INSTRUCTIONS

EZ-Link® NHS-SS-Biotin

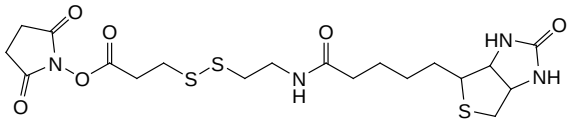
21441

1774.1

Number	Description
21441	**EZ-Link NHS-SS-Biotin** (succinimidyl-2-(biotinamido)ethyl-1,3-dithiopropionate), 50mg

Molecular Weight: 504.65

Spacer Arm: 24.3Å



Storage: Upon receipt store at -20°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific EZ-Link NHS-SS-Biotin is a thiol-cleavable amine-reactive biotinylation reagent with an extended spacer arm, which reduces steric hindrances associated with avidin binding. This reagent is soluble in organic solvents such as DMSO or DMF, allowing it to penetrate membranes of intact cells for biotinylation at the surface and the interior of the cell. Once dissolved in an organic solvent, the reagent is further diluted in a non-amine containing aqueous buffer.

N-Hydroxysuccinimide (NHS) ester-activated biotins are the most popular type of biotinylation reagent. NHS esters react efficiently with primary amino groups (-NH_2) in pH 7-9 buffers to form stable amide bonds. Because antibodies and other proteins generally contain multiple lysine (K) residues in addition to the N-terminus of each polypeptide, they have multiple primary amines available as targets for labeling with NHS-activated biotin reagents.

Unlike other biotin-labeling reagents, EZ-Link NHS-SS-Biotin contains a disulfide bond in its spacer arm, enabling labeled proteins to be cleaved from the biotin group by treatment with dithiothreitol (DTT) or other reducing agents. This feature is especially useful in affinity purification experiments where it is necessary to elute the biotinylated protein from its bound state to avidin or streptavidin.

Important Product Information

- NHS-SS-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.
- Dissolve NHS-SS-Biotin immediately before use. The NHS-ester moiety readily hydrolyzes and becomes non-reactive; therefore, weigh and dissolve only a small amount of the reagent at a time and do not prepare stock solutions for storage. Discard any unused reconstituted reagent.
- Avoid buffers containing primary amines (e.g., Tris or glycine) as these 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.
- Avoid buffers containing reducing agents during the labeling reaction to prevent cleavage of the reagent disulfide bond.

Additional Materials Required

- Phosphate-buffered Saline (PBS): 0.1M phosphate, 0.15M sodium chloride; pH 7.2 (Product No. 28372) or other non-amine containing buffer at pH 7.0-8.0
- Quenching Buffer: Tris-buffered saline (TBS; 25mM Tris, 0.15M sodium chloride; pH 7.2; Product No. 28376), glycine or other amine-containing buffer
- Water-miscible organic solvent such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF)

- Method for removing non-reacted Biotin (buffer exchange): Thermo Scientific Slide-A-Lyzer MINI Dialysis Units for 10-100µL sample volumes; Slide-A-Lyzer® Dialysis Cassette Kit for 0.1-30.0mL sample volumes; or Zeba Spin Desalting Columns for sample volumes ranging from < 10µL to 4mL
- Reducing Agent: Use either DTT (Product No. 20290), 2-mercaptoethanol (Product No. 35602), TCEP (Product No. 20490) or other reducing agent for cleaving the disulfide bond

Procedure for Biotinylating IgG with NHS-SS-Biotin

A. Calculations

The extent of biotin labeling depends on the size and distribution of amino groups on the protein and the amount of reagent 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. Typically using a 20-fold molar excess of biotin reagent to label 1-10mg/mL antibody (IgG) results in 4-6 biotin groups per antibody molecule. Adjust the molar ratio of Sulfo-NHS-SS-Biotin to protein to obtain the desired level of incorporation.

1. Calculate millimoles of NHS-SS-Biotin to add to the reaction for a 20-fold molar excess:

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

2. Calculate microliters of 10mM NHS-SS-Biotin (prepared in Step B.3) to add to the reaction:

$$\text{mmol Biotin} \times \frac{505\ \text{mg}}{\text{mmol Biotin}} \times \frac{1{,}000\ \mu\text{L}}{5.0\ \text{mg}} = \mu\text{L Biotin}$$

- 20 = Molar fold excess of biotin
- 505 = Molecular weight of NHS-SS-Biotin
- 1000 = Microliters of water in which ~5.0mg of NHS-SS-Biotin is dissolved for a 10mM solution

Example: For 1mL of a 2mg/mL IgG (150,000 MW) solution, 26.8µL of 10mM NHS-SS-Biotin will be added.

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

$$0.000266\ \text{mmol Biotin} \times \frac{505\ \text{mg}}{\text{mmol Biotin}} \times \frac{1{,}000\ \mu\text{L}}{5.0\ \text{mg}} = 26.8\ \mu\text{L NHS-SS-Biotin}$$

B. Biotin Labeling Reaction

For reaction volumes from 10µL to 100µL, the buffer exchange and biotinylation may be conveniently performed in a single Slide-A-Lyzer MINI Dialysis Unit. For reaction volumes from 0.1mL to 30mL, Slide-A-Lyzer Dialysis Cassettes may be used. Alternatively, Zeba Spin Desalting Columns can be used for a faster buffer exchange.

1. Equilibrate the vial of NHS-SS-Biotin to room temperature before opening in Step 3.
2. Dissolve 1-10mg protein in 0.5-2mL of PBS according to the calculation made in Section A.
3. Immediately before use, prepare a 10mM solution of NHS-SS-Biotin by adding 5mg to 1mL of DMSO or DMF.
4. Add the appropriate volume of the NHS-SS-Biotin solution (see Calculations section) to the protein solution, making sure that the volume of organic solvent does not exceed 10% of the final reaction volume.
5. Incubate reaction on ice for two hours or at room temperature for 30-60 minutes.
6. Remove the non-reacted NHS-SS-Biotin by dialysis or gel filtration. See instructions provided with the preferred buffer exchange product.
7. Store the biotinylated protein using the same condition that is optimal for the non-biotinylated protein.
8. To cleave the disulfide bond in the spacer arm, incubate sample in 50 mM DTT or 2-mercaptoethanol for 2 hours at room temperature for 30 minutes at 50°C, or by boiling for 5 minutes. Alternatively, add a molar excess of TCEP•HCl and incubate for 10 minutes at room temperature.

Example Procedure for Intra-cellular Biotinylation

The following protocol is an example application for this product. Specific applications require optimization.

1. Wash cells three times with ice-cold PBS (pH 8.0) to remove any contaminating proteins or culture media.
2. Suspend cells at a concentration of 25×10^6 cells/mL in PBS (pH 8.0).

   Note: Other cell concentrations may be used. Scale the concentration of biotinylation reagent up or down based on cell concentration, size or type.
3. Immediately before use, prepare a 10mM solution of NHS-SS-Biotin by adding 5mg to 1mL of DMSO or DMF.
4. Add 100µL of 10mM NHS-SS-Biotin per milliliter of reaction volume.
5. Incubate reaction at room temperature for 30 minutes.

   Note: Longer reaction time may be necessary to ensure significant diffusion of NHS-SS-Biotin into the cells; otherwise, most labeling may occur at the cell surface.
6. Wash cells three times with ice-cold PBS (pH 8.0) to remove non-reacted NHS-SS-Biotin. Alternatively, use 25-50mM Tris (pH 8.0) for the initial wash to quench any non-reacted biotinylation reagent.

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
	Hydrolysis of the NHS ester	Allow reagent to equilibrate to room temperature before opening and use reagent immediately upon reconstitution
	Incomplete removal of primary amines	Dialyze or desalt into a buffer free of primary amines
Protein is not functional	Excessive biotinylation	Reduce molar excess of biotinylation reagent, or reduce time or temperature for biotinylation
		Choose a biotinylation reagent that targets different groups

Additional Information

A. Determination of Biotin Incorporation

Biotin incorporation can be estimated using HABA [2-(4´-hydroxyazobenzene)-benzoic acid]. This method is based on the ability of the HABA dye to bind avidin forming a complex with maximal absorption at 500nm. Biotin is then added to the solution and because of its higher affinity for avidin, biotin displaces the HABA and the absorption at 500nm decreases proportionately to the dissociation of avidin from the HABA dye. The absorbance of the HABA-avidin solution is measured before and after adding the biotin-containing sample. The change in absorbance relates to the amount of biotin in the sample (see Related Thermo Scientific Products section).

B. Please visit the web site for additional information on this product including the following:

- Tech Tip #14: Perform labeling and other reactions in Slide-A-Lyzer Dialysis Cassettes
- HABA Calculator

Related Thermo Scientific Products

28005	**Pierce® Biotin Quantitation Kit**
21331	**EZ-Link Sulfo-NHS-SS-Biotin,** 100mg
20351	**Streptavidin Agarose Columns**, 5 × 1mL
20219	**Pierce Avidin Agarose,** 5mL
29200	**NeutrAvidin® Agarose Resin**, 5mL
20290	**Dithiothreitol (DTT),** 5g
20291	**No-Weigh DTT,** 48 × 7.7mg microtubes
35602	**2-Mercaptoethanol (2-BME),** 10 × 1mL
20490	**TCEP•HCl,** 1g
77720	**Bond-Breaker™ TCEP Solution, Neutral pH,** 5mL
77712	**Immobilized TCEP Reducing Gel,** 5mL
28372	**BupH Phosphate Buffered Saline Packs,** 40 packs
28376	**BupH Tris Buffered Saline Packs,** 40 packs
23010	**Aminoethyl-8; converts sulfhydryls to amines,** 1g

General References

Bruneau, N., *et al.* (2003). Lectin-like Ox-LDL receptor is expressed in human INT-407 intestinal cells: involvement in the transcytosis of pancreatic bile salt-dependent lipase. *Mol Biol Cell* **14:**2861-75.

Chaiet, I. and Wolf, F.J. (1964). The properties of streptavidin, a biotin-binding protein produced by *Streptomyces*. *Arch Biochem Biophys* **106:**1-5.

Gitlin, G., *et al.* (1987). Studies of the biotin-binding site of avidin. *Biochem J* **242:**923-6.

Green, N.M. (1965). A spectrophotometric assay for avidin and biotin based on binding dyes by avidin. *Biochem J* **94:**23c-24c.

Green, N.M. (1975). Avidin: Advances in Protein Chemistry. Academic Press, New York. **29:**85-133.

Gretch, D.R., *et al.* (1987). The use of biotinylated monoclonal antibody and streptavidin affinity chromatography to isolate herpes virus hydrophobic proteins or glycoproteins. *Anal Biochem* **163:**270-7.

Selo, I., *et al.* (1996). Preferential labeling of α-amino N-terminal groups in peptides by biotin: application to the detection of specific anti-peptide antibodies by enzyme immunoassays. *J Immunol Method* **199**:127-38.

Shimkus, M., *et al.* (1985). A chemically cleavable biotinylated nucleotide: Usefulness in the recovery of protein-DNA complexes from avidin affinity columns. *Proc Natl Acad Sci* **82:**2593-7.

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