INSTRUCTIONS

Thermo SCIENTIFIC

EZ-Link Sulfo-NHS-LC-Biotin

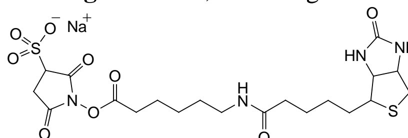
MAN0016133
Rev. B.0
Pub. Part No. 2161855

A39257 21335

Number	Description
21335	**EZ-Link Sulfo-NHS-LC-Biotin,** 100mg
A39257	**EZ-Link Sulfo-NHS-LC-Biotin, No-Weigh Format**, 10 × 1mg

Molecular Weight: 556.59

Spacer Arm: 22.4Å



Storage: Upon receipt store desiccated at -20°C. EZ-Link Sulfo-NHS-LC-Biotin is shipped at ambient temperature. No-Weigh Format EZ-Link Sulfo-NHS-LC-Biotin is 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™ Sulfo-NHS-LC-Biotin (sulfosuccinimidyl-6-[biotin-amido]hexanoate) enables simple and efficient labeling of antibodies, proteins and any other primary amine-containing molecules. Specific labeling of cell surface proteins is another common application for this water-soluble and membrane impermeable reagent. The Thermo Scientific™ No-Weigh™ Format EZ-Link Sulfo-NHS-LC-Biotin consists of convenient single-use vials, eliminating difficulties associated with weighing small quantities of reagent.

Biotin is a vitamin that binds with high affinity to avidin and streptavidin proteins. Because it is small (244Da), biotin can be conjugated to many proteins without altering their biological activities. Labeled proteins can be purified from unlabeled proteins using immobilized streptavidin and avidin and detected in ELISA, dot blot 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-LC-Biotin is water soluble, enabling reactions to be performed in the absence of organic solvents such as DMSO or DMF.

Cell-surface biotinylation has emerged as 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-Biotin for cell-surface labeling has been demonstrated in these applications.[1,2] Because Sulfo-NHS-LC-Biotin dissolves readily in polar solutions and is charged by the sodium sulfonate group on the succinimidyl ring, it cannot permeate the cell membrane. As long as the cell remains intact, only primary amines exposed on the surface will be biotinylated.

Important Product Information

- Sulfo-NHS-LC-Biotin is moisture-sensitive. Store the vial of biotin reagent at -20°C with desiccant. To avoid moisture condensation onto the product, equilibrate vial to room temperature before opening.
- No-Weigh format handling: Immediately before use, unscrew the vial, add water and mix by pipetting up and down. Alternatively, the vial can be vortexed for a few seconds to ensure a homogeneous solution. Store the unused vials in the foil pouch provided.

- As directed in the procedure, dissolve the biotin reagent immediately before use. The NHS-ester moiety readily hydrolyzes and becomes non-reactive; therefore, do not prepare stock solutions for storage. Discard any unused reconstituted reagent.
- Avoid buffers containing primary amines (e.g., Tris or glycine) as these will compete with the intended reaction. If necessary, dialyze or otherwise desalt to exchange the protein sample into an amine-free buffer such as phosphate-buffered saline (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. A 10mL desalting column is best suited for processing reactions involving 1-10mg of protein in approximately 0.5-2mL. For smaller protein amounts or reaction volumes, both the biotinylation reaction and subsequent buffer exchange can be performed in a single Thermo Scientific Slide-A-Lyzer MINI Dialysis Unit. For reaction volumes too large for processing with a desalting column, either split the sample between two columns or use an appropriate Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cassette. For processing small volumes (i.e., 10-150µL) of peptides and other low molecular weight molecules, Thermo Scientific™ Pierce™ C18 Spin Columns (Product No. 89870 or 89873) may be used.

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, protein concentration and the amount of reagent used. 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. Experiments performed at Pierce that used a 20-fold molar excess of biotin reagent to label 1-10mg antibody (in 0.5-2mL) resulted in 4-6 biotin groups per antibody molecule. Experiments that used a 50-fold molar excess of biotin reagent to label 50-200µg of antibody (in 200-700µL) resulted in 1-3 biotin groups per antibody molecule. Adjust the molar ratio of Sulfo-NHS-LC-Biotin 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:

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

   - 20 = Molar fold excess of biotin

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

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

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

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

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

B. Biotin Labeling Reaction

1. Remove vial of Sulfo-NHS-LC-Biotin from freezer and equilibrate it to room temperature before opening in Step 3.
2. Prepare protein in 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:
   - Product No. 21335 (100mg vial): Add 360µL of ultrapure water to 2.0mg of reagent.
   - Product No. A39257 (No-Weigh format): Add 180µL of ultrapure water to the 1mg vial. **The maximum useable volume of the vial 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, for optimal performance and stability, purify the labeled protein using desalting or dialysis. If the Thermo Scientific™ Pierce™ Biotin Quantitation Kit (HABA assay; Product No. 28005) 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 General 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-Biotin to all surfaces of the cells will be limited, and labeling will occur predominately on the exposed surface. Culture media must be washed from 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 high 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 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 1.0mg of Sulfo-NHS-LC-Biotin reagent per mL of cell suspension (results in ~2mM biotin reagent). Alternatively, add 200µL 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 + 100mM glycine to quench and remove excess biotin reagent and byproducts.

Additional Information Available on the Web

- 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,** 10 units plus float
66382	**Slide-A-Lyzer Dialysis Cassette Kit, 10K MWCO,** 3mL
66807	**Slide-A-Lyzer Dialysis Cassette Kit, 10K MWCO,** 12mL
89892	**Zeba™ Spin Desalting Columns, 5mL, 7K MWCO,** 25 columns
28005	**Pierce™ Biotin Quantitation Kit**
20347	**Streptavidin Agarose**, 2mL
20228	**Pierce™ Monomeric Avidin Agarose,** 5mL
21126	**Streptavidin, Horseradish Peroxidase Conjugated**, 1mg
21445	**EZ-Link™ Sulfo-NHS-SS-Biotinylation Kit**
21935	**EZ-Link™ Micro Sulfo-NHS-LC-Biotinylation Kit**
89870	**Pierce C18 Spin Columns,** 25 columns

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