MSD GOLD™ SULFO-TAG NHS-Ester Conjugation
Quick Guide

Introduction

MSD GOLD SULFO-TAG™ NHS-Ester is an N-hydroxysuccinimide ester that readily couples to primary amine groups of proteins. SULFO-TAG conjugated proteins are 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 MSD GOLD 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 before conjugation, a 2-hour incubation step, and a simple post-conjugation buffer exchange 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 the 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	MSD GOLD SULFO-TAG NHS-Ester label for coupling to antibodies and other proteins
Spin Column*	2–8°C	0.7 mL	20 columns	Size exclusion chromatography columns for the purification of proteins larger than 40,000 Da
	RT	2 mL	10 columns	
Filter, 0.22 µm	RT	N/A	10 each	Filter for use during purification
Syringe*	RT	1 mL	10 each	Syringe for use during purification
		3 mL		
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

**MSD GOLD SULFO-TAG Conjugation Pack 1 includes 20 columns of Bio-Rad's Micro Bio-Spin P-30 Column, 0.7 mL capacity and 10 syringes of 1 mL size; and MSD GOLD SULFO-TAG Conjugation Pack 2 includes 10 columns of Thermo Fisher's Zeba Spin Desalting Column, 40K MWCO, 2 mL capacity and 10 syringes of 3 mL size.*
‡Some components may ship at a different temperature than the recommended storage temperature. Upon receipt, store each reagent at the temperature recommended in the table.

Additional Materials (not provided)

1. Polypropylene microfuge tubes and 15 mL conical tubes
2. Protein assays such as BCA, Bradford, or Lowry
3. Concentrator (optional) (e.g., MilliporeSigma BIOMAX-50, AMICON Ultra-4, or AMICON Ultra-15 concentrators)
4. Additional spin columns are available in various sizes from Thermo Fisher Scientific (catalog numbers A57761-A57766) and Bio-Rad (catalog numbers 732-6223/732-6224)

Quick Guide

Conjugation Protocol

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

Pre-Conjugation Procedure

1. Prepare a 1-2 mg/mL protein solution to be conjugated in the supplied Conjugation Buffer. Protein solutions may be concentrated or buffer exchanged using spin columns that have been equilibrated with Conjugation Buffer **Note:** For Conjugation Pack 1, depending on sample volume (>100 µL), conjugation at low concentration (~1 mg/mL) may require additional spin columns.
   - ❑ 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 spin 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. **Note:** Reaction volumes larger than the capacity of a spin column should be distributed over multiple spin columns and pooled before filtration.
   - ❑ Measure the concentration of the protein solution to be conjugated. Protein concentration can be calculated from an OD280 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 MSD GOLD 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 before use, reconstitute the vial containing 150 nmol MSD GOLD SULFO-TAG NHS-Ester with 50 µL of cold distilled water to generate a stock solution of 3 nmol/µL. Gently vortex. Reconstituted MSD GOLD SULFO-TAG NHS-Ester may be kept for up to 10 minutes on ice before use. **Note:** For conjugation of <100 µg protein, prepare an intermediate dilution of MSD GOLD SULFO-TAG NHS-Ester by adding 100 µL of Conjugation Buffer to a 3 nmol/µL stock solution MSD GOLD SULFO-TAG NHS-Ester prepared in step 1. This will provide 1 nmol/µL solution of MSD GOLD SULFO-TAG NHS-Ester.
2. Add the calculated volume of reconstituted MSD GOLD SULFO-TAG NHS-Ester (Pre-conjugation Procedure, Step 3) to the protein solution and vortex immediately. Discard any remaining MSD GOLD 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).

Quick Guide

Conjugation Protocol

Post-Conjugation Procedure

1. Prepare spin column. 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) in a centrifuge with a swinging bucket rotor (for 2, 5, and 10 mL spin columns) or a tabletop microfuge (for 0.7 mL spin column).

Table 1: Specifications for Spin Columns

		Bio-Rad	Thermo Fisher Scientific		
Size of Column		**0.7 mL**	**2 mL**	**5 mL**	**10 mL**
Spin Temperature		2–4°C	Room temperature (20–25°C)		
Sample Volume Range		20–50 µL	200–500 µL	400–700 µL	800–2,500 µL
Wash Buffer Volume		500 µL	1 mL	2.5 mL	5 mL
Centrifugation Speed		1,000 x g	700 x g	700 x g	700 x g
Centrifugation Time (Min)	Storage Solution Removal	2	2	2	2
	Wash 1	2	2	2	2
	Wash 2	4	3	3	3
	Wash 3	4	4	4	4
	Sample Recovery	4	3	3	3

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. **Note:** Reaction volumes larger than the capacity of a spin column should be distributed over multiple spin columns and pooled before filtration.
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 ratio. MSD SULFO-TAG conjugated proteins may be sensitive to extended exposure to light and should be stored in dark, amber, or opaque vials. Antibody conjugates are usually stable for at least 2 years at 2–8°C in Conjugate Storage Buffer. The stability of other proteins needs to be determined by the user.

Quick Guide

Worksheet

Pre-Conjugation Calculations

1,000 x Protein conc. (mg/mL) x Challenge ratio x Vol. of protein solution (µL) = nmol of SULFO-TAG reagent reqd.
Protein MW (Da)

nmol of SULFO-TAG reagent required = µL of SULFO-TAG stock solution required for the conjugation reaction Conc. of SULFO-TAG stock solution (nmol/µL)

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

Protein conc. (mg/mL) = _____M(A) $\frac{OD_{455}}{15{,}400 \text{ (extinction coefficient) x optical path length (cm)}}$ = _____M (B)
Protein MW (Da)

Conjugation ratio (SULFO-TAG label:protein) = (B/A) _____

Contact Information

MSD Customer Service

Phone: 1-240-314-2795
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support

Phone: 1-240-314-2798
Fax: 1-240-632-2219 attn: Scientific Support
Email: ScientificSupport@mesoscale.com

MK-DS-227-v4-2024Jan