INSTRUCTIONS

Sulfo-SBED Biotin Label Transfer Kit – Western Blot Application

33073

1497.4

Number	Description
33073	**Sulfo-SBED Biotin Label Transfer Kit – Western Blot Application,** sufficient reagents for eight label transfer reactions for subsequent Western blot analysis

Kit Contents:

Sulfo-SBED (sulfosuccinimidyl-2-[6-(biotinamido)-2-(*p*-azidobenzamido) hexanoamido] ethyl-1,3'-dithiopropionate), 8 × 1 mg microtubes

- Molecular weight: 879.97
- Biotin spacer arm: 19.1 Å
- Sulfo-NHS ester spacer arm: 13.7 Å
- Aryl azide spacer arm: 9.1 Å

BupH™ Phosphate Buffered Saline, 1 pack, results in 0.1 M sodium phosphate, 0.15 M NaCl; pH 7.2 when reconstituted with 500 ml of ultrapure water

Label Transfer Buffer (20X), 200 ml, the 1X buffer is equal to 4 L of 50 mM HEPES, 150 mM NaCl; pH 7.3 ±0.2

Streptavidin-Horseradish Peroxidase Conjugate, 1 mg, contains 10 mM sodium phosphate and 150 mM sodium chloride; pH 6.8 when reconstituted with 1 ml of water

Dithiothreitol (DTT), 8 × 7.7 mg microtubes

Slide-A-Lyzer® MINI Dialysis Units Plus Float, 10K MWCO, 10 units/pk

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

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Introduction

The Thermo Scientific Sulfo-SBED Biotin Label Transfer Kit provides key components needed to detect and identify protein:protein interactions. The tri-functional crosslinking reagent Sulfo-SBED (Figure 1) contains a sulfonated *N*-hydroxysuccinimide (Sulfo-NHS) active ester, a photoactivatable aryl azide and a biotin, which can be used for identification, affinity purification and localization of target molecules. The linkage containing the active ester has a cleavable disulfide bond that makes this reagent ideal for protein:protein interaction studies using the label transfer method.

Studying protein:protein interactions is a difficult process because the complex is usually transient during analysis. The use of a covalent crosslinker to stabilize the interacting groups has made it easier to identify associated partners and how they

interact. The label transfer method uses the NHS ester of Sulfo-SBED to biotinylate a protein. An interacting protein is then captured by the photoreactive aryl azide moiety. Upon reduction of the disulfide bond, the biotin "label" is "transferred" to the interacting protein, usually referred to as the "prey" protein. The biotinylated prey protein can be detected by Western blot using streptavidin-HRP and an appropriate substrate and detection system.

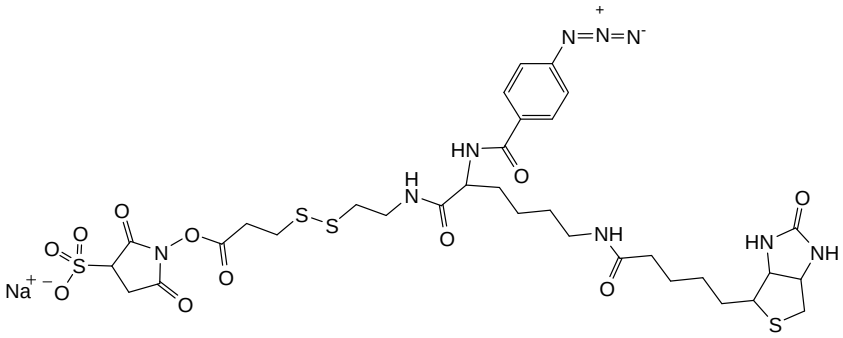

Figure 1. Chemical structure of Sulfo-SBED.

Procedure Summary

Note: Sulfo-SBED is light-sensitive. Perform all reaction steps in subdued indirect light. Cover all reaction vessels during incubation steps until after photoactivation.

1. React the purified protein (bait) with Sulfo-SBED for 30 minutes at room temperature.
2. Dialyze the reaction mixture against 1X Label Transfer Buffer or other suitable buffer.
3. Add the SBED-labeled protein to the sample containing the interacting target and incubate for 30 minutes.
4. Photoactivate the reaction mixture for 0.5-15 minutes with a UV light source.
5. Analyze sample mixture by electrophoresis, followed by Western blotting.
6. Probe for the biotin label using streptavidin-HRP and detect using a chemiluminescent or colorimetric substrate.

Note: When analyzing weak protein interactions, purification or enrichment of biotinylated complexes can be performed using immobilized monomeric avidin. Reducing the disulfide bond while the complex is bound to the monomeric avidin will release the bait protein. The captured biotinylated target protein is then eluted using mild conditions. Immobilized monomeric avidin is available as a kit containing all necessary purification buffers (Product No. 20227) or by itself (Product No. 20228).

Important Product Information

- Sulfo-SBED is light-sensitive. Perform all reaction and incubation steps in subdued indirect light. Cover all reaction vessels with aluminum foil or use containers impermeable to light until after photoactivation.
- Store Sulfo-SBED at 4°C protected from light and moisture. Do not store Sulfo-SBED in solution because the NHS ester will hydrolyze and become nonreactive. 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 soluble 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.
- 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. Phosphate, borate, carbonate and HEPES are acceptable buffers. Avoid amine-containing buffers such as Tris or glycine.
- Sulfo-SBED-modified proteins may precipitate in solution at concentrations lower than expected. If a precipitate forms in the final conjugate, dilute conjugate before use. Some applications may require filtering 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.

Photolysis Information

- Use a shallow reaction vessel for maximum efficiency. Irradiation efficiency decreases as the distance light must penetrate the solution increases. Choose a low protein-binding vessel for maximum sample recovery.
- For photolysis use a UV lamp that irradiates at 300-370 nm (see **Note** below). High-wattage lamps are more effective and require shorter exposure times than low-wattage lamps. Suggestions for lamps include the Stratalinker 2400 (5 × 15 watt bulbs, emission at either 312 nm or 365 nm), mercury vapor lamps (180 watt, 350 watt, between 300 nm to 360 nm), XeCl excimer laser (150 mJ, 308 nm) and UV Spectroline lamps (medium/long wavelength lamps). Using low-wattage hand-held lamps, such as 6 watt lamps, will result in lower conjugation efficiencies.

  Note: Avoid UV lamps that emit light at 254 nm; this wavelength causes proteins to photodestruct. Filters that remove light at wavelengths below 300 nm are ideal. Using a second filter that removes wavelengths above 370 nm could be beneficial but is not essential.
- Position the UV lamp 5-10 cm from the reaction. For lamps > 150 watts use a distance of 10 cm. For lower powered lamps, use a distance of 5 cm. Perform photolysis by placing the lamp above the reaction as the reaction vessel may impede irradiation by filtering some of the UV light.

Procedure for Label Transfer

The following protocol is an example application of this product. Specific applications and systems require optimization.

A. Additional Materials Required

- DMF (Product No. 20673) or DMSO (Product No. 20684)
- Suitable UV light source to initiate photolysis – see **Photolysis Information** for considerations and guidelines
- Non-Reducing Sample Buffer such as Lane Marker Non-Reducing Sample Buffer (5X) (Product No. 39001)
- Nitrocellulose (Product No. 88018) or PVDF (Product No. 88118) membrane
- Western blot blocking buffer such as Thermo Scientific StartingBlock Blocking Buffer (Product No. 37542)
- Thermo Scientific SuperSignal West Dura Chemiluminescent Substrate (Product No. 34075) or other Western blotting HRP substrate
- X-ray film (Product No. 34090 or 34091) or other documentation system for chemiluminescent detection

B. Labeling Bait Protein with Sulfo-SBED

1. Reconstitute contents of the BupH Phosphate Buffered Saline (PBS) Pack with 500 ml of ultrapure water. Filter sterilize unused PBS and store at 4°C.
2. Prepare the purified bait protein at 0.1-10 mg/ml in PBS. Typical reaction volumes range from 20 to 500 µl. Transfer the prepared bait protein to an amber or foil-covered polypropylene microcentrifuge tube.
3. Calculate the amount of Sulfo-SBED needed to yield a 1- to 5-molar excess over the purified protein.

   Note: Higher molar ratios may result in protein aggregation and precipitation.

$$\text{Sulfo-SBED MW (880)} \times \frac{\mu\text{g of Protein}}{\text{Protein MW}} \times \text{desired fold molar excess} = \mu\text{g of Sulfo-SBED needed}$$

4. Puncture the foil covering on one microtube of Sulfo-SBED and add 25 µl of DMF or DMSO. Use a pipette tip to gently mix the contents until all of the Sulfo-SBED is in solution. The concentration of Sulfo-SBED will be 40 µg/µl.

   Note: If less than 40 µg (1 µl) of Sulfo-SBED is needed, 50-100 µl of DMF can be used to dissolve the Sulfo-SBED.
5. For every microgram of Sulfo-SBED needed, add 0.025 µl of the dissolved Sulfo-SBED solution to the bait protein. Gently mix and cover the reaction tube with aluminum foil or place in a dark location for 30 minutes at room temperature or for 2 hours at 4°C.
6. Prepare the 1X Label Transfer Buffer by diluting 15 ml of the 20X Label Transfer Buffer with 285 ml of ultrapure water.

   Note: The pH of the 20X Label Transfer Buffer will decrease from pH 7.8 to 7.3 when diluted to 1X. Alternative buffers may be used to optimize a specific protein:protein interaction.

7. Carefully pipette 10-100 µl of the labeling reaction into one MINI Dialysis unit and cap the unit. If the reaction is greater than 100 µl, use multiple dialysis units. Insert the dialysis units containing samples into the provided float and then place the float into a 500 ml beaker containing the 1X Label Transfer Buffer.

   Note: Desalting columns (Product No. 89849) equilibrated with 1X Label Transfer Buffer also may be used to remove nonreacted crosslinker.

8. Cover the beaker with foil and dialyze for at least 4 hours or overnight at 4°C.

   Note: If the sample volume increased during overnight dialysis, correct for this volume increase when performing Step C.1 below by adding additional sample containing the SBED-labeled protein.

9. After dialysis, the SBED-labeled protein is ready for interaction with the prey protein sample(s) for the label-transfer reaction. Divide any unused SBED-labeled protein into single-use aliquots and store protected from light at -80°C. Avoid multiple freeze/thaw cycles of the protein.

   Note: Label incorporation may be assessed by several methods. To quantify biotinylation, use the Biotin Quantitation kit (Product No. 28005); or perform a dot blot using Streptavidin-HRP as the probe; or perform a Western blot containing a control lane of the labeled bait protein before it has been crosslinked to the prey protein.

C. Protein Interaction and Crosslinking

1. Add the SBED-labeled protein to the sample containing the prey protein. The ratio of labeled protein to prey protein sample to use is dependent on each specific system being investigated. For best results, test different sample concentrations and ratios of SBED-labeled protein to sample.
2. Incubate the SBED-labeled protein with the target sample for 60 minutes at room temperature. Protect the reaction from light until ready to perform crosslinking.
3. Photoactivate the SBED conjugate with an UV light source. Although photoactivation may be performed at room temperature, for best results when using high-wattage lamps, place samples on ice to prevent sample heating. Exposure time varies depending on the intensity of the light source. Suggested exposure times are as follows:

   15 minutes for 6 watt hand-held lamps at distance of 5 cm

   8 minutes for a device with 2 × 15 watt lamps at distance of 5 cm

   5 minutes for a device with 5 × 15 watt lamps at distance of 5 cm

   5 minutes for a 180 watt lamp at distance of 10 cm

   1.5 minutes for a 350 watt lamp at distance of 10 cm

   Note: For information about selecting and using UV light sources for photoactivation, see the Important Product Information – Photolysis Information Section.

D. Western Blot Analysis

1. Puncture the foil covering of one DTT microtube with a pipette tip and add 50 µl of ultrapure water to yield 1 M DTT.
2. Add 50 µl of the 1 M DTT to 50 µl of Lane Marker Non-reducing Sample Buffer or other non-reducing sample buffer.
3. To two new microcentrifuge tubes, add 10 to 20 µl of sample. To one sample, add 1/5 volume of reducing sample buffer (prepared in Step 2). The final concentration of DTT must be 100 mM for complete disulfide bond reduction. To the other tube, add 1/5 volume of non-reducing sample buffer. Vortex both tubes.
4. Heat samples at 70°C for 5 minutes. Apply samples to a polyacrylamide gel and perform gel electrophoresis. Transfer proteins to a nitrocellulose or PVDF membrane.
5. Block the membrane with blocking buffer for 30 minutes at room temperature.
6. For detection with SuperSignal West Dura Substrate, use the primary antibody at 0.2 µg/ml and the secondary antibody at 4-20 ng/ml, or follow manufacturer's recommendations for the specific substrate system being used.
7. Following detection, the membrane may be stripped, using Restore™ Stripping Buffer (Product No. 21059), and re-probed with Streptavidin-HRP (4-20 ng/ml). Reconstitute the Streptavidin-HRP with 1 ml of ultrapure water to produce a 1 mg/ml stock solution. Store stock solution at 4°C for ≤ 1 month or prepare single-use volumes and store at -20°C.

Note: Streptavidin-biotin interactions are difficult to strip from membranes; therefore, use Streptavidin-HRP as the last probe.

Troubleshooting

Problem	Possible Cause	Solution
Precipitation forms after initial labeling reaction	Over labeling of the protein	Use a lower molar ratio of Sulfo-SBED to protein
	Improper storage of labeled protein	Use labeled protein immediately after dialysis or store frozen as single use aliquots; avoid multiple freeze-thaw cycles
Western blot has too many bands	Nonspecific binding of probe	Try a different blocking agent
		Target sample may contain other proteins which bind to labeled ligand – add Tween®-20 (0.005%, v/v) the 1X Label Transfer Buffer
		Enrich complex sample using immobilized monomeric avidin
	Too much protein loaded on gel	Apply less protein to the gel
No detectable signal in Western blot	Poor labeling of bait protein, poor photoactivation or concentration of target (prey) protein is too low	Determine label incorporation of the bait protein, before photoactivation with the prey protein, by Western blot
		Check UV light sources – see Photolysis Information Section for guidelines
		Enrich target protein system if possible
		Optimize antibody concentrations used in the Western blot
	Protein interaction is blocking the epitope, which may occur with monoclonal antibodies and peptide directed antibodies	Use an antibody from a different source or probe with Streptavidin-HRP
Good labeling of bait protein but poor conjugation to target	Crosslinking caused steric hindrance	Lower the ratio of Sulfo-SBED used in the initial labeling of bait protein or add low levels of Tween-20 to the 1X Label Transfer Buffer (0.005%, v/v)
	Poor protein interaction conditions	Optimize Label Transfer Buffer conditions, incubation times and temperature
	Poor photoactivation	Check UV light sources – see Photolysis Information Section for guidelines

Related Thermo Scientific Products

Please see the catalog or website for a complete listing of products for Western blotting and sample preparation.

25200-25244 **Precise™ Protein Gels** (see catalog or web site for a complete listing)

89849 **Protein Desalting Spin Columns,** 25/pkg

89862 **Protein Desalting Spin Columns,** 50/pkg

28005 **EZ Biotin Quantitation Kit**

39001 **Lane Marker Non-Reducing Sample Buffer (5X),** 5 ml

39000 **Lane Marker Reducing Sample Buffer (5X),** 5 ml

34080 **SuperSignal West Pico Chemiluminescent Substrate,** 500 ml

34075 **SuperSignal West Dura Extended Duration Substrate,** 100 ml

34095 **SuperSignal West Femto Maximum Sensitivity Substrate,** 100 ml

21059 **Restore Western Blot Stripping Buffer,** 500 ml

34090 **CL-XPosure™ Film (5" × 7"),** 100 sheets

34091 **CL-XPosure Film (8"× 10"),** 100 sheets

21065 **Pierce® Background Eliminator Kit,** for eliminating background from X-ray film

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