

Meso Scale Discovery

MSD SULFO-TAG™ NHS-Ester



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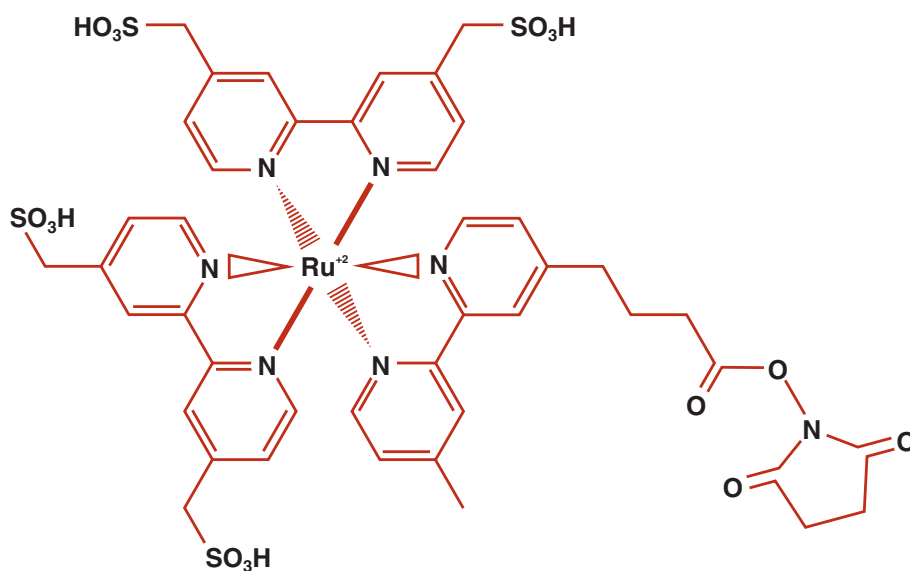
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This protocol details the labeling procedure for proteins of a molecular weight (MW) $\geq 10,000$ Daltons using MSD SULFO-TAG NHS Ester. The straightforward procedure involves a buffer exchange (if necessary), a 1 hour incubation, and the use of a spin column to quickly isolate the labeled protein. MSD SULFO-TAG NHS-Ester (Figure 1) is an amine-reactive, N-hydroxysuccinimide ester which readily couples to primary amine groups of proteins under mildly basic conditions to form a stable amide bond.

MSD SULFO-TAG-labeled conjugates are stable and may be used at low concentrations. These features minimize time, costs, and labor as large batches of a stable conjugate can be prepared, validated, and used for long periods of time. Its excellent performance characteristics and simple labeling procedure make MSD SULFO-TAG NHS-Ester a popular conjugate for labeling molecules that contain primary amines (e.g. lysine-containing proteins). MSD SULFO-TAG NHS-Ester offers low non-specific binding, resulting in highly sensitive detection when used in conjunction with MSD's SECTOR™ instruments and SECTOR PR™ readers.

Figure 1: MSD SULFO-TAG NHS-Ester



Preparation of MSD SULFO-TAG Protein Conjugates



preparation of MSD SULFO-TAG protein conjugates

General Notes

In order to minimize hydrolysis of the NHS-Ester, the MSD SULFO-TAG reagent should be dissolved in cold distilled water just prior to its addition to the protein solution. If necessary, the stock SULFO-TAG solution can be kept on ice for up to 10 minutes. The reconstituted solution is unstable and any unused material should be discarded. Consider labeling more than one protein at the same time to maximize the use of the SULFO-TAG reagent.

The optimal SULFO-TAG reagent:protein labeling ratio should be empirically determined for each specific application. For immunoassays, typical MSD SULFO-TAG reagent:antibody (IgG) molar challenge ratios are 6:1, 12:1, and 20:1. If reagents are limiting, a 12:1 molar challenge ratio is recommended. The best challenge ratios to use with other protein types may vary and depend on various factors including the protein size and the number of lysines available for coupling. Standard labeling conditions using a 2 mg/mL IgG solution result in a label incorporation of about 30-40%. Optimal assay performance using a SULFO-TAG labeled antibody is often obtained with a SULFO-TAG reagent:protein molar incorporation ratio between 2:1 and 10:1. Excessively high labeling ratios may increase non-specific binding and inactivate the binding site. Labeling with higher protein concentrations and slightly alkaline PBS (pH 7.9) solution without preservatives yields the best labeling efficiencies. Maintaining consistent labeling conditions (protein concentration, buffer type, label concentration, incubation time, shaking, and temperature) are important when preparing multiple batches of labeled protein in order to achieve consistent assay results.

This protocol describes the SULFO-TAG labeling procedure for proteins with a MW of $\geq 10,000$ Da. If the protein/polypeptide to be labeled has a lysine and a MW $\leq 10,000$ Da, the SULFO-TAG reagent may still be used with modifications to the labeling and separation conditions. For example, an alternative separation method independent of molecular size may be required. MSD offers a variety of services for the custom labeling of reagents including proteins, peptides, and non-proteinaceous molecules.

Materials Required

1. Phosphate-buffered saline (PBS), pH 7.9, preservative-free
2. PBS pH 7.4 + 0.05-0.1% Sodium Azide
3. Polypropylene microfuge tubes
4. Shaker (optional)
5. Spin columns (MSD recommends the use of Quick Spin™ High Capacity Columns from Roche Diagnostics, catalog number 03117928001, or alternative Sephadex® G-50 spin columns. The use of Sephadex G-25 based columns is not recommended.)
6. Spectrophotometer capable of an OD₄₅₅ measurement
7. Protein assay such as BCA, Bradford, or Lowry

Protocol

1. Prepare a purified 2 mg/mL solution of the protein to be labeled in preservative-free PBS, pH 7.9. Antibodies in a storage buffer with preservatives such as sodium azide or EDTA must be buffer-exchanged before the labeling reaction. It is recommended that dilute protein solutions be concentrated to at least 0.5 mg/mL. Protein solutions should be concentrated and/or buffer-exchanged using the spin columns described above or an alternative centrifugal filtration/concentration unit that has been prepared with preservative-free PBS, pH 7.9. The concentration of the protein solution to be labeled should be confirmed prior to beginning the labeling reaction.
2. Calculate the amount of MSD SULFO-TAG NHS-Ester stock solution required for the labeling reaction using the formula seen below and on the attached worksheet.

Notes:

Other buffers can be used for the labeling reaction provided they are free of amine-containing molecules (i.e., no tris- or glycine-containing buffers) and preservatives. Affinity-purified antibodies are commonly eluted with high molarity glycine solutions; therefore it is important that they are properly desalted prior to labeling.

Lower protein concentrations may be used in labeling reactions, however lower incorporation efficiencies may be observed.

CALCULATIONS

$$1000 \times \frac{\text{Protein Conc. (mg/mL)}}{\text{Protein MW}} \times \text{Challenge ratio} \times \text{Vol. of protein in soln. (\mu\text{L})} = \text{nmol SULFO-TAG reagent required}$$

Using this value, calculate the volume of SULFO-TAG stock solution required for the reaction. Step 3 of this protocol details the reconstitution instructions for MSD SULFO-TAG label to generate a stock solution in nmol/ μL .

$$\frac{\text{nmol SULFO-TAG reagent required}}{\text{concentration of MSD SULFO-TAG stock solution (nmol/\mu\text{L})}} = \mu\text{L MSD SULFO-TAG stock solution required for labeling reaction}$$

EXAMPLE

- 500 μL of 2 mg/mL antibody
- 12:1 challenge ratio
- SULFO-TAG stock = 3 nmol/ μL

$$1000 \times \frac{2 \text{ mg/mL}}{150,000 \text{ Da}} \times 12 \times 500 \mu\text{L} = 80 \text{ nmol}$$

$$\frac{80 \text{ nmol SULFO-TAG reagent}}{3 \text{ nmol/\mu\text{L} SULFO-TAG stock}} = 26.7 \mu\text{L MSD SULFO-TAG stock solution required for labeling reaction}$$

Protocol (continued)

Notes:

3. Reconstitute MSD SULFO-TAG NHS-Ester immediately prior to use with cold distilled water. For the 500 and 150 nmol sizes of SULFO-TAG NHS-Ester, dissolve with 50 μL to generate stock solutions of 10 and 3 nmol/ μL , respectively. Gently twirl the vial to ensure dissolution of all lyophilized material.
4. Add the calculated volume (from Step 2) of reconstituted SULFO-TAG NHS-Ester to the protein solution and vortex immediately. Discard any remaining unused SULFO-TAG NHS-Ester.
5. Incubate at room temperature for 2 hours. Shield the reaction from light by covering the tube with aluminum foil or placing it in a dark area (e.g. a closed drawer). Take care to maintain consistent labeling conditions between multiple preparation lots to ensure the best results.
6. During this incubation period, prepare spin columns with PBS-0.05% Sodium Azide, pH 7.4, by filling the columns with buffer and draining by gravity. Repeat 3 times. Immediately prior to the end of the reaction incubation, centrifuge the columns with their attached reservoirs, at 1000 x g for 4 minutes at 4°C, using a swinging bucket rotor to remove residual buffer from the resin. Remove buffer from the reservoirs.
7. In a drop-wise manner, apply labeling solution to spin column (250 μL maximum volume per column if using recommended columns listed above). Centrifuge the columns with their attached reservoirs, at 1000 x g for 6 minutes at 4°C, using a swinging bucket rotor to separate the SULFO-TAG labeled protein from the unconjugated SULFO-TAG reagent. Retain the labeled material in the reservoirs; the columns can be discarded.
8. Determine the protein concentration of the labeled protein using a standard colorimetric protein assay such as BCA, Bradford, or Lowry.
9. Measure the absorbance of the MSD SULFO-TAG protein conjugate at 455 nm using a 1 cm path cuvette. Divide the measured value by the extinction coefficient of the label (15,400 $\text{M}^{-1} \text{cm}^{-1}$) to obtain the MSD SULFO-TAG label concentration in moles per liter. For reference, a formula calculation worksheet page is attached.
10. To calculate the MSD SULFO-TAG label:protein ratio, divide the SULFO-TAG label concentration value determined in step 9, by the protein concentration value determined in step 8.
11. Stabilize dilute protein solutions (< 0.1 mg/mL) by supplementing with 1-3% (w/v) bovine serum albumin or other appropriate serum protein. Antibody conjugates are usually stable 1 year at 4°C; stability of other protein types should be determined. Many proteins require storage at -20°C. For long-term storage, labeled conjugates should be stored in amber or opaque vials. Conjugate aliquots can be stored frozen as long as the protein is stable to freeze-thaw cycles.

Shaking the solution is optional, and may increase the reaction efficiency.

If using the Roche spin columns recommended above, a reaction volume of 250 μL (maximum) can be loaded per column. Prepare a sufficient amount of columns based on the volume of the labeling reaction.

The unconjugated SULFO-TAG reagent should appear as a yellow band retained in the spin column, with a clear separation between the free SULFO-TAG band and the labeled material in the reservoirs.

Do not use an OD_{280} absorbance reading as MSD SULFO-TAG will absorb light at this wavelength.

III Storage, Handling, and Stability



storage, handling, and stability

MSD SULFO-TAG NHS Ester is supplied as a dry orange-red lyophilized solid. The vials should be stored frozen (-10 to -30°C), desiccated, and shielded from light. Following reconstitution with water, any remaining unused material should be discarded.

Note: MSD SULFO-TAG reagent is light-sensitive. For long term storage of SULFO-TAG protein conjugates, keep material in the dark or in light-shielded containers. Short term exposure of conjugates to ambient light during conjugate preparation or when carrying out assays is generally not a concern.

IV Support

support

MSD Customer Support is available 9 AM - 5 PM Monday through Friday, excluding holidays.

Ordering Information

ordering information

| Product | Size | Catalog Number |
|-------------------------|------------|----------------|
| MSD SULFO-TAG NHS Ester | 150 nmoles | R91AN-1 |
| | 500 nmoles | R91AN-2 |

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The products referenced in this technical note are for research use only, and are not for use in diagnostic or therapeutic procedures.

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Quick Spin is a trademark of the Roche Group.

Sephadex is a trademark of Amersham Biosciences.

Date: _____

Materials

Protein to be labeled: _____

Concentration: _____ Vendor: _____

Catalog Number: _____ Lot Number: _____

Sample Preparation: _____

Method: _____ Buffer: _____

Lot Number: _____ Date: _____

Columns/Concentrators: _____

Lot Number: _____

MSD-SULFO-TAG NHS-Ester Reconstitution: _____

Size: _____ Lot Number: _____

Distilled Water: _____

Lot Number: _____ Date: _____

Volume of water added to vial: _____

Stock Concentration (nmol/ μ L): _____

Separation and Calculations: _____

Buffer: _____

Lot Number: _____ Date: _____

Columns: _____

Lot Number: _____

Protein Assay Kit: _____

Type: _____ Lot Number: _____

Pre-Labeling Calculations

$$1000 \times \frac{(\text{Protein Conc., mg/mL})}{\text{MW of protein}} \times (\text{Challenge ratio}) \times (\text{Volume of protein, } \mu\text{L}) = \text{nmol SULFO-TAG NHS-Ester required for reaction}$$

$$\frac{\text{nmol SULFO-TAG reagent required for reaction}}{\text{nmol}/\mu\text{L MSD SULFO-TAG stock solution}} = \mu\text{L MSD SULFO-TAG stock solution required}$$

Labeling Procedure

Sample Preparation: _____
Concentration/Buffer Exchange: _____
Notes: _____
Volume of SULFO-TAG stock solution added to protein: _____
Time reaction started: _____ Time reaction completed: _____ Shaking: Y / N
Separation of Labeled Material: _____
Columns: _____
Centrifuge: _____
Time: _____ Temp: _____ Speed: _____
Buffer: _____

Post-Labeling Procedure

Protein Assay: _____
Vendor: _____
Catalog Number: _____ Lot Number: _____
Protein Concentration: _____
OD₄₅₅: _____

Post-Labeling Calculations

$$\frac{(\text{Protein conc, mg/mL})}{\text{MW of protein}} = \text{_____ M (A)} \quad \frac{\text{OD}_{455}}{15,400 \text{ (Extinction Coefficient)}} = \text{_____ M (B)}$$

Labeling Incorporation Ratio (SULFO-TAG label:protein) = **B/A**

Storage Information

Aliquot size: _____ Storage temperature: _____
Location: _____ Date: _____
Notes: _____

