INSTRUCTIONS

Pierce Premium Grade Sulfo-NHS-SS-Biotin

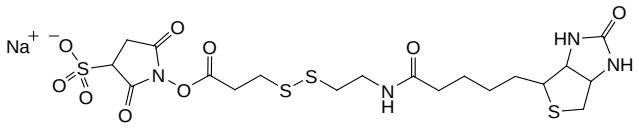
PG82077 PG82078

2538.1

Number	Description
PG82077	**Premium Grade Sulfo-NHS-SS-Biotin**, 100mg
PG82078	**Premium Grade Sulfo-NHS-SS-Biotin**, 1g

Molecular Weight: 606.69

Spacer Arm: 24.3Å



Storage: Upon receipt store product at -20°C protected from moisture. Product is shipped at ambient temperature.

Introduction

Thermo Scientific™ Pierce™ Premium Grade Reagents are high-quality formulations of selected chemical modification reagents, specially characterized for applications where product integrity and risk minimization are critical. Compared to standard grade equivalents, Pierce Premium Grade Reagents provide more clearly defined quality and product support by including (a) increased analytical testing and product characterization, (b) greater batch-specific information and quality assurance review, (c) extensive lot sample retention, and (d) change control notification.

Thermo Scientific™ Pierce™ Premium Grade Sulfo-NHS-SS-Biotin (sulfosuccinimidyl-2-[biotinamido]ethyl-1,3-dithiopropionate) is a thiol-cleavable amine-reactive biotinylation reagent that contains an extended spacer arm to reduce steric hindrances associated with avidin binding. This reagent is water-soluble (to approximately 10mM) enabling biotinylation in the absence of organic solvents such as DMSO or DMF. Water solubility is advantageous for applications that cannot tolerate solvents or when inclusion of solvents complicates the protocol. The *N*-hydroxysulfosuccinimide (NHS) ester group on this reagent reacts with the ε-amine of lysine residues to produce a stable product. Although α-amine groups present on the N-termini of peptides react with NHS esters, α-amines on proteins are seldom accessible for conjugation. NHS esters react with primary amines in the deprotonated form and, therefore, the reaction requires neutral to basic pH values to proceed. Primary amines react with NHS esters by nucleophilic attack and *N*-hydroxysulfosuccinimide is released as a by-product. Hydrolysis of the NHS-ester competes with the reaction in aqueous solution. The rate of hydrolysis increases with increasing pH and occurs more readily in dilute protein solutions.

There is considerable flexibility in conditions used for conjugating Sulfo-NHS-SS-Biotin to a protein or peptide. NHS-ester reactions proceed at 4-37°C, reaction mixture pH values of 7-9 and at incubation times ranging from a few minutes to overnight. A particular set of conditions will result in different degrees of label incorporation. Because of protein variability, especially with regard to the number of amines available for conjugation, a particular set of conjugation conditions that is optimal for one protein may not yield optimal results when applied to a different protein.

Important Product Information

- Pierce Premium Grade Sulfo-NHS-SS-Biotin is moisture-sensitive. Store reagent at -20°C in the original container with desiccant. To avoid moisture condensation onto the product, vial must be equilibrated to room temperature before opening.
- Avoid buffers containing primary amines (e.g., Tris or glycine) as these will compete with the reaction.
- Avoid reducing agents during conjugation to prevent cleavage of the disulfide bond within the spacer arm.
- Prepare the 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.

Example Procedure for Biotinylating IgG with Pierce Premium Grade Sulfo-NHS-SS-Biotin

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

A. Additional Materials Required

- Reaction Buffer: Phosphate-buffered saline (e.g., 0.1M phosphate, 0.15M sodium chloride; pH 7.2; e.g., Thermo Scientific, Product No. 28372), or other non-amine-containing buffer at pH 7.0-8.5
- Method for removing non-reacted biotin (buffer exchange): Thermo Scientific™ Slide-A-Lyzer™ MINI Dialysis Units for 10-100µL samples (Product No. 69576); Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cassette Kit for 0.5-3.0mL samples (Product No. 66382); or Dextran Desalting Columns, 5K MWCO, for samples up to 1.25mL (Thermo Scientific, Product No. 43230)
- Reducing Agent: DTT (e.g., Thermo Scientific, Product No. 20290) or 2-mercaptoethanol (e.g., Thermo Scientific, Product No. 35602) for cleaving the disulfide bond

B. Calculations

The amount of Pierce Premium Grade Sulfo-NHS-SS-Biotin Reagent to use for each reaction depends on the amount of the protein to be labeled and the protein concentration. By using the appropriate molar ratio of biotin to the protein, the extent of labeling can be controlled. When labeling dilute protein solutions (e.g., 2mg/mL) a greater molar fold excess of biotin is used compared to a concentrated protein solution (e.g., 10mg/mL). For example, for best results use ≥ 12-fold molar excess of biotin for a 10mg/mL IgG solution or ≥ 20-fold molar excess of biotin for a mg/mL IgG solution.

1. Calculate millimoles of Sulfo-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 Sulfo-NHS-SS-Biotin (prepared in Step C.2) to add to the reaction:

$$\text{mmol Biotin} \times \frac{607\text{mg}}{\text{mmol Biotin}} \times \frac{1000\,\mu\text{L}}{6.0\text{mg}} = \mu\text{L Biotin}$$

- 20 = Recommended molar-fold excess of biotin for 2mg/mL IgG sample
- 607 = Molecular weight of Sulfo-NHS-SS-Biotin
- 1000 = Microliters of water in which 6.0mg of Sulfo-NHS-SS-Biotin is dissolved for a 10mM solution

Example: For 1mL of IgG (150,000 MW) at 2mg/mL, 26.9µL of 10mM Sulfo-NHS-SS-Biotin will be added.

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

$$0.000266\text{mmol Biotin} \times \frac{607\text{mg}}{\text{mmol Biotin}} \times \frac{1000\,\mu\text{L}}{6.0\text{mg}} = 26.9\,\mu\text{L Sulfo-NHS-SS-Biotin}$$

C. Biotinylation

For reaction volumes from 10-100µL, the buffer exchange and biotinylation may be conveniently performed in a single Slide-A-Lyzer MINI Dialysis Unit. For reaction volumes from 0.1-12mL, Slide-A-Lyzer Dialysis Cassettes may be used.

1. Dissolve or buffer exchange IgG into Reaction Buffer.

2. Immediately before use, prepare a 10mM solution of Sulfo-NHS-SS-Biotin as follows by adding 6mg to 1mL of ultrapure water.
3. Add the appropriate volume of the Sulfo-NHS-SS-Biotin solution (see Calculations section) to the IgG solution.
4. Incubate reaction on ice for two hours or at room temperature for 30 minutes.
5. Remove the non-reacted Sulfo-NHS-SS-Biotin by dialysis or gel filtration. See instructions provided with preferred buffer exchange product.
6. Store the biotinylated protein using the same condition that is optimal for the non-biotinylated protein.
7. To cleave the disulfide bond in the spacer arm, incubate sample in 50mM 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.

Example Procedure for Biotinylating Cell Surface Proteins

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

1. Wash cells three times with ice-cold PBS (pH 8.0) to remove any contaminating proteins.
2. Suspend cells at a concentration of 25×10^6cells/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 Sulfo-NHS-SS-Biotin by adding 6mg to 1mL of ultrapure water.
4. Add ~80µL of 10mM Sulfo-NHS-SS-Biotin per milliliter of reaction volume.
5. Incubate reaction at room temperature for 30 minutes.
6. Wash cells three times with ice-cold PBS (pH 8.0) to remove non-reacted biotinylation reagent. Alternatively, 25-50mM Tris (pH 8.0) may be used for the initial wash to quench any non-reacted biotinylation reagent.

Troubleshooting

Problem	Cause	Solution
Lack of biotinylation	No amines available on molecule of interest	Use a biotinylation reagent that targets a different functional group or convert sulfhydryl to amine using Thermo Scientific™ Aminoethyl-8 (Product No. 23010)
	Buffer contains primary amines	Use a non-amine containing buffer
	Reagent not reactive due to hydrolysis of the NHS ester	Allow reagent vial 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 lost function	Excessive biotinylation	Reduce molar excess of biotinylation reagent, or reduce time or temperature for biotinylation
		Choose biotinylation reagent that targets different groups

Additional Information

A. Determination of Biotin Incorporation

Biotin incorporation can be estimated using HABA [2-(4´-hydroxyazobenzene)-2-carboxylic 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. 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 Products section.)

Related Thermo Scientific Products

21328	**EZ-Link™ Sulfo-NHS-SS-Biotin, No-Weigh™ Format,** 8 × 1mg microtubes
21331	**EZ-Link Sulfo-NHS-SS-Biotin,** 100mg
PG82075	**Pierce Premium Grade Sulfo-NHS-LC-Biotin,** 100mg
PG82076	**Pierce Premium Grade Sulfo-NHS-LC-Biotin,** 1g
28005	**Pierce Biotin Quantitation Kit,** contains HABA and a biotinylated standard
33033	**Sulfo-SBED Biotin Label Transfer Reagent,** 10mg, for protein:protein interaction studies
20228	**Pierce Monomeric Avidin Agarose,** reversible biotin-binding resin
20227	**Pierce Monomeric Avidin Kit,** complete kit for purifying biotinylated molecules
20347	**Streptavidin Agarose Resin,** 2mL
Alternative EZ-Link Biotinylation Products:	
21445	**Sulfo-NHS-SS-Biotinylation Kit,** ~10 reactions with 1-10mg of protein
21945	**Micro Sulfo-NHS-SS-Biotinylation Kit,** 8 reactions with 50-200µg of protein
21925	**Micro Sulfo-NHS-Biotinylation Kit,** 8 reactions with 50-200µg of protein
21935	**Micro Sulfo-NHS-LC-Biotinylation Kit,** 8 reactions with 50-200µg of protein
21327	**Pierce Sulfo-NHS-LC-Biotin, No-Weigh Format,** 8 × 1mg microtubes
21326	**Pierce Sulfo-NHS-Biotin, No-Weigh Format,** 8 × 1mg microtubes
21329	**Pierce NHS-PEG$_4$-Biotin, No-Weigh Format,** 8 × 2mg microtubes

General References

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