

MESO SCALE DISCOVERY

MSD SULFO-TAG NHS-Ester

150 nMoles R91AN-1

500 nMoles R91AN-2



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MSD SULFO-TAG NHS-Ester

This package insert must be read in its entirety before using this product.

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MESO SCALE DISCOVERY®

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Ordering Information

ordering information

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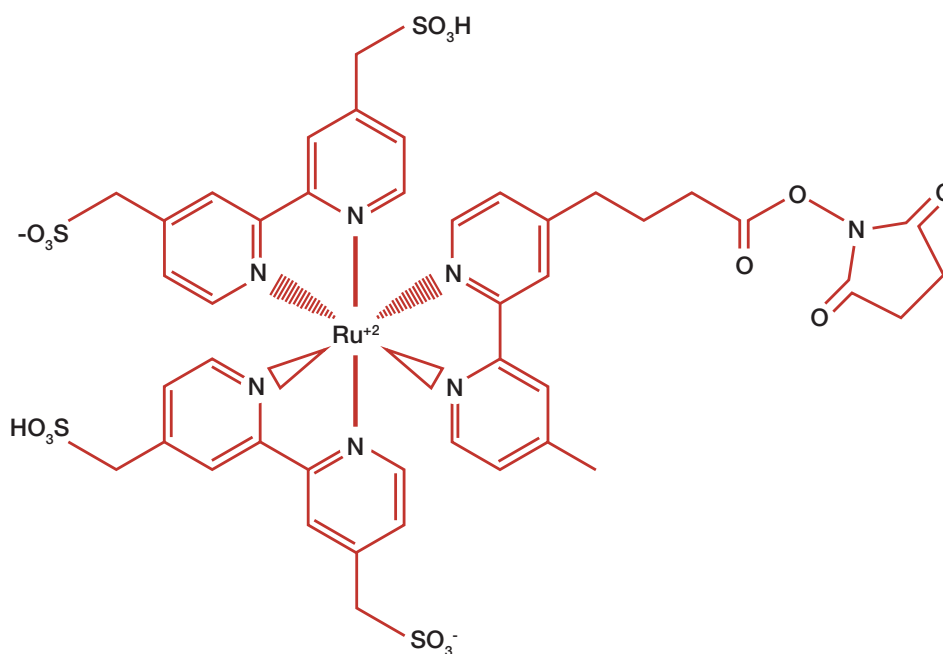
Introduction

introduction

This protocol details the labeling procedure for proteins of molecular weight (MW) > 40000 Daltons using MSD® SULFO-TAG NHS Ester. The straightforward procedure involves a buffer exchange (if necessary), an incubation step for 2 hours, 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™ conjugates are stable and may be used at low concentrations. These features minimize time, cost, 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 the product of choice for molecules that contain primary amines (e.g. lysine-containing proteins). MSD SULFO-TAG 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 conjugating more than one protein at the same time to maximize the use of the SULFO-TAG reagent. The kit provides 150 nmol of SULFO-TAG NHS-Ester, sufficient for conjugation of up to 1 mg of IgG.

The Conjugation Ratio is the number of molecules of SULFO-TAG conjugated to each molecule of protein. Optimal conjugation ratios for a SULFO-TAG labeled protein should be determined empirically for each specific application. For most applications using IgG antibodies (MW~ 150000), optimal performance is obtained with conjugation ratios between 2:1 and 10:1. In this range, assay signals typically show a linear dependence on conjugation ratio. Conjugation ratios significantly higher than 10:1 can be counterproductive and may lead to elevated background signals or loss of binding activity. For proteins that are significantly smaller than IgGs, lower conjugation ratios (between 1:1 and 5:1) may provide better assay performance.

The Challenge Ratio is the number of moles of SULFO-TAG per mole of protein in the conjugation reaction mixture. The challenge ratio required to achieve a specific conjugation ratio depends on a number of factors including pH, temperature, protein concentration, protein size and the number of lysines available for coupling. Labeling a 2 mg/mL IgG solution using the standard conditions described in this protocol will typically result in a label incorporation of about 50%, i.e., a challenge ratio of 10:1 will result in a conjugation ratio of about 5:1. Labeling efficiencies for other proteins may be different. In general, labeling with high protein concentrations on the order of 1-2mg/mL in slightly alkaline PBS (pH 7.9) in the absence of preservatives yields the best labeling efficiencies. Maintaining consistent labeling conditions (protein concentration, buffer type, SULFO-TAG NHS-Ester concentration, incubation time, shaking and temperature) is important when preparing multiple batches of labeled protein in order to achieve consistent assay results.

When developing immunoassays, MSD recommends labeling antibodies using the standard labeling conditions outlined in this document and challenge ratios of 6:1, 12:1 and 20:1 to identify optimal labeling conditions. If evaluating different conditions is not possible due to limited reagent quantities, a challenge ratio of 20:1 will generally provide good performance. For immunogenicity applications where an antibody drug or protein therapeutic is used, the suggested challenge ratios are 12:1 and 6:1 SULFO-TAG:drug. If only one ratio is tested, a 10:1 challenge ratio is recommended. For details on building immunogenicity assays, please refer to the Bridging Immunogenicity Guidelines for Assay Development at www.mesoscale.com.

The protocol describes the SULFO-TAG labeling procedure for proteins with a MW > 40000 Da. Smaller proteins/polypeptides may also be labeled using SULFO-TAG NHS-Ester as long as they have an accessible lysine or the N-terminal amino group, however, alternative separation methods may be needed to remove unconjugated label. 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. Spin columns. MSD recommends the use of ZEBA™ Spin Desalting Columns, 40K MWCO of various sizes from Thermo Scientific, catalog numbers 87766 - 87773
5. 15 mL conical tubes for use with ZEBA Spin Desalting Columns, 40K MWCO, 2 mL column size
6. Protein assay such as BCA, Bradford, or Lowry
7. MSD Blocker A (optional)
8. 0.2 µm filter (optional)

Vendor	Catalog Number	Volume of Conjugated Protein
Whatman	AV125EAQU	≥ 2.0 mL
Millipore or Fisher Scientific (MILLEX®-GV)	SLGV004SL	0.2 - 0.5 mL

9. Concentrator (optional)

Vendor	Catalog Number	Range
Millipore BIOMAX-50 concentrator, 50 MWCO	UFV5BQK25	0.05 - 0.5 mL
AMICON® Ultra-4 concentrator, PLQK Ultracel-PL Membrane, 50 MWCO	UFC805008	0.5 - 4.0 mL
AMICON® Ultra-15 concentrator, PLQK Ultracel-PL Membrane, 50 MWCO	UFC905024	0.5 - 15.0 mL

10. Spectrophotometer capable of an OD₄₅₅ measurement

Note

The following table lists the catalog numbers of the ZEBA Spin Desalting Columns, 40K MWCO, from Thermo Scientific and the recommended sample volume for each column.

Table 1

Thermo Scientific Catalog Number	Number of Columns/pack	ZEBA Spin Desalting Column Volume	Recommended Volume of the Labeling Reaction
87766	25	0.5 mL	70-100 µL
87767	50	0.5 mL	70-100 µL
87768	5	2 mL	200-450 µL
87769	25	2 mL	200-450 µL
87770	5	5 mL	300-1000 µL
87771	25	5 mL	300-1000 µL
87772	5	10 mL	1000-2000 µL
87773	25	10 mL	1000-2000 µL

Protocol

1. Prepare a 1-2 mg/mL solution of the protein to be conjugated 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 conjugation reaction. It is recommended that dilute protein solutions are concentrated to at least 1 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 equilibrated with preservative-free PBS, pH 7.9. The concentration of the protein solution to be conjugated should be confirmed prior to beginning the labeling reaction.
2. Equilibrate the protein to be conjugated with SULFO-TAG NHS-Ester at the conjugation temperature of 23°C. A temperature range of 20°C to 25°C is acceptable. The equilibration can take between 10-30 minutes depending on the volume of protein.
3. Calculate the amount of MSD SULFO-TAG NHS-Ester stock solution required for the conjugation 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 4 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

$$1000 \times \frac{2 \text{ mg/mL}}{150000 \text{ Da}} \times 12 \times 500 \mu\text{L} = 80 \text{ nmol}$$

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

Protocol (continued)

Notes:

- Centrifuge the MSD SULFO-TAG NHS-Ester vial by pulse spinning for 1 minute or by gently tapping on a soft surface in order to collect lyophilized material at the bottom of the vial. 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 μ L to generate stock solutions of 10 and 3 nmol/ μ L respectively. Gently vortex the vial to ensure complete dissolution of all lyophilized material.
- Add the calculated volume (from Step 3) of reconstituted SULFO-TAG NHS-Ester to the protein solution and vortex immediately. Discard any remaining unused SULFO-TAG NHS-Ester.
- Incubate at 23°C for 2 hours; a temperature range of 20°C to 25°C is acceptable. 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 reproducibility in labeling.
- Prepare ZEBRA Spin Desalting Columns, 40K MWCO, towards the end of the incubation period. Remove the column's bottom closure and loosen the cap. Do not remove the cap. Place the column in a collection tube to remove the storage buffer and wash the column 3 times with PBS. Each preparation step should be carried out by centrifuging the columns, and their respective collection tubes, in a centrifuge with a swinging bucket rotor at 2-8°C. Refer to table 2 for the recommended sample volume, wash buffer volumes, collection tube sizes, and centrifugation times for each preparation step. Alternatively, the desalting columns may be equilibrated in a buffer other than PBS if the end application requires storage of the labeled protein in a non-PBS buffer.
- Apply the labeling reaction to the spin column in a drop-wise manner, following the recommendations in Table 2. Using a swinging bucket rotor, centrifuge the columns in clean new collection tubes to separate the SULFO-TAG conjugated protein from the unconjugated SULFO-TAG reagent. The SULFO-TAG conjugated protein will be captured in the conical tubes. Retain the conjugated material in the conical tubes, and discard the columns.

Reconstituted SULFO-TAG NHS-Ester may be kept for up to 10 minutes on ice prior to use.

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

Reaction volumes larger than the capacity of a ZEBRA column can be distributed over several ZEBRA columns.

The unconjugated SULFO-TAG reagent should appear as a yellow band in the spin column, that is clearly separated from the labeled material in the collection tube.

Table 2

Centrifugation speeds, times and volumes for the Wash Buffer and Sample					
ZEBRA Spin Desalting Column Size (mL)	0.5	2	5	10	
Labeling Reaction Sample Volume Range (μ L)	70-100	200-450	300-1000	1000-2000	
Wash/Equilibrium Buffer Volume	300 μ L	1 mL	2.5 mL	5 mL	
Collection Tubes	1.5 mL microcentrifuge tubes	15 mL conical tubes	15 mL conical tubes	50 mL conical tubes	
Centrifuge Speed (x g)	1500	1000	1000	1000	
Centrifugation Time (min)	Storage Solution Removal	1	2	2	2
	Wash 1	1	4	4	4
	Wash 2	1	2	2	2
	Wash 3*	3	5	5	5
	Sample Recovery**	4	6	6	6
* After the 3rd wash, the resin should have an opaque white uniform color. If the resin color is not uniform, additional spin time may be needed. If the top layer shows granules, over centrifugation has occurred and the 3rd wash can be repeated.					
** Additional sample recovery spin time is allowed if needed to ensure maximum recovery of sample. Overdrying the resin at this stage will not harm the protein, therefore, spinning for up to 8 minutes is allowed.					

Protocol (continued)

9. It is recommended to filter the conjugated protein using a 0.2 μm filter. Filtration may cause some loss of the protein. Please refer to page 3 for the recommended filter units.
10. Determine the molar protein concentration of the conjugated protein using a standard colorimetric protein assay such as BCA, Bradford, or Lowry.
11. Measure the absorbance of the MSD SULFO-TAG protein conjugate at 455 nm using a 1 cm path cuvette or other suitable nano-spectrophotometer. Divide the measured value by the extinction coefficient of the label (15400 $\text{M}^{-1}\text{cm}^{-1}$) to obtain the MSD SULFO-TAG label concentration in moles per liter. For reference, a formula calculation worksheet page is attached. If the absorbance pathlength is not 1 cm first divide the absorbance reading by the pathlength in cm, please refer to your spectrophotometer manual for details. The absorbance of the purified labeled antibody should be measured without dilution of the solution.
12. To calculate the MSD SULFO-TAG label: protein conjugation ratio, divide the SULFO-TAG label concentration value determined in step 11 by the molar protein concentration value determined in step 10.
13. Antibody conjugates are usually stable for at least 1 year at 2-8°C at a concentration of 1-2 mg/mL; stability of other protein types should be determined. For long term storage of conjugated protein at 2-8°C, 0.05% azide should be added to prevent any microbial growth. Conjugated proteins may be stored frozen at $\leq -20^\circ\text{C}$ or $\leq -70^\circ\text{C}$, as long as the protein is stable to freeze-thaw cycles or stored in single use aliquots. MSD SULFO-TAG reagent is sensitive to extended exposure to light and conjugated material should be stored in the dark or in amber or opaque vials. Short term exposure of conjugates to ambient light during conjugate preparation or when carrying out assays is generally not a concern. If the protein concentration is low (< 0.1 mg/mL), consider adding a carrier protein, such as 0.1% MSD Blocker A.

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 ($\leq -10^\circ\text{C}$), desiccated, and shielded from light. The expiration date of SULFO-TAG NHS-Ester is indicated on the label. Following reconstitution with water, any remaining unused material should be discarded.

1. **What chemicals interfere with SULFO-TAG labeling?**

Primary amines and strong nucleophiles interfere with SULFO-TAG NHS-Ester labeling. Common reagents that can interfere with the amine coupling of NHS chemistry are:

1. Tris
2. Glycine
3. Histidine
4. Azide
5. Imidazole
6. Glutathione
7. Ammonium ions
8. Glycerol

2. **What are typical carrier proteins in antibody solutions?**

- a) BSA
- b) Gelatin

Antibodies should be obtained in carrier-protein free formulations for labeling with SULFO-TAG NHS-Ester. Carrier proteins will interfere with SULFO-TAG NHS-Ester labeling and cannot be removed with desalting columns.

3. **What is the minimum amount of material that can be conjugated?**

Generally, 50-100 µg can be labeled if the protein concentration is high enough, preferably 1-2 mg/mL, in PBS without interfering buffer components. Otherwise, microconcentrators may be used to concentrate the antibody solution following equilibration of the microconcentrator with PBS.

4. **What is an ideal challenge ratio for conjugating an antibody?**

A challenge ratio of 20:1 is recommended, especially for dilute antibodies that are close to 0.5 mg/mL in concentration. We do not recommend conjugating antibodies that are less than 0.5 mg/mL. If possible, dilute antibodies should be concentrated prior to conjugation with SULFO-TAG. Recommended challenge ratios for immunogenicity applications can be found in the Bridging Immunogenicity Guidelines for Assay Development on www.mesoscale.com.

5. **How can I purify the SULFO-TAG conjugated antibody after labeling?**

Post labeling purification of antibody can be done using Thermo Scientific ZEBRA Spin Desalting Columns, 40K MWCO (Catalog Numbers: 87766 - 87773). Refer to purification steps listed in the protocol for guidelines. Typical sample concentrations that are loaded on the column after SULFO-TAG labeling are 0.5 mg/mL to 2 mg/mL. We have found that the 7K MWCO to be less effective at removal of free SULFO-TAG after conjugation reactions.

6. **Are there alternatives to using Thermo Scientific ZEBRA Spin Desalting Column for purifying the SULFO-TAG conjugated antibody after labeling?**

- a) Users may purchase commercially available G-50 SEPHADEX® columns or prepare G-50 SEPHADEX columns at the bench. However some G-50 columns may not be efficient in complete removal of unconjugated material. The SEPHADEX grade is important. MSD recommends using fine grade SEPHADEX for preparing self packed gel filtration columns. Medium Grade SEPHADEX does not provide suitable separations and Superfine SEPHADEX does not allow an adequate flow rate without use of a pump. It is not recommended to use PD10 columns or G-25 SEPHADEX spin columns for purification of SULFO-TAG conjugated protein as these are not able to separate free SULFO-TAG reagent from labeled conjugates.

- b) Alternatively, CENTRICON® concentrators or similar microconcentrator products with adequate MWCO (for concentrator information please refer to page 3) can be used to remove unbound label. First resuspend the labeling mixture in a larger volume of PBS-0.05% azide, concentrate to a smaller volume and repeat the process as per the product instructions for desalting applications.
- c) Post labeling purification of proteins with MW < 40000 Da will require alternative procedures (such as High resolution size exclusion chromatography, HPLC, FPLC, etc.) because ZEBRA Spin Desalting Columns or G-50 columns will not provide adequate separation in this size range.

7. ***What is the molecular weight of SULFO-TAG?***

Unreacted SULFO-TAG NHS-Ester has a molecular weight of 1141 g/mol. After the labeling reaction, each conjugated SULFO-TAG adds 1027 g/mol to the protein.

8. ***What type of material can be labeled?***

MSD SULFO-TAG NHS-Ester is reactive with primary amines. Proteins and large peptides are easily labeled. Fab fragments have also been labeled successfully.

MSD Custom labeling may be used to label small molecule and peptides. Please contact MSD Scientific Support (Phone: 1-301-947-2025, Email: ScientificSupport@mesoscale.com) or your local MSD Application Scientist for details.

9. ***What labeling ratio is recommended for an IgM?***

A challenge ratio range from 8:1 to 12:1 may be used for labeling IgM antibodies. The molecular weight of an IgM is in the order of 900000 Da. IgMs can be unstable at higher pHs, therefore labeling at pH 7.0 to 7.2 may be better than the standard labeling buffer of pH 7.9 used for IgG.

10. ***Why should I need to label my protein in buffer instead of water?***

Labeling with SULFO-TAG requires a well-buffered solution because the SULFO-TAG NHS-Ester itself is acidic.

11. ***Will my antibody retain activity after labeling?***

The SULFO-TAG is a small hydrophilic molecule and generally does not affect the function of its conjugation partner, especially when labeling large proteins such as antibodies. With small molecule or peptide labeling, the addition of the SULFO-TAG may have an effect on binding affinities.

12. ***What is the stability of SULFO-TAG NHS-Ester?***

The recommended expiration date of SULFO-TAG is 2 years at $\leq -10^{\circ}\text{C}$. The reagent life can be prolonged by storage at $\leq -70^{\circ}\text{C}$. Reagent stability is lower at room temperature or at $2-8^{\circ}\text{C}$. At room temperature, there may be a 1/3 to 1/2 loss of active material in a month. Once the reagent is reconstituted, it should be used as soon as possible since the NHS-ester hydrolyzes in water. After reconstitution, the solution may be kept up to 10 minutes on ice with minimal loss of activity.

13. ***What if the protein to be conjugated does not have any primary amine groups?***

Alternative linking chemistry options available as custom products from MSD which allow non-amine containing molecules to be successfully labeled. These include Thiol-Reactive linker (SULFO-TAG Iodoacetamide), Carboxyl (-COOH) Reactive linker (SULFO-TAG Amine) and Carbohydrate Reactive SULFO-TAG. Please contact MSD Scientific Support (Phone: 1-301-947-2025, Email: ScientificSupport@mesoscale.com) or your local MSD Application Scientist for details.

14. ***I have an IgG purified antibody from my protein production group which has been eluted into PBS. Should I desalt before labeling?***

Yes. Tris-glycine is a major component of antibody elution buffers used in the purification procedure. On many occasions a single desalting into PBS is insufficient. Hence it is recommended to repeat the desalting step into PBS to remove any trace quantities of Tris-glycine that can hinder conjugation with SULFO-TAG.
15. ***How do I conjugate a high concentration protein solution with SULFO-TAG?***

The labeling reaction will be more efficient at high protein concentrations. It is recommended to use a lower challenge ratio for labeling to compensate for the increased efficiency.
16. ***How do I conjugate a low concentration protein solution with SULFO-TAG?***

MSD recommends the protein concentration to be at least 0.5 mg/mL. However, if concentrating the protein solution is not feasible; labeling can be done at a lower concentration. This may result in a lower labeling efficiency. Therefore, the conjugation reaction should be performed at a higher challenge ratio, likely 20:1 or higher.
17. ***How do I conjugate small proteins with SULFO-TAG?***

Proteins with MW < 40000 Da can be labeled by the same chemistry as antibodies; lower challenge ratios may be required for SULFO-TAG labeling of small proteins and peptides than for IgGs. The NHS-Ester will react with primary amine such as lysine residues and the N-terminus of proteins and peptides. If there is no primary amine available a different chemistry will be necessary. Post labeling purification of small proteins will require alternative procedures (such as HPLC, FPLC, etc.) because small proteins are not resolved by G-50 columns. If MSD custom labeling is desired, Customer Service can provide a Custom Labeling Form in order to generate an appropriate quote.
18. ***Why can't I use a spectrophotometer at 280 nm to determine labeled protein concentration?***

SULFO-TAG strongly absorbs at 280 nm and will interfere with any measurement of protein concentration at this wavelength.
19. ***What is the stability of SULFO-TAG conjugated proteins?***

The SULFO-TAG labeled protein is generally as stable as the unlabeled protein, provided that it is stored in the appropriate buffer, concentration and storage temperature. The labeled protein should be stored in the dark, either at 2-8°C or frozen in aliquots. Azide should be added for long term storage at 2-8°C to prevent any microbial growth. If the protein concentration is low, consider adding a carrier protein, such as 0.1% MSD Blocker A.
20. ***My antibody did not label very well. What are the possible reasons?***

The presence of preservatives, carrier protein or residual Tris-glycine or other interfering substances in the labeling buffer (see Q 1 and 2) can reduce labeling efficiency of the protein. Very low concentration of the starting material (below 0.5 mg/mL) may also reduce labeling ratios. It is also been observed that some IgGs label more efficiently than others.
21. ***What components can be removed by buffer exchange or dialysis?***

Salt, azide, glycerol, buffering agent (e.g. Tris), carbohydrates (e.g. trehalose), amino acids (e.g. histidine, glycine) can be successfully removed by buffer exchange method.
22. ***Who should I contact if I have any questions on SULFO-TAG labeling?***

Please contact MSD Scientific Support (Phone: 1-301-947-2025, Email: ScientificSupport@mesoscale.com) or your local MSD Application Scientist for details.

Worksheet

worksheet

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/μL): _____

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

$$\frac{\text{(Protein conc, mg/mL)}}{\text{MW of protein}} = \text{_____ M (A)} \quad \frac{\text{OD}_{455}}{15400 \text{ (Extinction Coefficient)}} = \text{_____ M (B)}$$

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

Storage Information

Aliquot size: _____ Storage temperature: _____

Location: _____ Date: _____

Notes: _____
