INSTRUCTIONS

EZ-Link Sulfo-NHS-LC-LC-Biotin, No-Weigh Format

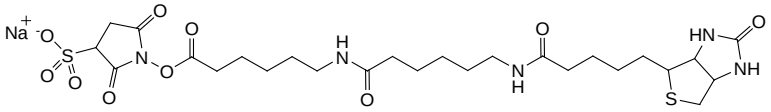
MAN0017097
Rev. A.0
Pub. Part No. 2162631.0

A35358

Number	Description
A35358	**EZ-Link Sulfo-NHS-LC-LC-Biotin, No-Weigh™ Format** 10 × 1mg

Molecular Weight: 669.75

Spacer Arm: 30.5Å



Storage: Upon receipt store desiccated at -20°C. Product is shipped on ice.

For Research Use Only. Not for use in diagnostic procedures.

Introduction

Thermo Scientific™ EZ-Link™ Sulfo-NHS-LC-LC-Biotin (sulfosuccinimidyl-6-[biotinamido]-6-hexanamido hexanoate) enables simple and efficient biotin labeling of antibodies, proteins and any other primary amine-containing macromolecules. Specific labeling of cell surface proteins is another common application for these uniquely water-soluble and membrane impermeable reagents.

Thermo Scientific™ No-Weigh™ products are specialty reagents provided in a pre-aliquoted format. The pre-weighed packaging prevents the loss of reagent reactivity and contamination over time by eliminating the repetitive opening and closing of the vial. The format enables use of a fresh vial of reagent each time, eliminating the hassle of weighing small amounts of reagents and reducing concerns over reagent stability.

Biotin is a small, naturally occurring vitamin that binds with high affinity to avidin and streptavidin proteins. Because it is so small (244Da), biotin can be conjugated to many proteins without altering their biological activities. Labeled proteins may be purified from unlabeled proteins using immobilized streptavidin and avidin affinity gels (see Related Thermo Scientific Products), and they may be detected easily in ELISA, dotblot or Western blot applications using streptavidin or avidin-conjugated 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. 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 with NHS-activated biotin reagents. Several different NHS esters of biotin are available, with varying properties and spacer arm lengths. Sulfo-NHS ester reagents are water soluble, enabling reactions to be performed in the absence of organic solvents such as DMSO or DMF.

Cell surface biotinylation is an important tool for studying the expression and regulation of receptors and transporters, differentiation of plasma membrane proteins from those localized to organelle membranes, and distribution of membrane proteins in polarized epithelial cells. The specificity of Sulfo-NHS-LC-LC-Biotin reagents for cell surface labeling has been demonstrated in these applications.[1,2] Because these molecules dissolve readily in polar solutions and are charged by the sodium sulfonate group on the succinimidyl ring, they cannot permeate the cell membrane. As long as the cell remains intact, only primary amines exposed on the surface will be biotinylated with these Sulfo-NHS-LC-LC-Biotin reagents.

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Important Product Information

- Sulfo-NHS-LC-LC-Biotin is moisture-sensitive. Store the vials of reagent at -20°C with desiccant. To avoid moisture condensation onto the product, equilibrate vials to room temperature before opening.
- As directed in the procedure, dissolve the biotin reagent immediately before use. The NHS-ester moiety readily hydrolyzes and becomes non-reactive. Discard any unused reconstituted reagent.
- Avoid buffers containing primary amines (e.g., Tris or glycine) as these will compete with the reaction (see Figure 2). If necessary, dialyze or otherwise desalt to exchange the protein sample into an amine-free buffer such as phosphate buffered saline (PBS; see Related Thermo Scientific Products).
- When biotinylating proteins in solution, excess non-reacted biotin and reaction byproducts are easily removed by size exclusion using either desalting columns or dialysis (See Additional Information and Related Thermo Scientific Products). A 10mL desalting column is best suited for processing biotinylation reactions involving 1-10mg of protein in approximately 0.5-2mL. For smaller amounts of protein and/or smaller reaction volumes, both the biotinylation reaction and subsequent buffer exchange may be performed in a single Thermo Scientific™ Slide-A-Lyzer™ MINI Dialysis Unit. For larger reaction volumes than can be processed with a desalting column, either split the sample between two columns or use an appropriate Slide-A-Lyzer Dialysis Cassette for buffer exchange steps.

Additional Materials Required

- Phosphate-buffered Saline (PBS) or other amine-free buffer having pH 7-8 for use as reaction buffer (see Important Product Information and Related Thermo Scientific Products)
- Desalting columns or dialysis units for buffer exchange (see Important Product Information and Related Thermo Scientific Products)

Procedure for Biotinylating Proteins in Solution

A. Calculations

The extent of biotin labeling depends on the distribution of amino groups on the protein, and the concentration and amount of protein to be labeled. By using the appropriate molar ratio of biotin to protein, the extent of labeling can be controlled. Compared to reactions involving concentrated protein solutions, labeling reactions with dilute solutions require a greater fold molar excess of biotin reagent to achieve the same incorporation level. Generally, use ≥ 12-fold molar excess of biotin for proteins at 2-10mg/mL or ≥ 20-fold molar excess of biotin for proteins at ≤ 2mg/mL. Adjust the molar ratio of biotin reagent to protein to obtain the level of incorporation desired.

1. Calculate millimoles of biotin reagent to add to the reaction for a 20-fold molar excess:

$$\text{mL protein} \times \frac{\text{mg protein}}{\text{mL protein}} \times \frac{\text{mmol protein}}{\text{mg protein}} \times \frac{\text{20 mmol Biotin}}{\text{mmol protein}} = \text{mmol Biotin}$$

   - 20 = Molar fold excess of biotin for 2 mg/ml protein sample

2. Calculate microliters of 10mM biotin reagent solution (prepared in Step B.3) to add to the reaction:

$$\text{mmol Biotin} \times \frac{1{,}000{,}000\ \mu\text{L}}{\text{L}} \times \frac{\text{L}}{\text{10 mmol}} = \mu\text{L Biotin}$$

Example: For 1mL of a 2mg/mL IgG (150,000 MW) solution, ~27µL of 10mM biotin reagent will be added.

$$1\text{mL IgG} \times \frac{2\text{ mg IgG}}{1\text{mL IgG}} \times \frac{1\text{ mmol IgG}}{150{,}000\text{ mg IgG}} \times \frac{20\text{ mmol Biotin}}{1\text{ mmol IgG}} = 0.000266\text{ mmol Biotin}$$

$$0.000266\text{ mmol Biotin} \times \frac{1{,}000{,}000\ \mu\text{L}}{\text{L}} \times \frac{\text{L}}{\text{10 mmol}} = 26.6\ \mu\text{L Biotin Reagent}$$

B. Biotin Labeling Reaction

1. Remove a vial of biotin reagent from freezer and equilibrate it to room temperature before opening in step 3.
2. Dissolve 1-10mg protein in 0.5-2.0mL PBS according to the calculation made in section A.

   Note: Protein that is already dissolved in amine-free buffer at pH 7.2-8.0 may be used without buffer exchange or dilution with PBS. Proteins in Tris or other amine-containing buffers must be exchanged into a suitable buffer.
3. Immediately before use, prepare a 10mM solution of the biotin reagent by adding 150µL of ultrapure water to each vial. Dissolve by repeated pipetting of the solution or by vortex with the cap in place. The maximum useable volume of each tube is 800µL.
4. Add the appropriate volume (see Calculations in section A) of 10mM biotin reagent solution to the protein solution.
5. Incubate reaction on ice for two hours or at room temperature for 30 minutes.

   Note: Other than the possibility of ordinary protein degradation or microbial growth, there is no harm in reacting longer than the specified time.
6. Protein labeling is complete at this point, and 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 proper function and labeling of the protein has been confirmed, the labeled protein may be purified for optimal performance and stability using desalting or dialysis. If the Thermo Scientific™ Pierce™ Biotin Quantitation Kit (HABA assay; see Related Thermo Scientific Products) will be performed to determine the level of biotin incorporation, the protein first must be desalted or dialyzed to remove non-reacted biotin.

Procedure for Biotinylating Cell Surface Proteins

Many variations of this procedure exist in the literature (see Product References). Labeling may be performed on cells in suspension or on adherent cells in culture plates. In the latter situation, diffusion of the Sulfo-NHS-LC-LC-Biotin Reagent to all surfaces of the cells will be limited, and labeling will occur predominately on the exposed surface. Culture media must be washed from the cells; otherwise, amine-containing components will compete and quench the reaction to cell surface proteins. Using a more concentrated cell suspension is most effective because less biotin reagent is required in the reaction. Generally, a final concentration of 2-5mM Biotin Reagent is effective. NHS reactions occur more rapidly at higher pH; therefore, pH 8.0 is used in the following example so that labeling can be completed as quickly as possible.

1. Wash cells three times with ice-cold PBS (pH 8.0) to remove amine-containing culture media and proteins from the cells.
2. Suspend cells at a concentration of ~25×10^6 cells/mL in PBS (pH 8.0).
3. Add 1 vial (1.0mg) of Sulfo-NHS-LC-LC-Biotin reagent per mL of cell suspension (results in ~1.5mM biotin reagent). Alternatively, add 150µL of the 10mM biotin reagent solution (see step B.3 on previous page) per mL of cell suspension.
4. Incubate reaction mixture at room temperature for 30 minutes.

   Note: Performing this incubation at 4°C may reduce active internalization of the biotin reagent.
5. Wash cells three times with PBS plus 100mM glycine to quench and remove excess biotin reagent and by products.
6. Lyse and/or analyze biotin-labeled cells as required for the research method.

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
- HABA Calculator for computing the results associated with the HABA assay measurements

Related Thermo Scientific Products

28372	**BupH™ Phosphate Buffered Saline Packs,** 40 pack
69576	**Slide-A-Lyzer MINI Dialysis Unit Kit**
66382	**Slide-A-Lyzer Dialysis Cassette Kits**
89890	**Zeba™ Spin Desalting Columns, 7K MWCO, 2mL,** 25 columns
89892	**Zeba Spin Desalting Columns, 7K MWCO, 5mL,** 25 columns
89894	**Zeba Spin Desalting Columns, 7K MWCO, 10mL,** 25 columns
28005	**Pierce Biotin Quantitation Kit**
20347	**Streptavidin Agarose Resin**, 2mL
20228	**Pierce™ Monomeric Avidin Agarose**, 5mL
21126	**Streptavidin, Horseradish Peroxidase Conjugated**, 1mg
21445	**EZ-Link Sulfo-NHS-SS-Biotinylation Kit**
21945	**EZ-Link Micro Sulfo-NHS-SS-Biotinylation Kit**
21925	**EZ-Link Micro Sulfo-NHS-Biotinylation Kit**
21935	**EZ-Link Micro Sulfo-NHS-LC-Biotinylation Kit**
21326	**EZ-Link Sulfo-NHS-Biotin, No-Weigh™ Format**, 8 × 1mg microtubes
21328	**EZ-Link Sulfo-NHS-SS-Biotin, No-Weigh Format**, 8 × 1mg microtubes
21329	**EZ-Link NHS-PEO_4-Biotin, No-Weigh Format,** 8 × 2mg microtubes
21327	**EZ-Link Sulfo-NHS-LC-Biotin, No-Weigh Format**, 8 × 1mg microtubes

Cited References

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