# Quick Guide

## Introduction

SULFO-TAG™ NHS-Ester is an N-hydroxysuccinimide ester which readily couples to primary amine groups of proteins. SULFO-TAG conjugated proteins can be used as detection reagents in MSD® immunoassays. The conjugates are stable, may be used at low concentrations, have low non-specific binding, and result in highly sensitive detection. The exceptional performance characteristics and simple conjugation procedure of SULFO-TAG NHS-Ester make it the product of choice for molecules that contain primary amines (e.g., lysine in proteins).

This guide describes the SULFO-TAG conjugation protocol for proteins with a molecular weight (MW) > 40,000 Da. The straightforward procedure involves an optional buffer exchange prior to conjugation, a 2-hour incubation step, and a buffer exchange post-conjugation to quickly isolate the conjugated protein using a spin column. Smaller proteins/polypeptides may also be conjugated if they have an accessible lysine or N-terminal amino group; however, alternative conjugation parameters and post-conjugation separation methods may be needed to remove unconjugated SULFO-TAG label.

### MSD GOLD SULFO-TAG NHS-Ester Conjugation Pack Components (Catalog # R31AA)

| Reagent | Storage | Size | Quantity | Description |
|---|---|---|---|---|
| MSD GOLD SULFO-TAG NHS-Ester | ≤-70°C | 150 nmol | 5 vials | SULFO-TAG NHS-Ester label for coupling to antibodies and other proteins |
| Zeba Spin Desalting Column, 40K MWCO* | 2–8°C | variable | 10 columns | Size exclusion chromatography columns for the purification of proteins larger than 40,000 Da |
| Filter, 0.22 µm | RT | N/A | 10 each | Filter for use during purification |
| Syringe | RT | N/A | 10 each | Syringe for use during purification |
| Conjugation Buffer | RT | 40 mL | 1 bottle | 100 mM Phosphate Buffer, pH 7.9 |
| Conjugate Storage Buffer | RT | 40 mL | 1 bottle | Phosphate-buffered saline (PBS), pH 7.4, with 0.05% sodium azide |

*SULFO-TAG Conjugation Pack 1 includes 10 columns of 0.5 mL capacity and 10 syringes of 1 mL size, and SULFO-TAG Conjugation Pack 2 includes 10 columns of 5 mL capacity and 10 syringes of 3 mL size.

### Additional Materials (not provided)

1. Polypropylene microfuge tubes and 15 mL conical tubes
2. Protein assay such as BCA, Bradford, or Lowry
3. Concentrator (optional) (e.g., MilliporeSigma BIOMAX-50, AMICON Ultra-4, or AMICON Ultra-15 concentrators)
4. Additional Zeba Spin Desalting Columns, 40K MWCO are available in various sizes from Thermo Fisher Scientific (catalog numbers 87766–87773)

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# Conjugation Protocol

For additional guidance on SULFO-TAG conjugation, please refer to the SULFO-TAG NHS-Ester product insert available at www.mesoscale.com.

## Pre-Conjugation Procedure

1. Prepare a 1–2 mg/mL solution of the protein to be conjugated in the supplied Conjugation Buffer. Protein solutions may be concentrated or buffer exchanged using Zeba Spin Desalting Columns that have been equilibrated with Conjugation Buffer.
   - ❑ Preservatives such as sodium azide or EDTA, buffer components containing primary amines (e.g., Tris, glycine), and glycerol must be removed by buffer-exchange using the supplied Zeba Spin Desalting Columns before starting the conjugation reaction. Note: Conjugate Storage Buffer should not be used at this stage.
   - ❑ Filter the protein using a 0.2 µm filter.
   - ❑ Measure the concentration of the protein solution to be conjugated. Protein concentration can be calculated from an $OD_{280}$ absorbance or with a colorimetric protein concentration assay.
2. Equilibrate the protein to be conjugated at the conjugation temperature of 23°C (20–25°C is acceptable).
3. Calculate the amount of SULFO-TAG NHS-Ester stock solution required using the formula provided below.

### Calculations

$$\frac{1{,}000 \times \text{Protein conc. (mg/mL)} \times \text{Challenge ratio} \times \text{Vol. of protein solution (µL)}}{\text{Protein MW (Da)}} = \text{nmol of SULFO-TAG reagent reqd.}$$

Using this value, calculate the volume of SULFO-TAG stock solution required for the reaction.

$$\frac{\text{nmol of SULFO-TAG reagent required}}{\text{Conc. of SULFO-TAG stock solution (nmol/µL)}} = \text{µL of SULFO-TAG stock solution required for conjugation reaction}$$

## Conjugation Procedure

1. Gently tap the SULFO-TAG NHS-Ester vial or quick spin for 1 minute at 1,000 x *g* to collect lyophilized material at the bottom of the vial. Immediately prior to use, reconstitute the vial containing 150 nmol SULFO-TAG NHS-Ester with 50 µL of cold distilled water to generate a stock solution of 3 nmol/µL. Gently vortex. Reconstituted SULFO-TAG NHS-Ester may be kept for up to 10 minutes on ice prior to use.
   **Note:** For conjugation of <100 µg protein, prepare an intermediate dilution of SULFO-TAG NHS-Ester by adding 100 µL of Conjugation Buffer to 3 nmol/µL stock solution SULFO-TAG NHS-Ester prepared in step 1. This will provide 1 nmol/µL solution of SULFO-TAG NHS-Ester.
2. Add the calculated volume of reconstituted SULFO-TAG NHS-Ester (Pre-conjugation Procedure, Step 3) to the protein solution and vortex immediately. Discard any remaining SULFO-TAG NHS-Ester.
3. Incubate at 23°C for 2 hours (20–25°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).

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# Conjugation Protocol

## Post-Conjugation Procedure

1. Prepare Zeba Spin Desalting Columns. 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 MSD Conjugate Storage Buffer. Each preparation step should be carried out by centrifuging the columns (and their respective collection tubes) at 2–8°C in a centrifuge with a swinging bucket rotor (for 2, 5, and 10 mL Zeba columns) or a tabletop microfuge (for 0.5 mL Zeba columns).

**Table 1: Specifications for Zeba Spin Desalting Columns, 40K MWCO**

| **Size of Column** | | **0.5 mL** | **2 mL** | **5 mL** | **10 mL** |
|---|---|---|---|---|---|
| **Sample Volume Range** | | 70–100 µL | 200–450 µL | 300–1,000 µL | 1,000–2,000 µL |
| **Wash Buffer Volume** | | 300 µL | 1 mL | 2.5 mL | 5 mL |
| **Sample Volumes to use as stacker*** | | N/A | <350 µL | <750 µL | <1,500 µL |
| **Optional Stacker Volume** | | N/A | 40 µL | 100 µL | 200 µL |
| **Centrifugation Speed** | | 1,500 x *g* | 1,000 x *g* | 1,000 x *g* | 1,000 x *g* |
| **Centrifugation Time (Min)** | Storage Solution Removal | 1 | 2 | 2 | 2 |
| | Wash 1 | 1 | 4–8 | 4–8 | 4–8 |
| | Wash 2 | 1 | 2–8 | 2–8 | 2–8 |
| | Wash 3** | 3 | 5–8 | 5–8 | 5–8 |
| | Sample Recovery | 3–4 | 6–8 | 6–8 | 6–8 |

* When using the indicated sample volumes, use a stacker volume to achieve highest recovery. Stacker volume should be added after addition of the protein to the column.

** If column is not mostly white after the third wash, the column may be spun for an additional 1–3 minutes.

2. Apply the conjugation reaction to the center of the spin column in a drop-wise manner (refer to Table 1 for sample volume). Centrifuge the columns in clean, new collection tubes to purify the SULFO-TAG conjugated protein. The SULFO-TAG conjugated protein will be present in the eluate. Retain the purified conjugated material in the collection tubes and discard the columns.
3. Filter the conjugated protein using a 0.2 µm filter.
4. Determine the molar protein concentration of the conjugated protein using a standard colorimetric protein assay such as BCA, Bradford, or Lowry. Do not use an $OD_{280}$ absorbance reading as SULFO-TAG will absorb light at this wavelength.
5. Measure the absorbance of the MSD SULFO-TAG protein conjugate at 455 nm using a spectrophotometer. Divide the measured value by the pathlength in cm, and then divide by the extinction coefficient of the label (15,400 $M^{-1}cm^{-1}$) to obtain the MSD SULFO-TAG label concentration in moles per liter. For reference, a formula calculation worksheet page is attached.
6. Follow the calculations in the worksheet to determine the SULFO-TAG label:protein conjugation ratio. MSD SULFO-TAG conjugated proteins may be sensitive to extended exposure to light and should be stored in the dark or in amber or opaque vials. Antibody conjugates are usually stable for at least 2 years at 2–8°C in conjugate storage buffer. Stability of other proteins needs to be determined by the user.

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# Worksheet

## Pre-Conjugation Calculations

$$1{,}000 \times \frac{\text{Protein conc. (mg/mL)}}{\text{Protein MW (Da)}} \times \text{Challenge ratio} \times \text{Vol. of protein solution (µL)} = \text{nmol of SULFO-TAG reagent reqd.}$$

$$\frac{\text{nmol of SULFO-TAG reagent required}}{\text{Conc. of SULFO-TAG stock solution (nmol/µL)}} = \text{µL of SULFO-TAG stock solution required for conjugation reaction}$$

## Conjugation Procedure

Sample concentration: _____ Buffer exchange: Y / N
Volume of SULFO-TAG stock solution added to protein: _____
Time reaction started: _____Time reaction completed: _____
Separation of conjugated material: _____
Column size: _____ Buffer: _____

## Post-Conjugation Procedure

Protein assay: _____ Protein conc. (mg/mL): _____ $OD_{455}$: _____

## Post-Conjugation Calculations

$$\frac{\text{Protein conc. (mg/mL)}}{\text{Protein MW (Da)}} = \text{_____ M (A)}$$

$$\frac{OD_{455}}{\text{15,400 (extinction coefficient) x optical path length (cm)}} = \text{_____ M (B)}$$

Labeling incorporation ratio (SULFO-TAG label:Protein) = (B / A)_____

## Contact Information


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