EZ-Link™ Micro Sulfo-NHS-SS-Biotinylation Kit

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Thermo Scientific™ EZ-Link™ Micro Sulfo-NHS-SS-Biotinylation Kit provides the required reagents for labeling macromolecules containing primary amino groups and desalting columns for purifying the labeled molecule. The kit is structured for labeling 50-200 µg of protein in 200–700 µL. Thermo Scientific™ No-Weigh™ Sulfo-NHS-SS-Biotin is packaged in convenient single-use tubes, 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 (244 Da), 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, and western blot applications 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 (-NH2) in pH 7-9 buffers to form stable amide bonds (Fig. 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.

Unlike other bioin-labeling reagents, EZ-Link™ Sulfo-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.

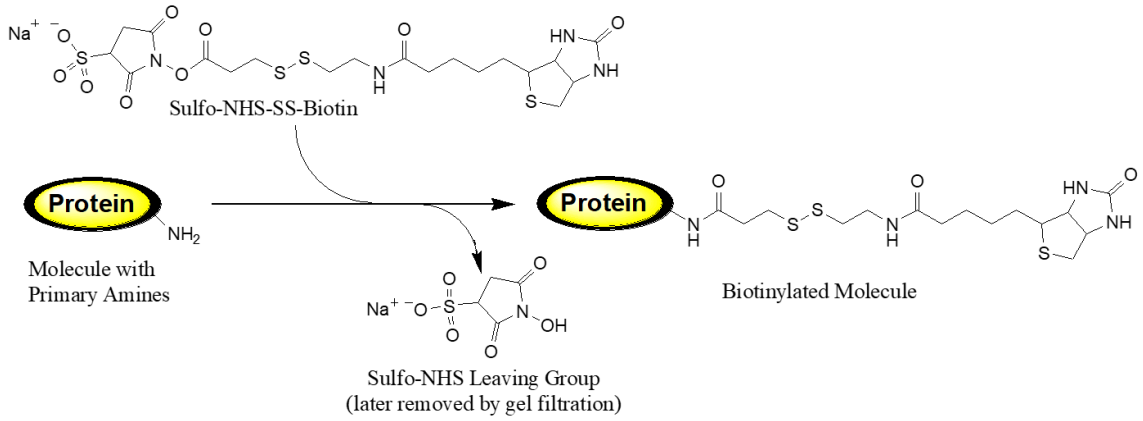

Figure 1 Reaction of Sulfo-NHS-SS-Biotin with primary amine. If drawn to scale, the oval representing the protein would be many times larger than the reagent structures and would likely contain several amino groups. Note that Sulfo-NHS is a leaving group (byproduct) in the reaction. The leaving group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

Contents and storage

Kit Component	Amount[1]	Storage
No-Weigh™ Sulfo-NHS-SS-Biotin	10 × 1 mg screw cap tubes Molecular weight: 606.9	–20°C
BupH™ Phosphate Buffered Saline Pack	1 pack 0.1 M sodium phosphate, 0.15 M NaCl, pH 7.2 when reconstituted in 500 mL of ultrapure water	4°C
Zeba™ Spin Desalting Columns	10 columns for 200–700 µL samples, 7,000 MWCO	

[1] Sufficient components for 10 labeling reactions each containing 50–200 µg of antibody or other protein in 200–700 µL reaction volumes.

Additional information

- Use reconstituted Sulfo-NHS-SS Biotin immediately. The NHS-ester moiety readily hydrolyzes and becomes non-reactive; therefore, do not prepare 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 (see Fig. 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 involving 50–200 µg of protein in approximately 200–700 µL. For smaller amounts of protein or smaller reaction volumes, perform both the biotinylation reaction and buffer exchanges in a single Thermo Scientific™ Slide-A-Lyzer™ MINI Dialysis Unit. For reaction volumes larger than can be processed 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 ~5 mg/mL before adding to the reaction mixture. To avoid having to pipette volumes less than 1 µL 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.

Biotinylate proteins

Calculations

The extent of labeling depends on the size and distribution of amino groups on the protein, protein concentration 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 50–200 µg of antibody (human IgG) for 30 minutes at room temperature resulted in 1.5–5 biotin groups per antibody molecule. Adjust the molar ratio of Sulfo-NHS-SS Biotin to protein to obtain the level of incorporation desired.

1. Calculate the millimoles of Sulfo-NHS-SS 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 8 mM Sulfo-NHS-SS Biotin to add to the reaction:

$$\text{mmol Biotin} \times \frac{607\ \text{mg}}{\text{mmol Biotin}} \times \frac{200\ \mu\text{L}}{1\ \text{mg}} = \mu\text{L Biotin Solution}$$

   - 607 = Molecular weight of Sulfo-NHS-SS Biotin
   - 200 = Microliters of solvent in which 1 mg of Sulfo-NHS-SS Biotin is dissolved to make 8 mM

   Example: To 0.7 mL of 0.29 mg/mL IgG (150,000 MW) add ~8 µL of 8 mM Sulfo-NHS-SS Biotin.

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

$$0.0000676\ \text{mmol Biotin} \times \frac{607\ \text{mg}}{\text{mmol Biotin}} \times \frac{200\ \mu\text{L}}{1\ \text{mg}} = 8.2\ \mu\text{L Biotin Solution}$$

Label using biotin

1. Dissolve 50–200 µg of protein in 200–700 µL of phosphate-buffered saline (PBS) according to the calculation made in "Calculations" on page 2. Prepare the BupH™ PBS as directed on the package label.

   Note: Protein 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 200–700 µL samples by dialysis or using a desalting column.This kit contains 10 single-use desalting columns and 10 tubes of biotin reagent. The columns are needed to remove excess biotin reagent after performing the biotinylation reaction for each of the 10 applications.

2. To prepare an 8 mM solution of Sulfo-NHS-SS Biotin, add 200 µL of solvent (e.g., water, DMSO or DMF) and mix by pipetting up and down. Vortex until the solution becomes clear. More dilute solutions can be prepared. The screw cap vial can accommodate 700 µL of solvent.

3. Add the appropriate volume of Sulfo-NHS-SS Biotin solution (see "Calculations" on page 2) to the protein solution.

4. Incubate the reaction on ice for 2 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 "Exchange buffer and remove excess biotin" on page 3.

Exchange buffer and remove excess biotin

1. Prepare a Zeba™ Spin Desalting Column by breaking off the bottom plug and placing the column into a 15-mL collection tube. Centrifuge the column at 1,000 × *g* for 2 minutes, discard the storage buffer, and return the 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 the centrifuge with the mark facing outward in all subsequent centrifugation steps.

2. Equilibrate the column by adding 1 mL of PBS to the top of the resin bed and centrifuging at 1,000 × *g* for 2 minutes. Discard the flowthrough and repeat the step 2–3 times.

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

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

4. Centrifuge the column at 1,000 × *g* for 2 minutes. The collected flowthrough solution is the purified protein sample. Store the protein solution in appropriate conditions.

5. To cleave the disulfide bond in the spacer arm, incubate the sample in 50 mM DTT for 2 hours at room temperature or for 30 minutes at 50°C. Other reducing agents may also be used to cleave the disulfide bond.

Troubleshooting

Observation	Possible cause	Recommended action
Low level of biotinylation	Carrier protein was added to purified IgG for stabilization.	Remove carrier protein before biotinylation to reduce competition for labeling
No biotinylation	No amines were available on the molecule of interest.	Use a biotinylation reagent that targets a different functional group or convert sulfhydryl to amine using Aminoethyl-8 (Cat. No. 23010).
	Buffer contained primary amines.	Use a non-amine-containing buffer.
	Reagent was not reactive due to 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 protein recovery after desalting	No stacker was used.	Apply a stacker above sample.
	Unstable protein.	Equilibrate column in a suitable buffer.
Protein is non-functional	Excessive biotinylation.	Reduce the molar excess of biotinylation reagent or reduce time or temperature for biotinylation.

Related products

Product	Cat. No.
EZ-Link™ Micro Sulfo-NHS-Biotinylation Kit	21925
EZ-Link™ Micro Sulfo-NHS-LC-Biotinylation Kit	21935
EZ-Link™ Micro Sulfo-NHS-SS-Biotinylation Kit	21945
EZ-Link™ Micro NHS-PEG_4-Biotinylation Kit	21955
No-Weigh™ Sulfo-NHS-Biotin, 10 × 1 mg	A39256
No-Weigh™ Sulfo-NHS-LC-Biotin, 10 × 1 mg	A39257
No-Weigh™ Sulfo-NHS-SS-Biotin, 10 × 1 mg	A39258
No-Weigh™ NHS-PEG_4-Biotin, 8 × 2 mg	A39259
Streptavidin Agarose Resin, 2 mL	20347

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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Revision history: Pub. No. MAN0011569

Revision	Date	Description
B.0	30 June 2022	Updating formatting to current standards.
A.0	17 October 2015	Initial release.