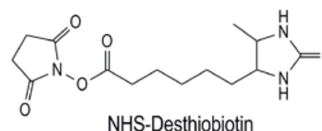
Thermo SCIENTIFIC

EZ-Link NHS-Desthiobiotin
EZ-Link Sulfo-NHS-LC-Desthiobiotin

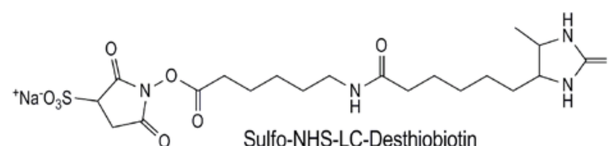
MAN0011869
Rev. B.0
Pub. Part No. 2162528

16129 A39265

Number	Description
16129	EZ-Link **NHS-Desthiobiotin**, 50mg
	Molecular Weight: 311.33
	Spacer Arm: 9.7Å
	Net Mass Addition: 197.13



Number	Description
A39265	**EZ-Link Sulfo-NHS-LC-Desthiobiotin No-Weigh Format,** 5 × 1mg
	Molecular Weight: 526.54
	Spacer Arm: 17.3Å
	Net Mass Addition: 310.20



Storage: NHS-Desthiobiotin and Sulfo-NHS-LC-Desthiobiotin are shipped with an ice pack. Upon receipt, store desiccated at -20°C.

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™ NHS-Desthiobiotin and EZ-Link Sulfo-NHS-LC-Desthiobiotin provide simple and efficient labeling of antibodies, proteins and any other primary amine-containing macromolecules in a solution. Differing in the spacer arm length and the ester moiety, these reagents offer researchers the possibility of optimizing labeling experiments where steric hindrance of desthiobiotin binding is an important factor. EZ-Link Sulfo-NHS-LC-Desthiobiotin is packaged in a No-Weigh™ format, which eliminates the difficulties associated with weighing small quantities of reagent and allows the customer to reseal the vials to reduce the risk of hydrolysis.

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.

Important Product Information

- *N*-Hydroxysuccinimide (NHS) esters and Sulfo-*N*-Hydroxysuccinimide (Sulfo-NHS) esters are among the most popular targeting chemistry for a wide variety of labeling reagents. NHS and Sulfo-NHS-activated desthiobiotin reagents react efficiently with primary amine groups ($-NH_2$) to form stable amide bonds. Proteins, including antibodies, generally have several primary amines in the side chain of lysine (K) residues and the N-terminus of each polypeptide that are available as targets for labeling. It is essential that the molecules to be labeled are contained in a buffer that is free of primary amines (e.g., Tris-HCl) with pH between 7-9. If necessary, dialyze or otherwise desalt the sample into an amine-free buffer such as phosphate-buffered saline (PBS).

- The NHS-containing reagents are very moisture sensitive and readily hydrolyze to become inactive. To maximize reliability and ease of handling, the Sulfo-NHS-LC-Desthiobiotin reagent is provided in a convenient No-Weigh screw-cap vial pack, which is designed to be solublized and used in the container provided. Dissolve NHS-Desthiobiotin and Sulfo-NHS-LC-Desthiobiotin in organic solvents such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF) just before use. This will reduce or eliminate any hydrolysis before addition to a labeling reaction. In addition, any unused solution will remain stable at -20°C for 2 months when stored with desiccant in the supplied foil pouch. Do not use old organic solvents, because they may become contaminated with trace amounts of water after long-term storage.

  Note: If required, Sulfo-NHS-LC-Desthiobiotin is also soluble in aqueous buffers, which can be used as an alternate to organic solvents; however, the reagent will begin hydrolyzing immediately and must be used quickly. Any unused reagent must be discarded.

Additional Materials Required

- Water-miscible organic solvent such as DMSO (Product No. 85190) or DMF (Product No. 20673)
- Elution buffer (4mM biotin, 20mM Tris and 50mM NaCl)
- Method for removal of non-reacted desthiobiotin reagent (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

A. Calculations

The extent of desthiobiotin labeling depends on the size and distribution of amino 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. However, adjustment for your specific sample may be desired. For example, compared to reactions involving concentrated protein 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 lysine residues, 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. For example, a lower molar excess is typically used for proteins with molecular weights below 30kDa. **Perform all calculations before starting an experiment.**

1. Calculate amount of desthiobiotin reagent 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 desthiobiotin reagent 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

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 desthiobiotin stock solution: 10mM. Desired molar excess: 15X

Example: (1mL sample) × (1mg/mL IgG) / (150,000mg IgG/mmol) × (15-fold excess) / (10mmol/L desthiobiotin solution) × 10^6µL/L = **10µL of 10mM desthiobiotin required**

B. Prepare Desthiobiotin Solution

Note: The NHS-containing reagents are very moisture sensitive and readily hydrolyze to become inactive. See the Important Product Information Section above for instructions on proper handling and storage. The following example uses Sulfo-NHS-LC-Desthiobiotin reagent; however, depending on the choice of reagent used, the exact calculations will have to be adjusted accordingly (using the correct molecular weight of the reagent being used).

1. Remove one 1mg vial of Sulfo-NHS-LC-Desthiobiotin. Return the unused vials of reagent to provided pouch and store desiccated at 4°C. Alternatively, weigh out the appropriate amount of NHS-Desthiobiotin.
2. Prepare a 10mM solution of the desthiobiotin reagent with the addition of an appropriate volume of DMSO or DMF (see calculations). **The maximum useable volume of the Sulfo-NHS-LC-Desthiobiotin 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. Use the following equation with 311.33 as the molecular weight of NHS Desthiobiotin and 526.54 as the molecular weight of the Sulfo-NHS-LC-Desthiobiotin.

   Final volume (XµL) = (1mg / 526.54mg/mmol) / (desired stock concentration mM) × 10^6µL/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 Sulfo-NHS-LC-Desthiobiotin solution to the protein solution to achieve the desired molar excess of labeling reagent (see calculations in Section A). Any unused Sulfo-NHS-LC-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.

   Note: When using low molecular-weight proteins, be sure to not exceed 0.1M of reagent in the labeling reaction, as this will result in excessive labeling and might make it difficult to remove the excess hydrolyzed label in Section D.
3. Incubate the reaction on ice for two hours or at room temperature for 30-60 minutes.

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

D. Buffer Exchange and Remove 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. Due to 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 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 × *g* for 2 minutes. Collected flow-through that 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 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	Possible Cause	Solution
Protein is not desthiobiotinylated	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 anhydrous organic solvents such as DMSO or DMF
	Protein sample contained secondary source of amines (e.g., Tris buffer, glycine)	Buffer exchange sample into an amine free buffer by dialysis or desalting
	Suboptimal reaction conditions for target protein concentration	Optimize molar excess of desthiobiotin reagent
	Limited free or accessible amines were available on the protein	Modify protein or choose alternative reactive chemistry
Binding capacity of streptavidin resin compromised	Non-reacted desthiobiotin was not effectively removed and is competing for resin binding	Desalt sample before performing assay. Be sure to not exceed capacity of the desalting columns
Interacting protein was not isolated	Weak or transient interaction	Wash conditions too stringent – lower the number of washes and ionic strength of wash buffer
	Poor expression level of prey protein	Apply more protein sample. Increase incubation time. Increase amount of bait protein used
	Excessive desthiobiotinylation hindered target protein binding site	Reduce molar excess of desthiobiotinylation reagent or use a reagent that targets a different functional group
	Binding or sample preparation conditions were insufficient to maintain or allow protein interactions	Try alternative buffers for sample preparation, binding or washing procedures

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High background or contaminating proteins	Isolation of naturally biotinylated proteins	Reduce elution time and temperature or dilute elution buffer 20-40%
		Pre-clear sample by incubating with unlabeled streptavidin resin
	Insufficient washing	Increase number of washes or add trace amounts (0.2%) of non-ionic solution such as NP-40 detergent to wash buffers

Additional Information Available on Our Website

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

Related Thermo Scientific Products

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

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

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

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

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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