Sulfo-SBED
Biotin Label Transfer Reagent

33033  33034

Number   Description

33033  Sulfo-SBED (Sulfosuccinimidyl-2-[6-(biotinamido)-2-(p-azidobenzamido) hexanoamido]ethyl-1,3’-dithiopropionate), 10 mg, supplied as a dry powder and packaged under nitrogen, store at -20°C protected from light and moisture

33034  Sulfo-SBED, No-Weigh™ Format, 8 × 1 mg microtubes, store at 4°C protected from light and moisture

Molecular Weight: 879.97
Spacer Arm Lengths:
• Biotin: 19.1 Å
• Sulfo-NHS ester: 13.7 Å
• Aryl azide: 9.1 Å

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

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Introduction
Sulfo-SBED 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 10 mg and is supplied as a dry powder packaged under nitrogen. No-Weigh™ Format Sulfo-SBED consists of convenient one-milligram microtubes, 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 (125 mM), DMF (170 mM), methanol (12 mM) and water (~5 mM). The concentration of Sulfo-SBED may vary from 0.1 to 3 mM in most buffers (~1 mM in 0.1 M 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 affects to the protein.

- To use the No-Weigh Format Sulfo-SBED, puncture the foil covering with a clean pipette tip. Add 22 µl of DMF or DMSO to one microtube, which results in a 50 mM solution. Gently mix the solution with a pipette tip to fully reconstitute the reagent. Use the Sulfo-SBED solution immediately. Discard any unused reconstituted Sulfo-SBED. Used microtubes may be cut off and discarded. Return unused microtubes to the foil pouch and keep seal closed between uses.

- 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**

- ~5 mg of soybean trypsin inhibitor (STI)
- Phosphate Buffered Saline (e.g., BupH™ Phosphate Buffered Saline Packs containing 0.1 M phosphate, 0.15 M 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)
- Zeba Desalt Spin Columns (Product No. 89891 and 89893) or other product for buffer exchange, such as Slide-A-Lyzer® Dialysis Cassettes
- Pierce BCA Protein Assay Kit (Product No. 23227) or other product to monitor protein
- TPCK Trypsin (Product No. 20233)
- 50 mM DTT (Product No. 20290) or 100 mM 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 ~5 mg of soybean trypsin inhibitor (STI) in 0.5 ml PBS in a microcentrifuge tube.

2. Immediately before use, dissolve 1.12 mg of Sulfo-SBED in 25 µl of DMSO or DMF. Alternatively, dissolve the contents of one No-Weigh Microtube of Sulfo-SBED with 22 µl of DMSO or DMF. Add 11 µl 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 solution.

4. Taking care to avoid the pellet, apply the reaction mixture to a 5 ml 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 5 mg of TPCK Trypsin dissolved in 0.5 ml PBS. Incubate at room temperature for 3 to 5 minutes.

2. Photoactivate the aryl azide using a long-wave UV lamp (365 nm) at a distance of 5 cm for 15 minutes.

3. Desalt using 10 ml desalting column equilibrated with PBS. Collect 1 ml 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 50 mM DTT or 100 mM 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.

B. Determination of Biotin Incorporation

Biotin incorporation can be estimated using the HABA (4'-hydroxyazobenzene-2-carboxylic acid] method (e.g., 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 500 nm. Biotin is then added to the solution and because of its higher affinity for avidin, biotin displaces the HABA and the absorption at 500 nm 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

20036  Bioconjugate Techniques (book), by Greg T. Hermanson, softcover

20291  Dithiothreitol (DTT), No-Weigh Format, 48 × 7.7 mg microtubes

21115  Biotinylated Protein Interaction Pull-Down Kit

21126  Streptavidin, Horseradish Peroxidase Conjugated, 1 mg

15120  Streptavidin Coated Plates, 5 plates (see catalog for a complete listing of plates)

20347  Streptavidin Agarose, 2 ml

General References


Label Transfer References


SBED is protected by U.S. Patent 5,532,379.

Sulfo-NHS Technology is protected by US Patents 5,872,261 and 5,892,057.

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