INSTRUCTIONS

EZ-Link® Micro Sulfo-NHS-LC-Biotinylation Kit

21935

1786.2

Number	Description
21935	**EZ-Link Micro Sulfo-NHS-LC-Biotinylation Kit**, sufficient reagents for 8 labeling reactions each containing 50-200µg of protein in 200-700µL reaction volumes

Kit Contents:

No-Weigh™ Sulfo-NHS-LC-Biotin, 8 × 1mg microtubes

Molecular Weight: 556.59

Spacer Arm: 22.4Å

BupH™ Phosphate Buffered Saline Pack, 1 pack, 0.1M sodium phosphate. 0.15M sodium chloride; pH 7.2 when reconstituted in 500mL of ultrapure water

Zeba™ Spin Desalting Column, 2mL, 10 columns, for 200-700µL samples, 7000 MWCO

Storage: Upon receipt store biotin reagent at -20°C. Store all other components at 4°C. Biotin reagent is shipped with an ice pack. All other components are shipped at ambient temperature.

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Introduction

The Thermo Scientific EZ-Link Micro Sulfo-NHS-LC-Biotinylation Kit contains the required reagents for labeling macromolecules containing primary amino groups and desalting columns for purifying the labeled molecule. The kit is structured for labeling 50-200µg of protein in 200-700µL. The No-Weigh Sulfo-NHS-LC-Biotin is packaged in convenient pre-measured microtubes, eliminating difficulties associated with weighing small quantities of reagent.

Biotin is a small naturally occurring vitamin that binds with high affinity to avidin and avidin-like proteins. Because biotin is small (244Da), it can be conjugated to many proteins without altering their biological activities. The labeled protein or other molecule can be detected in ELISA, dot blot or Western blot application using avidin or avidin-like probes.

N-Hydroxysuccinimide (NHS) esters of biotin are the most popular type of biotinylation reagent. NHS-activated biotins react efficiently with primary amino groups (-NH_2) in pH 7-9 buffers to form stable amide bonds (Figure 1). Proteins typically have many sites for labeling, including the primary amine in the side chain of lysine (K) residues and the N-terminus of each polypeptide. Several different NHS esters of biotin are available with varying properties and spacer arm lengths. The sulfo-NHS ester reagent in this kit is water-soluble, enabling reactions to be performed in the absence of organic solvents.

The EZ-Link Micro Sulfo-NHS-LC-Biotinylation Kit combines the basic reagents, tools and easy-to-follow instructions for biotin-labeling amine-containing macromolecules. With this kit, even researchers who have never before labeled antibodies or other proteins can expect to obtain results comparable to those obtained in commercial laboratories.

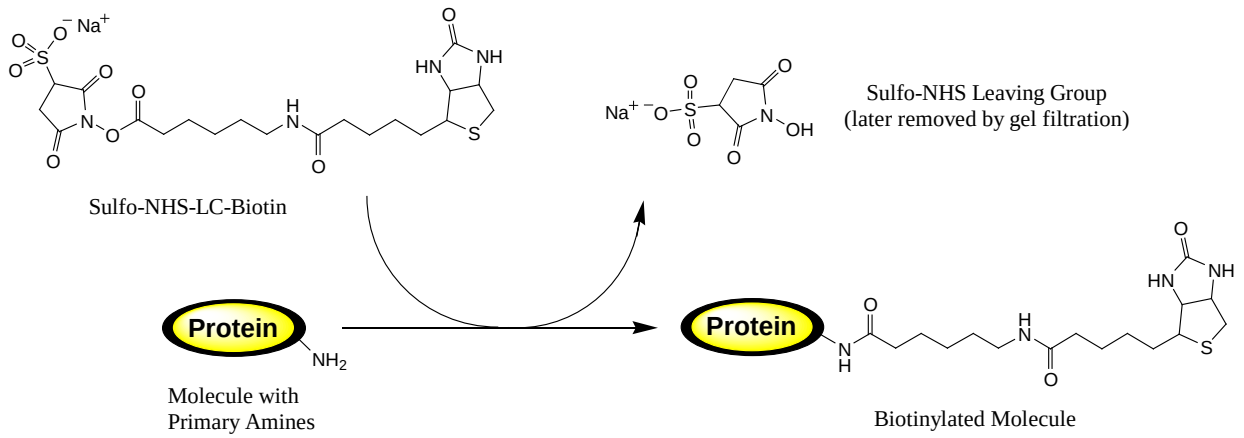

Figure 1. Reaction of Sulfo-NHS-LC-Biotin with primary amine. If drawn to scale, the oval representing the protein would be many times larger than the structures and would likely contain several amino groups. Note that NHS is a leaving group (byproduct) in the reaction. The leaving group and any non-reacted biotin reagent are removed during the desalting step.

Important Product Information

- Use reconstituted Sulfo-NHS-LC-Biotin immediately. The NHS-ester moiety readily hydrolyzes and becomes non-reactive; therefore, do not prepare solutions for storage. Discard any unused reconstituted reagent.
- Sulfo-NHS-LC-Biotin is moisture-sensitive. Immediately before use, puncture the microtube foil with a pipette tip, add solvent and mix by pipetting up and down. After use, cut the used microtube from the unused microtubes and discard. Store the microtube strip desiccated at -20ºC in the foil pouch provided.
- Avoid buffers containing primary amines (e.g., Tris or glycine), as these will compete with the intended reaction (see Figure 1). If necessary, dialyze or otherwise desalt to exchange the protein sample into an amine-free buffer such as phosphate-buffered saline (one packet is included in this kit).
- The desalting columns provided in this kit are best suited for processing biotinylation reactions containing 50-200µg of protein in approximately 200-700µL. For smaller amounts of protein or reaction volumes, perform both the biotinylation reaction and buffer exchanges in a single Thermo Scientific Slide-A-Lyzer MINI Dialysis Unit (see Additional Information and Related Thermo Scientific Products). For reaction volumes that are too large for processing with a desalting column, split the sample between two columns or use an appropriate Slide-A-Lyzer® Dialysis Cassette for buffer exchanges.
- The biotin reagent is first prepared at ~5mg/mL before adding to the reaction mixture. To avoid having to pipette volumes less than 1µL when biotinylating low protein amounts, dilute the biotin reagent further (e.g., 1:10 dilution). To minimize hydrolysis in such a dilute solution, dissolve the biotin reagent in either anhydrous DMSO or DMF and make dilutions with the same solvent.

Procedure for Biotinylating Proteins

A. Calculations

The extent of labeling depends on the size and distribution of amino groups on the protein and the reagent amount used. Compared to reactions involving concentrated protein solutions, labeling reactions with dilute protein solutions require a greater fold molar excess of biotin reagent to achieve the same incorporation level. Experiments that used a 50-fold molar excess of biotin reagent to label 50-200µg of antibody (human IgG) for 30 minutes at room temperature resulted in 1-4 biotin groups per antibody molecule. Adjust the molar ratio of Sulfo-NHS-LC-Biotin to protein to obtain the level of incorporation desired. Instructions for using Sulfo-NHS-LC-Biotin (Product No. 21335) for labeling cells are available from the website.

1. Calculate the millimoles of Sulfo-NHS-LC-Biotin to add to the reaction for a 50-fold molar excess:

$$\text{mL protein} \times \frac{\text{mg protein}}{\text{mL protein}} \times \frac{\text{mmol protein}}{\text{mg protein}} \times \frac{50\ \text{mmol Biotin}}{\text{mmol protein}} = \text{mmol Biotin}$$

- 50 = Recommended molar fold excess of biotin per protein sample

2. Calculate microliters of 9mM Sulfo-NHS-LC-Biotin (prepared in Step B.3) to add to the reaction:

$$\text{mmol Biotin} \times \frac{557\text{ mg}}{\text{mmol Biotin}} \times \frac{200\,\mu\text{L}}{1.0\text{ mg}} = \mu\text{L Biotin Solution}$$

- 557 = Molecular weight of Sulfo-NHS-LC-Biotin
- 200 = Microliters of solvent in which 1.0mg of Sulfo-NHS-LC-Biotin is dissolved to make 9mM

Example: For 0.7ml of a 0.29mg/mL IgG (150,000 MW) solution, 8µL of 9mM Sulfo-NHS-LC-Biotin will be added.

$$0.7\text{ mL IgG} \times \frac{0.29\text{ mg IgG}}{1\text{ mL IgG}} \times \frac{1\text{ mmol IgG}}{150{,}000\text{ mg IgG}} \times \frac{50\text{ mmol Biotin}}{1\text{ mmol IgG}} = 0.0000676\text{ mmol Biotin}$$

$$0.0000676\text{ mmol Biotin} \times \frac{557\text{ mg}}{\text{mmol Biotin}} \times \frac{200\,\mu\text{L}}{1.0\text{ mg}} = 7.5\,\mu\text{L Biotin Solution}$$

B. Biotin-labeling Reaction

1. Dissolve 50-200µg of protein in 200-700µL of phosphate-buffered saline (PBS) according to the calculation made in Section A. Prepare the Thermo Scientific BupH PBS as directed on the package label.

   Note: Protein already dissolved in amine-free buffer at pH 7.2-8.0 may be used without buffer exchange. Proteins in Tris or other amine-containing buffers must be exchanged into PBS. Perform buffer exchange of 200-700µL samples by dialysis or using a desalting column included in this kit. This kit contains 10 single-use desalting columns and eight microtubes of biotin reagent. Two columns may be used for buffer exchange, but the remaining eight columns are needed to remove excess biotin reagent after performing the biotinylation reaction for each of the eight reactions.

2. Immediately before use, cut off one microtube of Sulfo-NHS-LC-Biotin from the No-Weigh Microtube Strip. Return the unused strip of microtubes to its pouch and store desiccated at -20ºC.

3. With a pipette tip, puncture the foil top on the biotin reagent microtube. To prepare 9 mM, add 200 µl of solvent (e.g., water, DMF or DMSO; see the Important Product Information Section) to the tube and mix by pipetting up and down.

4. Add the appropriate volume of Sulfo-NHS-LC-Biotin solution (see calculations in Section A) to the protein solution.

5. Incubate the reaction on ice for two hours or at room temperature for 30-60 minutes. There is no harm in reacting longer than the specified time other than the possibility of ordinary protein degradation or microbial growth.

   Note: Although excess non-reacted and hydrolyzed biotin 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 the labeled protein for optimal performance and stability using the procedure in Section C.

C. Buffer Exchange and Excess Biotin Removal

1. Prepare a Zeba Spin Desalting Column by breaking off the bottom plug and placing the column into a 15mL 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 upward. Place column in centrifuge with the mark facing outward in all subsequent centrifugation steps.

2. Equilibrate the column by adding 1mL of PBS to the top of the resin bed and centrifuging at 1000 × *g* for 2 minutes. Discard the flow-through and repeat this step 2-3 times.

3. Place column into a new 15mL collection tube and apply protein sample directly onto the center of the resin bed. Allow sample to absorb into the resin.

   Note: For samples < 400µL, add 100µL ultrapure water stacker on top of the absorbed sample to maximize recovery.

4. Centrifuge the column at 1000 × *g* for 2 minutes. The collected flow-through solution is the purified protein sample. Store the protein solution in appropriate conditions.

Troubleshooting

Problem	Possible Cause	Solution
Lack of biotinylation	No amines were available on molecule of interest	Use a biotinylation reagent that targets a different functional group or convert sulfhydryl to amine using Aminoethyl-8 (Product No. 23010)
	Buffer contained primary amines	Use a non-amine-containing buffer
	Reagent was not reactive; caused by hydrolysis of the NHS ester	Use reagent immediately upon reconstitution
	Not enough biotin reagent was added to the reaction mixture	Increase the molar excess of biotin reagent to protein
Low level of biotinylation	Carrier protein was added to purified IgG to help stabilize it	Remove carrier protein before biotinylation to reduce competition for labeling
Protein is non-functional	Excessive biotinylation	Reduce the molar excess of biotinylation reagent, or reduce time or temperature for biotinylation
Low protein recovery after desalting	No stacker used	Apply a stacker above sample
	Unstable protein	Equilibrate column in a suitable buffer

Additional Information

Please visit the website for additional information including the following:

- Tech Tip #14: Perform labeling and other reactions in Slide-A-Lyzer Dialysis Cassettes
- Tech Tip #43: Protein stability and storage

Related Thermo Scientific Products

21925 **EZ-Link Micro Sulfo-NHS-Biotinylation Kit**

21326 **No-Weigh Sulfo-NHS-Biotin,** 8 × 1mg microtubes

21945 **EZ-Link Micro Sulfo-NHS-SS-Biotinylation Kit**

21327 **No-Weigh Sulfo-NHS-LC-Biotin,** 8 × 1mg microtubes

21445 **EZ-Link Sulfo-NHS-SS-Biotinylation Kit**

21328 **No-Weigh Sulfo-NHS-SS-Biotin,** 8 × 1mg microtubes

21955 **EZ-Link Micro NHS-PEG$_4$-Biotinylation Kit**

21329 **No-Weigh NHS-PEG$_4$-Biotin,** 8 × 2mg microtubes

20347 **Streptavidin Agarose Resin,** 2mL

General References

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Chyung, J.H. and Selkoe, D.J. (2003). Inhibition of receptor-mediated endocytosis demonstrates generation of amyloid beta-protein at the cell surface. *J Biol Chem* **278(51):**51035-43.

Frickel, E-M., *et al.* (2002). TROSY-NMR reveals interaction between Erp57 and the tip of the calreticulin P-domain. *PNAS* **99(4):**1954-9.

Li, H. and Pajor, A.M. (2003). Mutagenesis of the *N*-glycosylation site of hNaSi-1 reduces transport activity. *Am J Physiol Cell Physiol* **285:**C1188-96.

Ohnishi, T., *et al.* (2003). MD-2 is necessary for the toll-like receptor 4 protein to undergo glycosylation essential for its translocation to the cell surface. *Clin Diagn Lab Immunol* **10(3):**405-10.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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

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