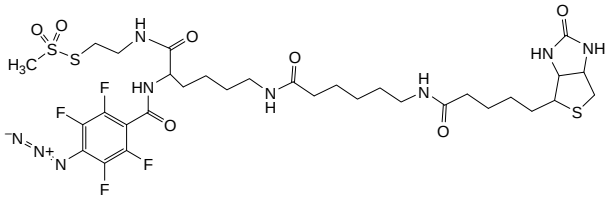
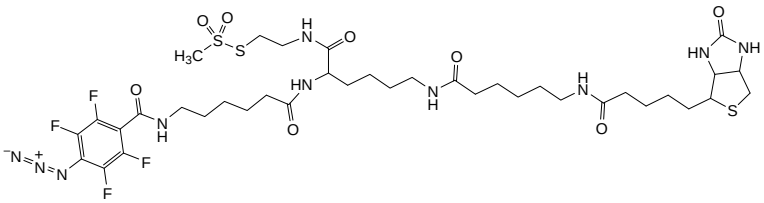
INSTRUCTIONS

ProFound™ Mts-Atf-Biotin Label Transfer Reagents

33093 33083

1744.0

Number	Description
33093	**Mts-Atf-Biotin** (2-[*N*2-(4-azido-2,3,5,6-tetrafluorobenzoyl)-*N*6-(6-biotinamidocaproyl)-L-lysinyl]ethyl methanethiosulfonate), 5 mg Molecular Weight: 839.95 Biotin-Mts Spacer Arm: 29.3 Å Biotin-Atf Spacer Arm: 30.7 Å Mts-Atf Spacer Arm: 11.1 Å 
33083	**Mts-Atf-LC-Biotin** (2-{*N*2-[*N*6-(4-azido-2,3,5,6-tetrafluorobenzoyl-6-aminocaproyl)-*N*6-(6-biotinamidocaproyl)-L-lysinylamido]}ethyl methanethiosulfonate), 5 mg Molecular Weight: 953.11 Biotin-Mts Spacer Arm: 29.3 Å Biotin-Atf Spacer Arm: 35.2 Å Mts-Atf Spacer Arm: 21.8 Å 

Storage: Upon receipt store reagent at -20°C desiccated and protected from light. Reagent is shipped at ambient temperature.

Introduction

The ProFound™ Mts-Atf-Biotin Label Transfer Reagents are trifunctional cross-linkers that contain a biotin, a sulfhydryl-reactive methanethiosulfonate (Mts) moiety and an efficient photoactivatable tetrafluorophenyl azide (Atf) moiety. The Atf groups, upon photoactivation, form short-lived nitrenes that can insert into carbon hydrogen bonds (C-H) and unsaturated carbon chains. The Mts groups, at physiological pH values, form disulfide bonds with available sulfhydryl groups. This disulfide bond is cleavable by reducing agents, such as dithiothreitol, 2-mercaptoethanol or TCEP, making these reagents ideal for protein:protein interaction studies using the label transfer method.

The objective of the label transfer method involves transfer of a biotin molecule from a purified bait protein to a binding partner designated as the prey protein (Figure 1). The bait protein, which must contain a free sulfhydryl group, is first reacted with the Mts moiety of the reagent. After removing excess nonreacted reagent, an interacting protein is captured by the photoactivatable Atf moiety. The interacting complex is then isolated and the disulfide bond subsequently reduced. Upon disulfide bond reduction, the biotin label is transferred to the interacting protein. The biotin-modified interacting protein can be detected by Western blot using Streptavidin-HRP and an appropriate substrate.

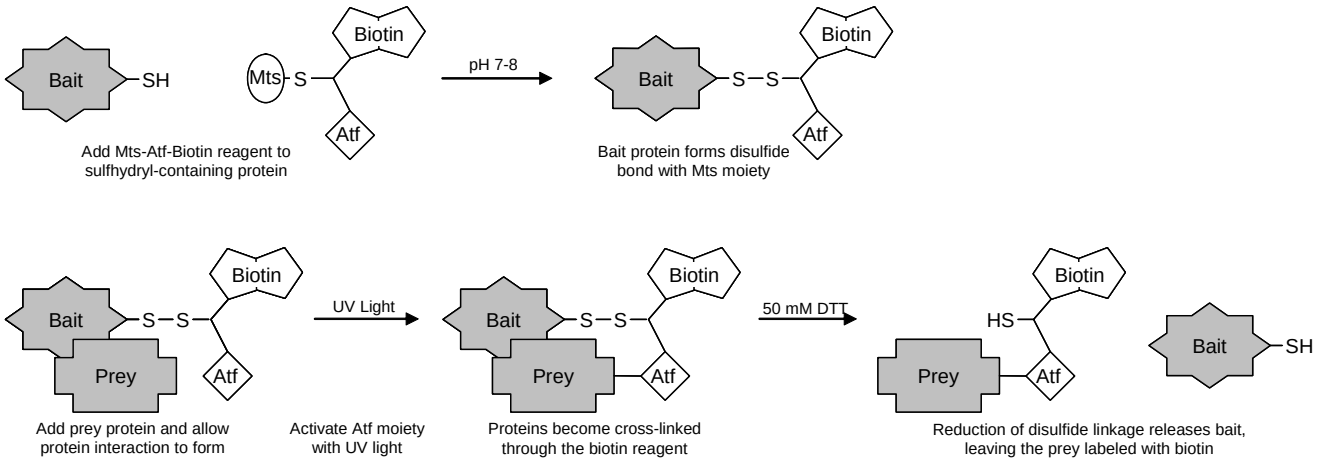


Figure 1. Schematic of the label transfer method. The bait protein is first biotinylated through the Mts moiety. An interacting prey protein is then captured by photoactivation of the Atf moiety. The interacting complex is isolated and the disulfide bond reduced, resulting in the biotin label being transferred to the interacting protein.

Important Product Information

- The following instructions are guidelines for protein:protein interaction studies using the label transfer method. When optimizing specific applications and systems, consider factors such as the strength and transience of the interaction, cofactor requirements, and amount of purified bait protein and the natural abundance of the prey protein.
- The bait protein must be purified and have free sulfhydryl group(s) available for conjugation. Cysteine-containing molecules often oxidize in solution and form disulfide bonds, which cannot react with the Mts moiety. Disulfide reducing agents used to produce free sulfhydryls must be removed before conjugation to the label transfer reagents. The Reduce-Imm™ Reducing Kit (Product No. 77700) and Immobilized TCEP Disulfide Reducing Gel (Product No. 77712) enable disulfide reduction and protein recovery in the absence of reducing agents.
- Sulfhydryls can be added to molecules using *N*-succinimidyl *S*-acetylthioacetate (SATA, Product No. 26102) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101). Both reagents modify primary amines.
- Prepare Label Transfer Reagent solutions immediately before use. Do not store these reagents in solution.
- These reagents are 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 photoactivation.
- Use an organic solvent, such as DMSO or DMF, to dissolve the label transfer reagents before adding to an aqueous buffer. Use the least amount of solvent as possible (1-10%) in the final reaction to minimize detrimental affects to the protein or conditions that could interfere with the intended interaction.

Photoactivation Information

- Use a shallow reaction vessel for maximum efficiency. Irradiation efficiency decreases as the distance between the reactants and the light source increases. Choose a low protein-binding vessel for maximum sample recovery.
- Use a UV lamp that irradiates at 300-370 nm (see **Note** below) for photoactivation. 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 and 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: The optimal wavelength for photoactivation of the Atf moiety is 320 nm. Avoid UV lamps that emit light at 254 nm as 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 photoactivation by placing the lamp above the open reaction vessel to avoid impeding irradiation by the vessel.

Procedure for Label Transfer using the Mts-Atf-Biotin Reagents

A. Materials Required

- Phosphate Buffered Saline (PBS) containing 0.1 M phosphate, 0.15 M NaCl; pH 7.2 (BupH™ Phosphate Buffered Saline Packs, Product No. 28372) or other amine-free buffer at pH 7.0-8.0
- DMSO (Product No. 20684) or DMF (Product No. 20673)
- Dialysis unit such as Slide-A-Lyzer® MINI Dialysis Unit or a desalting column such as the Zeba™ Desalt Spin Columns (i.e., Product No. 89889 for 200-700 µl samples or Product No. 89891for 500-2,000 µl samples) or other product for buffer exchange
- SDS-PAGE sample buffer such as Lane Marker Non-Reducing Sample Buffer (5X) (Product No. 39001) containing 50 mM DTT (Product No. 20290) or 100 mM 2-mercaptoethanol (Product No. 35602)
- Precise™ Protein Gel (Product No. 25200-25244, see catalog or web site for a complete listing)

B. Conjugation of the Mts Moiety with -SH group(s) on the Bait Protein

Note: Conduct all reagent manipulations in subdued light or in the dark to protect the Atf moiety from early photoactivation. Wrap reaction tube in foil or protect from light by some other means.

1. Dissolve or dilute the purified bait protein to 0.1-10 mg/ml in PBS. Typical reaction volumes are 20-500 µl. Transfer the prepared bait protein to an amber or foil-covered polypropylene microcentrifuge tube.
2. Calculate amount of label transfer reagent needed to achieve 1- to 5-fold molar excess over the purified bait protein as follows:

$$\text{Label Transfer Reagent MW} \times \frac{\mu\text{g of Protein}}{\text{Protein MW}} \times \text{desired fold molar excess} = \mu\text{g of Label Transfer Reagent needed}$$

3. Dissolve 1 mg of the label transfer reagent in 25 µl of DMSO or DMF. Mix gently until the reagent dissolves.
4. For each microgram of reagent needed, add 0.025 µl of the reagent solution to the bait protein. Gently mix reaction and incubate for 1 hour at room temperature or 4 hours at 4°C.
5. Remove nonreacted reagent by dialyzing against PBS for at least 4 hours at room temperature or overnight at 4°C. Place the dialysis container in the dark. Zeba™ Desalt Spin Columns may also be used to remove nonreacted reagent.
6. For storage, divide the labeled bait protein into single-use aliquots and store protected from light at -80°C. Avoid multiple freeze/thaw cycles of the protein.

   Note: Label incorporation can be assessed by several methods. To quantify biotinylation, use the EZ™ Biotin Quantitation Kit (see Additional Information Section) or perform a dot blot using Streptavidin-HRP as the probe.

C. Photoactivation of the Atf Moiety

1. Add the biotin-labeled bait protein to the prey protein sample. The ratio of biotin-labeled bait protein to prey protein is dependent on each specific system being investigated. For best results, test different sample concentrations and ratios.

   Note: Add any necessary binding cofactor(s) to the reaction before photoactivation. Avoid disulfide reducing agents.
2. Incubate reaction for 1 hour at room temperature. Protect reaction from light until photoactivation, which results in the capture of the bait:prey complex.

3. Photoactivate the Atf moiety with a UV light source (see the Photoactivation Information Section). Although photoactivation may be performed at room temperature, for best results place samples on ice when using high wattage lamps to prevent sample heating. Effective exposure time will vary depending on the intensity of the light source. Suggested exposure times are as follows:

   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: Proteins modified with these transfer reagents may precipitate. If a precipitate forms in the final conjugate, dilute or filter the solution before use.

D. Disulfide Reduction for Biotin Transfer

1. Add sample to the SDS-PAGE sample buffer. Use a final concentration of 50 mM DTT or 100 mM 2-mercaptoethanol for complete disulfide bond reduction.
2. Heat samples at 70°C for 5 minutes.
3. Apply samples to a polyacrylamide gel and perform gel electrophoresis.

Additional Information

Determination of Biotin Incorporation: The HABA [2-(4′-hydroxyazobenzene)-benzoic acid] method enables estimation of biotin incorporation. In solution, the HABA dye binds avidin, forming a complex with maximal absorption at 500 nm. When biotin is added to the solution, its higher affinity for avidin 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. The EZ™ Biotin Quantitation Kit (Product No. 28005) contains a premix of HABA and avidin and a biotinylated BSA control supplied in convenient No-Weigh™ Microtube packaging, which eliminates the difficulties associated with weighing small quantities of reagent.

Western Blotting: The prey protein can be detected by Western blot using a primary antibody directed against the prey protein and an appropriate secondary antibody-HRP conjugate. Alternatively, use Streptavidin-HRP (Product No. 21127) to detect the biotin label on the prey protein. For greatest sensitivity use chemiluminescence detection (see SuperSignal® Chemiluminescent Detection Products listed in Related Thermo Scientific Products Section).

Mass Spectrometry: The bait:prey complex or the biotinylated prey protein can be analyzed by mass spectrometry (MS) methods. To prepare a sample for liquid chromatographic separation or electrospray ionization MS, excise a band from a coomassie-stained gel and perform a digestion using the In-Gel Tryptic Digestion Kit (Product No. 89871). For MALDI-TOF MS analysis, further process the sample using PepClean™ C-18 Spin Columns (Product No. 89870).

Related Thermo Scientific Products

33033	**ProFound™ Label Transfer Sulfo-SBED Protein:Protein Interaction Reagent,** 10 mg
33034	**No-Weigh™ Sulfo-SBED,** 8 × 1 mg microtubes, store at 4°C protected from light and moisture
33073	**ProFound™ Sulfo-SBED Biotin Label Transfer Kit**
25200-25244	**Precise™ Protein Gels** (see catalog or web site for a complete listing)
24585	**MemCode™ Reversible Protein Stain Kit – for PVDF Membranes**
24580	**MemCode™ Reversible Protein Stain Kit – for Nitrocellulose Membranes**
88600	**Western Blotting Filter Paper, 8 cm × 10.5 cm,** 100 sheets
34080	**SuperSignal® West Pico Chemiluminescent Substrate,** 500 ml
34075	**SuperSignal® West Dura Extended Duration 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
34089	**CL-XPosure™ Film (18 × 24 cm),** 100 sheets
21065	**Erase-It® Background Eliminator Kit,** for removing background from X-ray film
78266	**B-PER® Bacterial Protein Extraction Reagent (in phosphate buffer),** 500 ml
78501	**M-PER® Mammalian Protein Extraction Reagent**, 250 ml

Slide-A-Lyzer® MINI Dialysis Unit Technology is protected by U.S. Patent # 6,039,871.

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

SuperSignal® Technology is protected by U.S. Patent # 6,432,662.

B-PER® Technology is protected by U.S. Patent # 6,174,704.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.