INSTRUCTIONS

EZ-Link® NHS-Biotin Reagents

20217 21336 21343

0237.5

Number **Description**

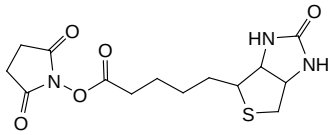
20217 **EZ-Link NHS-Biotin,** 100mg, *N*-hydroxysuccinimidobiotin

Molecular Weight: 341.38

Spacer Arm Length: 13.5Å

Net Mass Added: 226.08

Storage: Upon receipt store desiccated at room temperature.



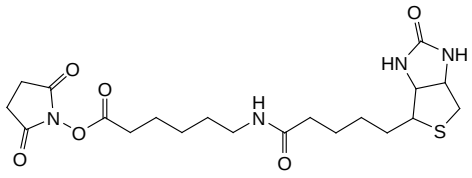
21336 **EZ-Link NHS-LC-Biotin,** 50mg, succinimidyl-6-(biotinamido)hexanoate

Molecular Weight: 454.54

Spacer Arm Length: 22.4Å

Net Mass Added: 339.16

Storage: Upon receipt store desiccated at 4°C. Product is shipped at ambient temperature.



21343 **EZ-Link NHS-LC-LC-Biotin,** 50mg, succinimidyl-6-(biotinamido)-6-hexanamido hexanoate

Molecular Weight: 567.70

Spacer Arm Length: 30.5Å

Net Mass Added: 452.24

Storage: Upon receipt store desiccated at 4°C. Product is shipped at ambient temperature.

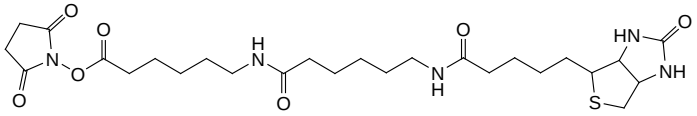


Table of Contents

Introduction

The Thermo Scientific EZ-Link NHS-Biotin Reagents enable simple and efficient biotin labeling of antibodies, proteins and any other primary amine-containing macromolecules in solution. Differing only in spacer arm lengths, the three reagents offer researchers the possibility of optimizing labeling and detection experiments where steric hindrance of biotin binding is an important factor.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce

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, 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. The three EZ-Link NHS-Biotin Reagents are not directly water soluble and must be dissolved in organic solvents such as DMSO or DMF before addition to aqueous solutions at the final concentration for the labeling reaction.

Biotinylation of intact cells 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. Sulfo-NHS-Biotin reagents (see Related Thermo Scientific Products) do not readily permeate cell membranes and are commonly used for specifically labeling the cell surface. By contrast, NHS-Biotin reagents are membrane permeable and may be used to biotinylate proteins inside intact cells. Parallel experiments with NHS- and Sulfo-NHS-Biotin analogs may help to localize particular proteins of interest.

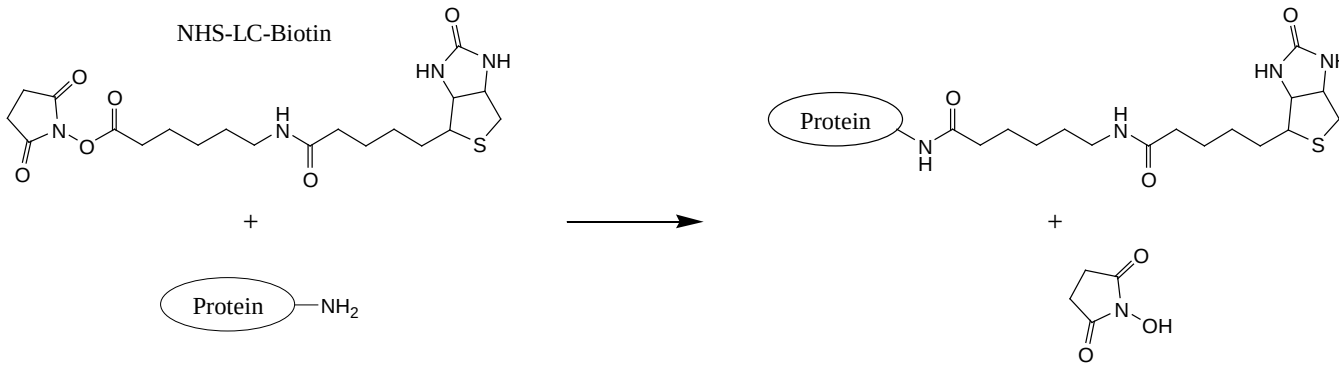


Figure 1. Reaction of NHS-LC-Biotin with primary amine. If drawn to scale, the oval representing the protein would be many times larger than the structures shown in this scheme and would likely contain several amino groups, each of which would be labeled in some proportion of the protein molecules in the reaction. Note that NHS is a leaving group (byproduct) in the reaction; this leaving group as well as any nonreacted biotin reagent is removed during the final desalting step in the procedure.

Important Product Information

- NHS-Biotin reagents are moisture-sensitive. If the vial of reagent has been stored cold, fully equilibrate vial to room temperature before opening to avoid moisture condensation inside the container.
- As directed in the procedure, dissolve the biotin reagent immediately before use. The NHS ester moiety readily hydrolyzes and becomes nonreactive; 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 will compete with the 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 (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

- Water-miscible organic solvent such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF)
- 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

The following procedure ordinarily will yield incorporation of 3-5 biotins per molecule of protein. Antibodies, which are large proteins, often will label with ~8-12 biotin molecules per molecule of IgG, especially when greater molar excesses of biotin reagent are used (see Calculations). The molar ratio of biotin reagent to protein may be adjusted to obtain the level of incorporation desired.

A. Calculations

The amount of biotin reagent to use for each reaction depends on the amount of protein to be labeled and its concentration. By using the appropriate molar ratio of biotin to protein, the extent of labeling can be controlled. When labeling more dilute protein solutions, a greater molar fold excess of biotin is necessary to achieve the same results. For best results, use ≥ 12-fold molar excess of biotin for a 10mg/mL protein solution or ≥ 20-fold molar excess of biotin for a 2mg/mL protein solution.

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 = Recommended molar fold excess of biotin for 2 mg/ml protein sample

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

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

Example Calculation:

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

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

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

B. Biotin Labeling Reaction

1. If the biotin reagent has been stored cold, remove the vial from storage and fully 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 in an organic solvent such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF):
   - For NHS-Biotin (Product No. 20217), dissolve 2.0mg reagent in 590µL of solvent.
   - For NHS-LC-Biotin (Product No. 21336), dissolve 2.3mg reagent in 500µL of solvent.
   - For NHS-LC-LC-Biotin (Product No. 21343), dissolve 2.0mg reagent in 350µL of solvent.
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 level of biotin incorporation will be determined using the Pierce Biotin Quantitation Kit (HABA assay; see Related Thermo Scientific Products), the protein first must be desalted or dialyzed to remove non-reacted biotin.

Procedure for Biotinylating Cells

Many variations of this procedure exist in the literature. Sulfo-NHS-Biotin reagents (see Related Thermo Scientific Products) do not readily permeate cell membranes and are commonly used for specifically labeling the cell surface. By contrast, NHS-Biotin reagents are membrane permeable and may be used to biotinylate proteins inside intact cells. Parallel experiments with NHS- and Sulfo-NHS-Biotin analogs may help to localize particular proteins of interest.

Labeling may be performed on cells in suspension or on adherent cells in culture plates. In the latter situation, diffusion of the NHS-Biotin reagent to all surfaces of the cells will be limited, and labeling will occur predominately on and through the exposed surface. Culture media must be washed from the cells, or amine-containing components will compete and quench the reaction to cell proteins. Using a more concentrated cell suspension is most effective since less biotin reagent will be required in the reaction. Generally, a final concentration of 2-5mM NHS-Biotin reagent is effective. NHS-Biotin 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. Prepare a 20mM solution of NHS-Biotin reagent by dissolving 4-5mg of reagent per 0.5mL of water-miscible organic solvent such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF).
4. Add 100µL of NHS-Biotin reagent solution to each 1mL of cell suspension (results in ~2mM biotin reagent).
5. Incubate reaction mixture at room temperature for 30 minutes.

   Note: Longer reaction time may be necessary to ensure significant diffusion of NHS-Biotin reagent into the cells; otherwise, most labeling may occur at the cell surface.
6. Wash cells three times with PBS plus 100mM glycine to quench and remove excess biotin reagent and byproducts.
7. Lyse and/or analyze biotin-labeled cells as required for the research method.

Additional Information

Visit the web site for additional information related to this product, including the Tech Tip procedure titled "Perform labeling and other reactions in Slide-A-Lyzer Dialysis Cassettes."

Related Thermo Scientific Products

21217	**EZ-Link Sulfo-NHS-Biotin,** 50mg
21335	**EZ-Link Sulfo-NHS-LC-Biotin,** 100mg
21338	**EZ-Link Sulfo-NHS-LC-LC-Biotin,** 50mg
28372	**BupH™ Phosphate Buffered Saline Packs,** 40 packs
69576	**Slide-A-Lyzer MINI Dialysis Unit Kit**
66382	**Slide-A-Lyzer Dialysis Cassette Kit**
89889	**Zeba™ Spin Desalting Columns,** 5 × 2mL columns
89891	**Zeba Spin Desalting Columns,** 5 × 5mL columns
28005	**Pierce® Biotin Quantitation Kit**
21450	**EZ-Link NHS-PEG$_4$-Solid Phase Biotinylation Kit**
20347	**Streptavidin Agarose Resin**, 2mL
20228	**Pierce Monomeric Avidin Kit**
21126	**Streptavidin, Horseradish Peroxidase Conjugated**, 1mg

Product References

Bessho, Y., *et al.* (2002). A tRNA aminoacylation system for non-natural amino acids based on a programmable ribozyme. *Nat Biotechnol* **20:**723-8.

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.

Fouassier, L., *et al*. (2000). Evidence for Ezrin-Radixin-Moesin-binding phosphoprotein 50 (EBP50) self-association through PDZ-PDZ interactions. *J Biol Chem* **275(32):**25039-45.

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

Yamauchi, T., *et al.* (2003). Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* **423:**462-9.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce