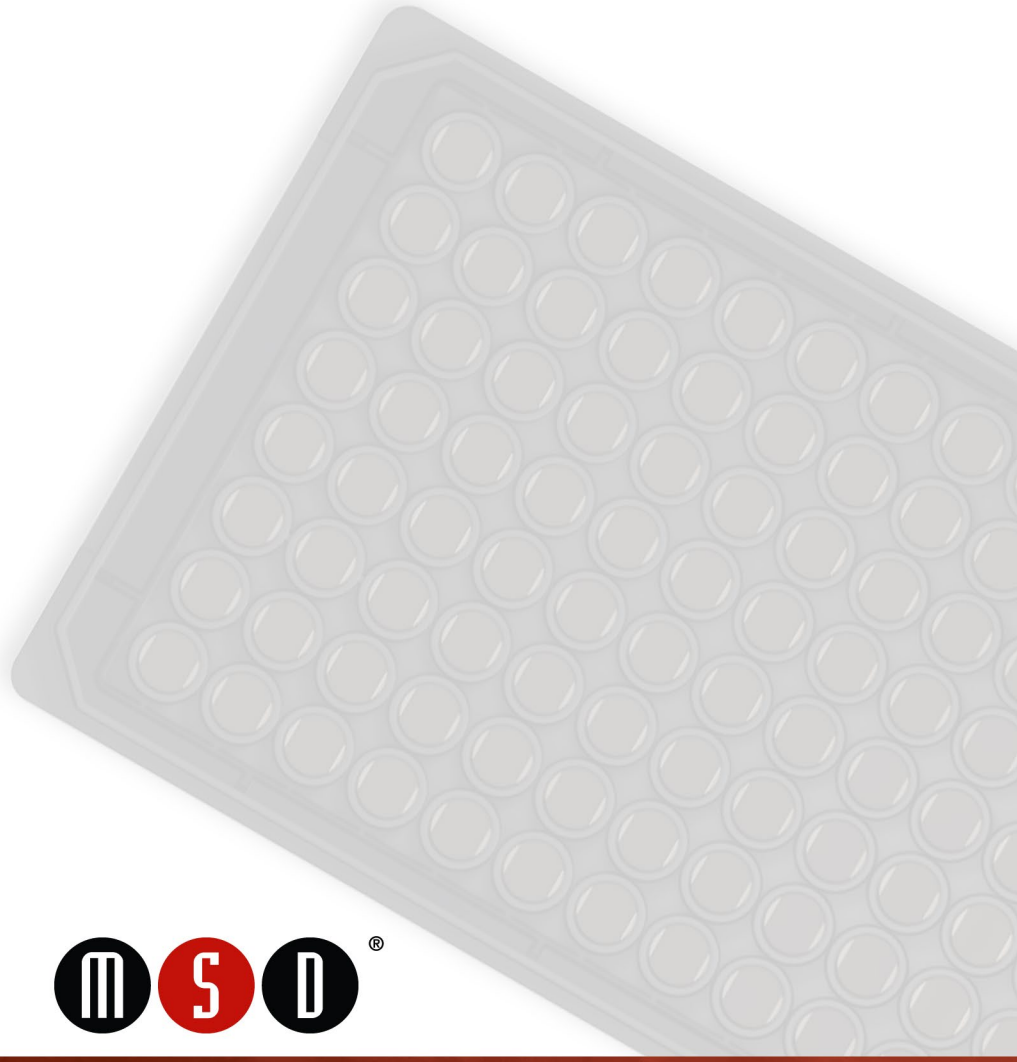
MSD GOLD™ SULFO-TAG NHS-Ester

MSD GOLD SULFO-TAG™ NHS-Ester

150 nmol (sufficient for conjugating 1 mg of IgG)	R91AO-1
2 µmol (sufficient for conjugating 10 mg of IgG)	R91AO-2

MSD GOLD SULFO-TAG NHS-Ester Conjugation Packs

Pack 1 (sufficient for conjugating 5 x 200 µg of IgG)	R31AA-1
Pack 2 (sufficient for conjugating 5 x 1 mg of IgG)	R31AA-2



MSD® Labeling Reagents

MSD GOLD SULFO-TAG NHS-Ester

For labeling amines

Notes:

The 150 nmol size of MSD GOLD SULFO-TAG NHS-Ester is sufficient for conjugating 1 mg of IgG and the 2 µmol size is sufficient for conjugating 10 mg of IgG at a challenge ratio of 20.

Each MSD GOLD SULFO-TAG NHS-Ester Conjugation Pack contains enough material for 5 reactions. At a challenge ratio of 20, Pack 1 is sufficient for conjugating 200 µg of IgG per reaction, and Pack 2 is sufficient for conjugating 1 mg of IgG per reaction.

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

This protocol details the conjugation procedure for proteins of molecular weight (MW) > 40,000 Daltons (Da) using MSD GOLD SULFO-TAG NHS-Ester label. The straightforward procedure involves an optional buffer exchange step, a 2-hour incubation step, and a mandatory buffer exchange step to quickly isolate the conjugated protein using a spin column. MSD GOLD SULFO-TAG NHS-Ester (Figure 1) is an amine reactive, N-hydroxysuccinimide ester that readily couples to primary amine groups of proteins under mildly basic conditions to form a stable amide bond.

MSD GOLD 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 conjugation procedure make MSD GOLD SULFO-TAG NHS-Ester the product of choice for molecules that contain primary amines (e.g., lysine-containing proteins). MSD GOLD SULFO-TAG offers low non-specific binding, resulting in highly sensitive detection when used in conjunction with MSD instruments.

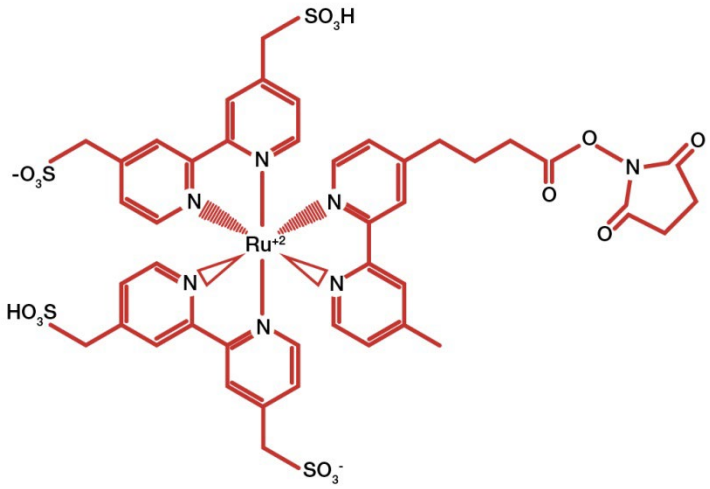

Figure 1. MSD GOLD SULFO-TAG NHS-Ester

Preparation of MSD GOLD SULFO-TAG Conjugates

General Notes

To minimize hydrolysis of MSD GOLD SULFO-TAG NHS-Ester, the reagent should be dissolved in cold distilled water just before its addition to the protein solution. If necessary, the stock MSD GOLD SULFO-TAG NHS-Ester 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 MSD GOLD SULFO-TAG NHS-Ester reagent. Generally, 150 nmol of MSD GOLD SULFO-TAG NHS-Ester is sufficient for conjugating up to 1 mg of IgG.

The conjugation ratio is the number of molecules of MSD GOLD SULFO-TAG conjugated to each molecule of protein. Optimal conjugation ratios for an MSD GOLD SULFO-TAG conjugated protein should be determined empirically for each specific application. For most applications using IgG antibodies (MW ~150,000), optimal performance is obtained with conjugation ratios between 2:1 and 20:1. In this range, assay signals typically show a linear dependence on the conjugation ratio. Conjugation ratios higher than 20:1 can be counterproductive and may lead to elevated background signals or loss of binding activity. Lower conjugation ratios (between 1:1 and 5:1) may provide better assay performance for proteins significantly smaller than IgGs.

The challenge ratio is the number of moles of MSD GOLD SULFO-TAG per mole of protein in the conjugation reaction mixture. The challenge ratio required to achieve a specific conjugation ratio depends on several factors, including pH, temperature, protein concentration, protein size, and the number of lysines available for coupling. Conjugating a 2 mg/mL IgG solution using the standard conditions described in this protocol will typically result in a label incorporation of approximately 50% (e.g., a challenge ratio of 10:1 will result in a conjugation ratio of about 5:1). Conjugation efficiencies for other proteins may be different. Conjugating with 1–2 mg/mL protein concentrations in a slightly alkaline buffer (pH 7.9) without preservatives yields the best conjugation efficiencies. Maintaining consistent conjugation conditions (protein concentration, buffer type, MSD GOLD SULFO-TAG NHS-Ester concentration, incubation time, and temperature) is essential when preparing multiple batches of conjugated protein to achieve consistent assay results.

When developing immunoassays, MSD recommends conjugating antibodies using the standard conditions outlined in this document and challenge ratios of 6:1, 12:1, and 20:1 to identify optimal conjugation conditions. If evaluating different conditions is impossible due to limited reagent quantities, a challenge ratio of 20:1 will generally provide good performance. The suggested challenge ratios for immunogenicity applications where an antibody-drug or protein therapeutic is used are 12:1 and 6:1 MSD GOLD SULFO-TAG: drug. If only one ratio is tested, a 10:1 challenge ratio is recommended.

This protocol describes the MSD GOLD SULFO-TAG conjugation procedure for proteins with a MW > 40,000 Da. Smaller proteins/polypeptides may also be conjugated using MSD GOLD SULFO-TAG NHS-Ester as long as they have an accessible lysine or N-terminal amino group; however, alternative separation methods may be needed to remove the unconjugated label. MSD offers various services for the custom conjugation of reagents, including proteins, peptides, and non-proteinaceous molecules. For more information, please visit Conjugation Services on the MSD website.

MSD GOLD SULFO-TAG NHS-Ester Conjugation Packs

MSD offers conjugation packs that include the components and guidance necessary for conjugating and purifying detection reagents with MSD GOLD SULFO-TAG label. Two sizes of conjugation packs are offered: MSD GOLD SULFO-TAG Conjugation Pack 1 and Pack 2. The two packs enable the conjugation of different amounts of IgG. MSD GOLD SULFO-TAG NHS-Ester Conjugation Pack 1 contains materials for conjugation and purification of up to 200 µg of IgG per reaction, and MSD GOLD SULFO-TAG NHS-Ester Conjugation Pack 2 allows conjugation of up to 1 mg of IgG per reaction. Each pack contains enough material for 5 reactions (i.e., 5 vials of MSD GOLD SULFO-TAG NHS-Ester).

Table 1. Components of MSD GOLD SULFO-TAG Conjugation Packs

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 and Equipment

The following additional materials may be required. Some items (denoted with a *) are included in the MSD GOLD SULFO-TAG Conjugation Packs.

1. Conjugation Buffer* (100 mM Phosphate Buffer, pH 7.9)
2. Conjugate Storage Buffer* (PBS, pH 7.4, with 0.05% sodium azide)
3. Polypropylene microfuge tubes
4. Spin columns* MSD recommends the use of the following spin columns (additional information in Table 4)
    a. Zeba Spin Desalting Columns, 40K MWCO of various sizes (2 mL–10mL) from Thermo Fisher Scientific, Catalog # A57761–A57766
    b. Micro Bio-Spin P-30 Columns (MW limit: 40K) from Bio-Rad, Catalog # 732-6223
5. 15 mL and 50 mL conical tubes for use with Zeba Spin Desalting Columns, 40K MWCO, 2 mL, and 10 mL column sizes, respectively

6. Protein assays such as BCA, Bradford, or Lowry
7. MSD Blocker A (optional), Catalog # R93AA-2 (250 mL) and R93AA-1 (1 L)
8. Spectrophotometer capable of an OD_{455} measurement
9. 0.2 µm filter* (optional)

Table 2. Suggestions for 0.2 µm filter

Vendor	Catalog #	Volume of Conjugated Protein
Whatman	AV125EAQU	≥ 2.0 mL
MilliporeSigma or Thermo Fisher Scientific (MILLEX-GV)	SLGV004SL	0.2–1.0 mL

10. Concentrator (optional)

Table 3. Suggestions for concentrators

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

Note

The following table lists the catalog numbers of the Micro Bio-Spin P-30 Columns, from Bio-Rad and Zeba Spin Desalting Columns, 40K MWCO, from Thermo Fisher Scientific with the recommended sample volume for each column.

Table 4. Catalog numbers of Spin Columns

Manufacturer	Manufacturer Catalog #	Number of Columns	Spin Column Volume	Recommended Volume of the Conjugation Reaction
Bio-Rad	732-6223	25	0.7 mL	20–50 µL
	732-6224	100	0.7 mL	20–50 µL
	732-6226	1,000	0.7 mL	20–50 µL
Thermo Fisher Scientific	A57761	5	2 mL	200–500 µL
	A57762	25	2 mL	200–500 µL
	A57763	5	5 mL	400–700 µL
	A57764	25	5 mL	400–700 µL
	A57765	5	10 mL	800–2,500 µL
	A57766	25	10 mL	800–2,500 µL

Protocol

1. Prepare a 1-2 mg/mL protein solution to be conjugated in the supplied Conjugation Buffer. Antibodies in a storage buffer with preservatives such as sodium azide or EDTA must be buffer exchanged before the conjugation reaction. We recommend that dilute protein solutions be 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 Conjugation Buffer. Filter the protein using a 0.2 µm filter. The concentration of the protein solution to be conjugated should be confirmed before beginning the conjugation reaction.

   Notes:

   - For Conjugation Pack 1, depending on sample volume (>100 µL), conjugation at low concentration (~1 mg/mL) may require additional spin columns. Refer to Table 4 for more details.
   - Reaction volumes larger than the capacity of a spin column should be distributed over multiple spin columns and pooled before filtration.
   - Conjugation buffer, 0.2 µm filters (qty: 10), and syringes (qty: 10) are included in the MSD GOLD SULFO-TAG NHS-Ester Conjugation Packs. Use five syringes and five filters for the processing steps.

2. Equilibrate the protein to be conjugated 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 GOLD SULFO-TAG NHS-Ester stock solution required for the conjugation reaction using the formula below and on the attached worksheet.

Calculations

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

Using this value, calculate the volume of MSD GOLD SULFO-TAG stock solution required for the reaction. Step 4 of this protocol details the reconstitution instructions for MSD GOLD SULFO-TAG label to generate a stock solution in nmol/µL.

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

EXAMPLE

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

$$1{,}000 \times \frac{2\ \text{mg/mL}}{150{,}000\ \text{Da}} \times 12 \times 500\ \text{µL} = 80\ \text{nmol of MSD GOLD SULFO-TAG reagent required}$$

$$\frac{80\ \text{nmol of SULFO-TAG reagent}}{3\ \text{nmol/µL SULFO-TAG stock solution}} = 26.7\ \text{µL of SULFO-TAG stock solution required for the conjugation reaction}$$

4. Centrifuge the MSD GOLD SULFO-TAG NHS-Ester vial by pulse spinning for 1 minute or gently tap on a soft surface in order to collect lyophilized material at the bottom of the vial. Reconstitute MSD GOLD SULFO-TAG NHS-Ester immediately before use with cold distilled water. For the 2 µmol and 150 nmol sizes of MSD GOLD SULFO-TAG NHS-Ester, dissolve with 200 µL and 50 µL, respectively to generate stock solutions of 10 and 3 nmol/µL. Gently vortex the vial to ensure complete dissolution of all lyophilized material.

   Note: Reconstituted MSD GOLD SULFO-TAG NHS-Ester may be kept for up to 10 minutes on ice before use.

5. Add the calculated volume (from Step 3) of reconstituted MSD GOLD SULFO-TAG NHS-Ester to the protein solution and vortex immediately. Discard any remaining unused MSD GOLD SULFO-TAG NHS-Ester.
6. 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 conjugation conditions between multiple preparation lots to ensure conjugation reproducibility.
7. Prepare spin columns 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 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. Refer to Table 5 below for the recommended sample volume, wash buffer volumes, spin temperature, collection tube sizes, and centrifugation times for each preparation step.

   Note: Reaction volumes larger than the capacity of a spin column should be distributed over multiple spin columns.

8. Apply the conjugation reaction to the spin column dropwise, following the recommendations in Table 5. Centrifuge the columns in new collection tubes using a swinging bucket rotor to purify the MSD GOLD SULFO-TAG conjugated protein. The MSD GOLD SULFO-TAG conjugated protein will be captured in the collection tubes. Retain the conjugated material in the collection tubes and discard the columns.

   Note: The unconjugated MSD GOLD SULFO-TAG reagent will appear yellow in the spin column.

Table 5. Specifications for Spin Columns

Size of Column		Bio-Rad	Thermo Fisher Scientific		
		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

Note: After each spin, examine the spin column. The column resin may range from being slightly wet (resin is opaque and grey with white specks) to mostly dry (resin is mostly white). Any of these conditions are fine as long as no buffer remains on the top of the column.

9. We recommend filtering the conjugated protein using a 0.2 µm filter. Filtration may cause some loss of the protein. Please refer to page 7 for the recommended filter units.

   Notes:

   - Reaction volumes distributed over multiple spin columns should be pooled before filtration.
   - Use the remaining five syringes and five filters for the post-conjugation steps.

10. Determine the molar protein concentration of the conjugated protein using a standard colorimetric protein assay such as BCA, Bradford, or Lowry.

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

11. Measure the absorbance of the MSD GOLD SULFO-TAG protein conjugate at 455 nm using a spectrophotometer. Divide the measured value by the path length in cm, and then divide by the extinction coefficient of the label (15,400 $M^{-1}cm^{-1}$) to obtain the MSD GOLD SULFO-TAG label concentration in moles per liter. For reference, a formula calculation worksheet page is attached (page 14).

12. To calculate the MSD GOLD SULFO-TAG label:protein conjugation ratio, divide the MSD GOLD 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 2 years at 2–8°C at a concentration of 1–2 mg/mL; stability of other protein types should be determined. Conjugated proteins may be stored frozen at ≤-70°C, as long as the protein is stable to freeze-thaw cycles or stored in single-use aliquots. MSD GOLD SULFO-TAG conjugated proteins may be sensitive to extended exposure to light and should be stored in dark, amber, or opaque vials. Short-term exposure of conjugates to light when carrying out assays is 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.

Storage, Handling, and Stability

MSD GOLD SULFO-TAG NHS-Ester is supplied as a dry orange-red lyophilized solid. The vials should be stored at ≤-70°C. The expiration date of the product is indicated on the label. Following reconstitution, any remaining unused material should be discarded.

FAQs

1) **What chemicals interfere with MSD GOLD SULFO-TAG conjugation?**

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

a) Tris
b) Glycine
c) Histidine
d) Azide
e) Imidazole
f) Glutathione
g) Ammonium ions
h) 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 MSD GOLD SULFO-TAG NHS-Ester. Carrier proteins will interfere with MSD GOLD SULFO-TAG NHS-Ester conjugation 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 conjugated in PBS (without interfering buffer components) if the protein concentration is high enough (1–2 mg/mL). Otherwise, microconcentrators may be used to concentrate the antibody solution following equilibration of the microconcentrator with PBS.

4) **Are there alternatives to using Thermo Fisher Scientific Zeba Spin Desalting Columns and Bio-Rad Micro Bio-Spin P-30 Columns for purifying the MSD GOLD SULFO-TAG conjugated antibody after conjugation?**

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 MSD GOLD SULFO-TAG-conjugated protein as these are not able to separate free MSD GOLD SULFO-TAG reagent from labeled conjugates.

b) Alternatively, CENTRICON concentrators or similar microconcentrator products with adequate MWCO (for concentrator information please refer to page 7) can be used to remove unbound label. Resuspend the conjugation mixture in a larger volume of PBS-0.05% azide, concentrate to a smaller volume, and then repeat the process as per the product instructions for desalting applications.

c) Post-conjugation purification of proteins with MW < 40,000 Da will require alternative procedures (such as high-resolution size exclusion chromatography, HPLC, FPLC, etc.) because Zeba Spin Desalting Columns, Micro Bio-Spin P-30 Columns, or G-50 columns will not provide adequate separation in this size range.

5) What is the molecular weight of MSD GOLD SULFO-TAG?

Unreacted MSD GOLD SULFO-TAG NHS-Ester has a molecular weight of 1,141 g/mol. After the conjugation reaction, each conjugated MSD GOLD SULFO-TAG adds 1,027 g/mol to the protein.

6) What types of material can be conjugated?

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

MSD Conjugation Services may be used to conjugate small molecules and peptides. Please contact MSD Scientific Support (Phone: 1-240-314-2798, Email: ScientificSupport@mesoscale.com) or your local MSD Application Scientist for details.

7) Are there alternatives to using Conjugation Buffer for the conjugation reaction?

For best results, we recommend using Conjugation Buffer. However, other buffers can be used for the conjugation 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 before conjugation. Using alternative conjugation buffers may result in lower incorporation efficiencies.

8) What should I do if my application requires the conjugated protein to be in a different buffer?

The spin columns may be equilibrated in a buffer other than PBS if the end application requires storage of the conjugated protein in a non-PBS buffer.

9) Will my antibody retain activity after labeling?

MSD GOLD 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 MSD GOLD SULFO-TAG may have an effect on binding affinities.

10) What is the stability of MSD GOLD SULFO-TAG NHS-Ester?

The shelf life of MSD GOLD SULFO-TAG NHS-Ester is 3 years at ≤-70°C. The reagent can be stored for up to 2 years at ≤-10°C with minimal loss of activity. Reagent stability is lower at room temperature or at 2–8°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.

11) What if the protein to be conjugated does not have any primary amine groups?

Alternative linking chemistry options are available 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. For details, please contact MSD Scientific Support (Phone: 1-240-314-2798, Email: ScientificSupport@mesoscale.com) or your local MSD Application Scientist.

12) I have an IgG purified antibody from my protein production group which has been eluted into PBS. Should I desalt before conjugation?

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. We recommend repeating the desalting step into PBS to remove any trace quantities of Tris-glycine that can hinder conjugation with MSD GOLD SULFO-TAG.

13) How do I conjugate a high concentration protein solution with MSD GOLD SULFO-TAG?

The conjugation reaction will be more efficient at high protein concentrations. We recommend using a lower challenge ratio for conjugation to compensate for the increased efficiency.

14) How do I conjugate a low concentration protein solution with MSD GOLD SULFO-TAG?

MSD recommends the protein concentration to be at least 0.5 mg/mL. If concentrating the protein solution is not feasible, conjugation can be done at a lower concentration, which may result in lower conjugation efficiency. Therefore, the conjugation reaction should be performed at a challenge ratio of 20:1 or higher.

15) How do I conjugate small proteins with MSD GOLD SULFO-TAG?

Proteins with MW < 40,000 Da can be conjugated by the same chemistry as antibodies; lower challenge ratios may be required for MSD GOLD SULFO-TAG conjugation of small proteins and peptides than for IgGs. The NHS-Ester will react with primary amines 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-conjugation purification of small proteins will require alternative procedures (such as HPLC, FPLC, etc.) because small proteins are not resolved by G-50 SEPHADEX columns.

16) Why can't I use a spectrophotometer at 280 nm to determine conjugated protein concentration?

MSD GOLD SULFO-TAG strongly absorbs at 280 nm and will interfere with any measurement of protein concentration at this wavelength.

17) What is the stability of MSD GOLD SULFO-TAG conjugated proteins?

MSD GOLD SULFO-TAG conjugated protein is generally as stable as the unconjugated protein if it is stored in the appropriate buffer, concentration, and storage temperature. The conjugated 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.

18) My antibody did not conjugate very well. What are the possible reasons?

The presence of preservatives, carrier protein, or residual Tris-glycine or other interfering substances in the conjugation buffer (see FAQ 1 and 2) can reduce the conjugation efficiency of the protein. Very low starting material concentrations (below 0.5 mg/mL) may also reduce conjugation ratios. It has also been observed that some IgGs label more efficiently than others.

19) What components can be removed by buffer exchange or dialysis?

Salt, azide, glycerol, buffering agent (e.g., Tris), carbohydrates (e.g., trehalose), and amino acids (e.g., histidine, glycine) can be successfully removed by buffer exchange method.

20) Who should I contact if I have any questions on MSD GOLD SULFO-TAG conjugation?

For details, please contact MSD Scientific Support (Phone: 1-240-314-2798, Email: ScientificSupport@mesoscale.com) or your local MSD Application Scientist.

Worksheet

Date: _____

Materials

Protein to be conjugated

Concentration: _____ Vendor: _____

Catalog number: _____ Lot number: _____

Sample Preparation

Method: _____ Buffer: _____

Lot number:_____ Date: _____

Columns/Concentrators: _____ Lot number: _____

MSD GOLD 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-Conjugation Calculations

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

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

Conjugation Procedure

Sample preparation: _____

Concentration: _____ Buffer exchange: Y/N

Notes: _____

Volume of MSD GOLD SULFO-TAG stock solution added to protein: _____

Time reaction started: _____ Time reaction completed: _____ Shaking: Y/N

Separation of conjugated material: _____

Columns: _____

Centrifuge: _____

Time: _____ Temperature: _____ Speed: _____

Buffer: _____

Post-Conjugation Procedure

Protein assay: _____

Vendor: _____

Catalog number: _____ Lot number: _____

Protein concentration: _____ OD_{455}: _____

Post-Conjugation Calculations

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

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

Conjugation ratio (MSD GOLD SULFO-TAG label:Protein) = (B / A) _____

Storage Information

Aliquot size: _____ Storage temperature: _____

Location: _____ Date: _____

Notes: _____

_____

_____

_____