INSTRUCTIONS

Sulfo-SBED

Biotin Label Transfer Reagent

MAN0011296
Rev. B.0
Pub. Part No. 2160589

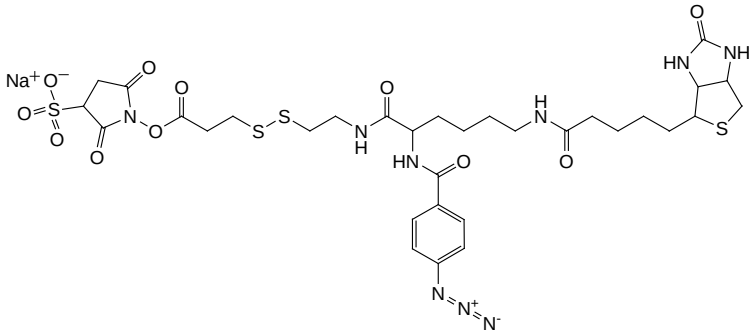
33033 A39260

Number	Description
33033	**Sulfo-SBED** (Sulfosuccinimidyl-2-[6-(biotinamido)-2-(*p*-azidobenzamido) hexanoamido]ethyl-1,3´-dithiopropionate)**,** 10mg, supplied as a dry powder and packaged under nitrogen, store at -20°C protected from light and moisture
A39260	**Sulfo-SBED, No-Weigh™ Format,** 10 × 1mg vials, store at 4°C protected from light and moisture

Molecular Weight: 879.97

Spacer Arm Lengths:

- Biotin: 19.1Å
- Sulfo-NHS ester: 13.7Å
- Aryl azide: 9.1Å



Storage: Upon receipt store product as indicated above. Product is shipped at ambient temperature.

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

Thermo Scientific™ Sulfo-SBED Transfer Reagent is a trifunctional crosslinking reagent containing a biotin, a sulfonated *N*-hydroxysuccinimide (Sulfo-NHS) active ester and a photoactivatable aryl azide. NHS esters react with primary amines at pH 7-9 to form covalent amide bonds. Upon photolysis, aryl azides form short-lived nitrenes that react nonspecifically or undergo ring expansion and react with nucleophiles, especially amines. The linkage containing the active ester has a cleavable disulfide bond, which makes this reagent ideal for protein:protein interaction studies using the label transfer method.

The label transfer method takes advantage of all the features built into Sulfo-SBED. The objective of the label-transfer method is to capture a protein interacting with another protein that has been biotinylated using Sulfo-SBED. An interacting protein is captured by the photoreactive aryl azide moiety. The interacting complex is then isolated and the disulfide bond subsequently reduced. Upon reduction of the disulfide bond, the biotin “label” is “transferred” to the interacting protein (See Additional Information Section). The biotin modified interacting protein can be detected by western blot using Streptavidin-HRP and an appropriate substrate. Sulfo-SBED is available in two formats. The standard format contains 10mg and is supplied as a dry powder packaged under nitrogen. Thermo Scientific™ No-Weigh™ Format Sulfo-SBED consists of convenient one-milligram per vial, eliminating difficulties associated with weighing small quantities of reagent.

Important Product information

- Do not store Sulfo-SBED in solution because the NHS ester will hydrolyze and become non-reactive. The half-life of The NHS ester moiety is ~20 minutes in phosphate buffer at room temperature. Discard any unused reconstituted crosslinker.
- Sulfo-SBED is soluble in DMSO (125mM), DMF (170mM), methanol (12mM) and water (~5mM). The concentration of Sulfo-SBED may vary from 0.1 to 3mM in most buffers (~1mM in 0.1M PBS). To solubilize Sulfo-SBED at higher concentrations, first dissolve it in a water-miscible organic solvent such as DMSO or DMF. Use 1-10% of solvent in the final reaction volume to minimize detrimental effects to the protein.

- To use the No-Weigh format Sulfo-SBED, uncap the vial immediately before use and add 22µL of DMF or DMSO to one vial, which results in a 50mM solution. Gently mix the solution with a pipette tip to fully reconstitute the reagent. Alternatively, the solution can be vortexed for a few seconds to ensure a homogeneous solution. Use the Sulfo-SBED solution immediately. Return unused vials to the foil pouch and keep seal closed between uses. **The maximum useable volume of the vial is 800µL.**
- For the Sulfo-NHS ester coupling reaction any buffer at pH 7-9 may be used provided it does not contain primary amines or sulfhydryls (e.g., phosphate, borate, carbonate and HEPES are acceptable buffers).
- Proteins modified with Sulfo-SBED may precipitate in solution at concentrations lower than expected. If a precipitate forms in the final conjugate, dilute conjugate before use if possible. For some applications it may be necessary to filter the conjugate before use.
- The disulfide bond of Sulfo-SBED may be cleaved by dithiothreitol or 2-mercaptoethanol, resulting in a biotin label attached to the protein conjugated by photoactivation. The biotinylated protein then may be used in such applications as immobilization of the protein, protein purification or an immunoassay.

Procedure for Coupling Trypsin and Soybean Trypsin Inhibitor with Sulfo-SBED

The following procedure is an example application for Sulfo-SBED. In this procedure, the primary amines on the soybean trypsin inhibitor (STI) are modified at 4-25°C in the dark. After removal of hydrolyzed and non-reacted crosslinker by gel filtration or dialysis, the modified protein then can be coupled by photoactivation to trypsin.

A. Materials Required

- ~5mg of soybean trypsin inhibitor (STI)
- Phosphate Buffered Saline (e.g., Thermo Scientific BupH Phosphate Buffered Saline Packs containing 0.1M phosphate, 0.15M NaCl, pH 7.2, Product No. 28372) or other buffer at pH 7.0-8.0
- DMSO (Product No. 20684) or DMF (Product No. 20673)
- Thermo Scientific™ Zeba™ Spin Desalting Columns (Product No. 89891 and 89893) or other product for buffer exchange, such as Thermo Scientific Slide-A-Lyzer Dialysis Cassettes
- Thermo Scientific™ Pierce™ BCA Protein Assay Kit (Product No. 23227) or other product to monitor protein
- TPCK Trypsin (Product No. 20233)
- 50mM DTT (Product No. 20290) or 100mM 2-mercaptoethanol (Product No. 35602)

B. NHS-Ester Reaction

Note: Perform Steps 1-5 in the dark to preserve the aryl azide group.

1. Dissolve ~5mg of soybean trypsin inhibitor (STI) in 0.5mL PBS in a microcentrifuge tube.
2. Immediately before use, dissolve 1.12mg of Sulfo-SBED in 25µL of DMSO or DMF. Alternatively, dissolve the contents of one No-Weigh™ vial of Sulfo-SBED with 22µL of DMSO or DMF. Add 11µL of the Sulfo-SBED solution to the STI.
3. Incubate at room temperature for 30 minutes or on ice for 2 hours. If a precipitate forms, centrifuge briefly (~1 minute) to remove hydrolyzed Sulfo-SBED from the solution.
4. Taking care to avoid the pellet, apply the reaction mixture to a 5mL desalting column equilibrated with PBS to remove the balance of the nonreacted Sulfo-SBED. Alternatively, use dialysis to remove the nonreacted Sulfo-SBED.

C. Conjugation of Biotinylated STI with Trypsin

1. Mix biotinylated STI with 5mg of TPCK Trypsin dissolved in 0.5mL of PBS. Incubate at room temperature for 3-5 minutes.
2. Photoactivate the aryl azide using a long-wave UV lamp (365nm) at a distance of 5cm for 15 minutes.
3. Desalt using a 10mL desalting column equilibrated with PBS. Collect 1mL fractions and pool protein-containing fractions.
4. The disulfide bond in the spacer arm originally attached to the Sulfo-NHS ester may be cleaved by incubating with 50mM DTT or 100mM 2-mercaptoethanol.

Additional Information

A. The Label Transfer Method

The objective of the label transfer method is to capture a protein (Y) interacting with another protein (X) that has been biotinylated using Sulfo-SBED. An interacting protein is captured by the photoreactive aryl azide moiety. The interacting complex is then isolated and the disulfide bond subsequently reduced. Upon reduction of the disulfide bond, the biotin is "transferred" to the interacting protein (Figure 1). The biotin-labeled interacting protein (Y) can be detected by Western blot using streptavidin-HRP and an appropriate substrate.

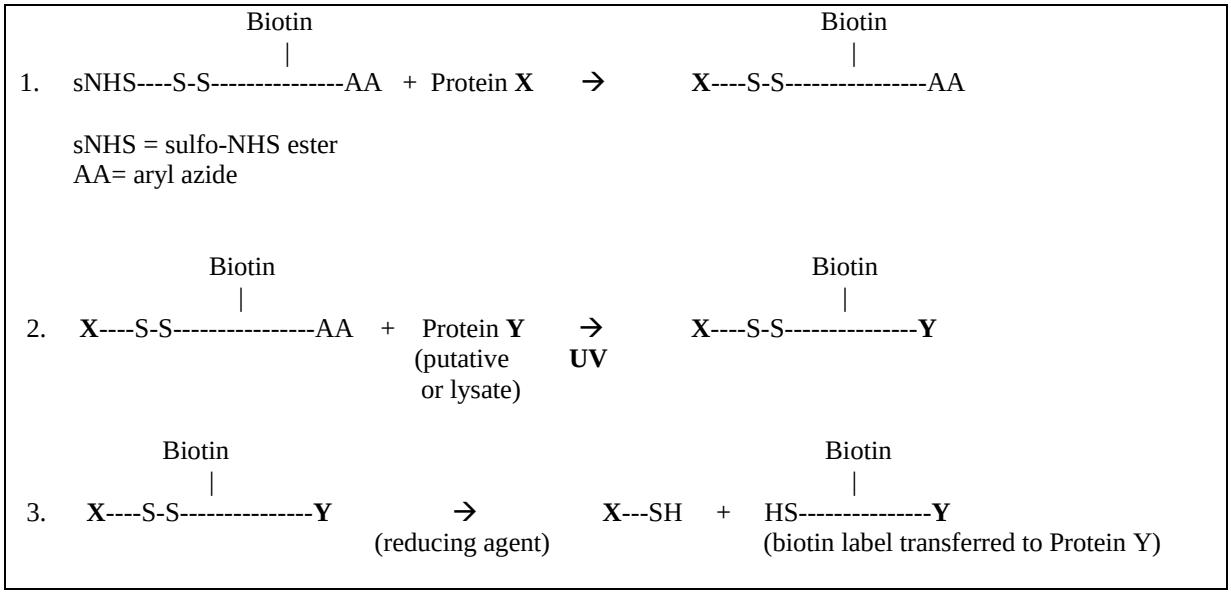


Figure 1. Label transfer using Sulfo-SBED. The protein (X) is biotinylated with Sulfo-SBED. An interacting protein (Y) is captured by the photoreactive aryl azide moiety. The interacting complex is isolated and the disulfide bond reduced resulting in the biotin "label" being "transferred" to the interacting protein.

B. Determination of Biotin Incorporation

Biotin incorporation can be estimated using the HABA (4´-hydroxyazobenzene-2-carboxylic acid] method (e.g., Thermo Scientific™ Pierce™ Biotin Quantitation Kit, Product No. 28005). 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.

Related Thermo Scientific Products

33073	**Sulfo-SBED Biotin Label Transfer Kit – Western Blot Application**
26166	**Bioconjugate Techniques (book),** by Greg T. Hermanson, softcover
A39255	**Dithiothreitol (DTT), No-Weigh Format,** 48 × 7.7mg
21115	**Biotinylated Protein Interaction Pull-Down Kit**
21126	**Streptavidin, Horseradish Peroxidase Conjugated,** 1mg
15120	**Streptavidin Coated Plates,** 5 plates (see catalog for a complete listing of plates)
20347	**Streptavidin Agarose Resin,** 2mL

General References

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Label Transfer References

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