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

Note:
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.

MESO SCALE DISCOVERY®
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# Ordering Information

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Introduction

This protocol details the conjugation procedure for proteins of molecular weight (MW) > 40,000 Daltons 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 which 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**

In order to minimize hydrolysis of MSD GOLD SULFO-TAG NHS-Ester, the reagent should be dissolved in cold distilled water just prior to 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 conjugation of up to 1 mg of IgG.

The labeling ratio is the number of molecules of MSD GOLD SULFO-TAG conjugated to each molecule of protein. Optimal conjugation ratios for a 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 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 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 a number of 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% (i.e., a challenge ratio of 10:1 will result in a conjugation ratio of about 5:1). Conjugation efficiencies for other proteins may be different. In general, conjugating with high protein concentrations of 1–2 mg/mL in slightly alkaline PBS (pH 7.9) in the absence of 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 important when preparing multiple batches of conjugated protein in order 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 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 MSD GOLD 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 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 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 conjugation of reagents including proteins, peptides, and non-proteinaceous molecules.
MSD GOLD SULFO-TAG NHS-Ester Conjugation Packs

MSD offers all-inclusive conjugation packs that include the components and guidance that may be 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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Size</th>
<th>Quantity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSD GOLD SULFO-TAG NHS-Ester</td>
<td>≤-70°C</td>
<td>150 nmol</td>
<td>5 vials</td>
<td>MSD GOLD SULFO-TAG NHS-Ester label for coupling to antibodies and other proteins</td>
</tr>
<tr>
<td>Zeba Spin Desalting Column, 40K MWCO*</td>
<td>2–8°C</td>
<td>0.5 mL or 5 mL</td>
<td>10 columns</td>
<td>Size exclusion chromatography columns for the purification of proteins larger than 40,000 Da</td>
</tr>
<tr>
<td>Filter, 0.22 µm</td>
<td>RT</td>
<td>N/A</td>
<td>10 each</td>
<td>Filter for use during purification</td>
</tr>
<tr>
<td>Syringe *</td>
<td>RT</td>
<td>N/A</td>
<td>10 each</td>
<td>Syringe for use during purification</td>
</tr>
<tr>
<td>Conjugation Buffer**</td>
<td>2–8°C</td>
<td>40 mL</td>
<td>1 bottle</td>
<td>Phosphate-buffered saline (PBS), pH 7.9, preservative-free</td>
</tr>
<tr>
<td>Conjugate Storage Buffer</td>
<td>RT</td>
<td>40 mL</td>
<td>1 bottle</td>
<td>PBS pH 7.4 + 0.05% Sodium Azide</td>
</tr>
</tbody>
</table>

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

**The pH of Conjugation Buffer is stable for up to 2 weeks after opening the bottle. For long term use, it is recommended to readjust the pH of the solution.

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* (Phosphate-buffered saline (PBS), pH 7.9, preservative-free)
2. Conjugate Storage Buffer* (PBS pH 7.4 + 0.05% 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 # 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), Catalog # R93AA-2 (250 mL) and R93AA-1 (1 L)
8. Spectrophotometer capable of an OD{sub} measurement
9. 0.2 µm filter* (optional)

Table 2. Suggestions for 0.2 µm filter

<table>
<thead>
<tr>
<th>Vendor</th>
<th>Catalog #</th>
<th>Volume of Conjugated Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whatman</td>
<td>AV125EQU</td>
<td>≥ 2.0 mL</td>
</tr>
<tr>
<td>Millipore or Fisher Scientific (MILLEX-GV)</td>
<td>SLGV004SL</td>
<td>0.2–1.0 mL</td>
</tr>
</tbody>
</table>

10. Concentrator (optional)

Table 3. Suggestions for concentrators

<table>
<thead>
<tr>
<th>Vendor</th>
<th>Catalog #</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Millipore BIOMAX-50 concentrator, 50 MWCO</td>
<td>UFV5BQK25</td>
<td>0.05–0.5 mL</td>
</tr>
<tr>
<td>AMICON Ultra-4 concentrator, PLQK Ultracel-PL Membrane, 50 MWCO</td>
<td>UFC805008</td>
<td>0.5–4.0 mL</td>
</tr>
<tr>
<td>AMICON Ultra-15 concentrator, PLQK Ultracel-PL Membrane, 50 MWCO</td>
<td>UFC905024</td>
<td>0.5–15.0 mL</td>
</tr>
</tbody>
</table>

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 4. Catalog numbers of Zeba Spin Desalting Columns from Thermo Scientific

<table>
<thead>
<tr>
<th>Thermo Scientific Catalog #</th>
<th>Number of Columns/pack</th>
<th>ZEBA Spin Desalting Column Volume</th>
<th>Recommended Volume of the Conjugation Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>87766</td>
<td>25</td>
<td>0.5 mL</td>
<td>70–100 µL</td>
</tr>
<tr>
<td>87767</td>
<td>50</td>
<td>0.5 mL</td>
<td>70–100 µL</td>
</tr>
<tr>
<td>87768</td>
<td>5</td>
<td>2 mL</td>
<td>200–450 µL</td>
</tr>
<tr>
<td>87769</td>
<td>25</td>
<td>2 mL</td>
<td>200–450 µL</td>
</tr>
<tr>
<td>87770</td>
<td>5</td>
<td>5 mL</td>
<td>300–1,000 µL</td>
</tr>
<tr>
<td>87771</td>
<td>25</td>
<td>5 mL</td>
<td>300–1,000 µL</td>
</tr>
<tr>
<td>87772</td>
<td>5</td>
<td>10 mL</td>
<td>1,000–2,000 µL</td>
</tr>
<tr>
<td>87773</td>
<td>25</td>
<td>10 mL</td>
<td>1,000–2,000 µL</td>
</tr>
</tbody>
</table>
1. Prepare a 1–2 mg/mL solution of the protein to be conjugated in Conjugation Buffer. 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 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 preservative-free PBS, pH 7.9. (Use Conjugation Buffer when using MSD GOLD SULFO-TAG NHS-Ester Conjugation Pack.) Filter the protein using a 0.2 µm filter. The concentration of the protein solution to be conjugated should be confirmed prior to beginning the conjugation reaction.

**Note:** Conjugation buffer, 0.2 µm filter, and syringes are included in the MSD GOLD SULFO-TAG NHS-Ester Conjugation Packs.

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

### Calculations

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

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

\[
1000 \times \frac{2 \text{ mg/mL} \times 12 \times 500 \text{ µL}}{150,000 \text{ Da}} = 80 \text{ nmol of MSD GOLD SULFO-TAG reagent required}
\]

\[
\frac{80 \text{ nmol of SULFO-TAG reagent}}{3 \text{ nmol/µL SULFO-TAG stock solution}} = 26.7 \text{ µL of SULFO-TAG stock solution required for conjugation reaction}
\]
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 prior to 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 prior to 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 Zeba 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 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 at 2–8°C. Refer to Table 5 below for the recommended sample volume, wash buffer volumes, collection tube sizes, and centrifugation times for each preparation step.

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

8. Apply the conjugation reaction to the Zeba column in a dropwise manner following the recommendations in Table 2. Using a swinging bucket rotor, centrifuge the columns in clean new collection tubes to purify the MSD GOLD SULFO-TAG conjugated protein. The MSD GOLD SULFO-TAG conjugated protein will be captured in the conical tubes. Retain the conjugated material in the conical tubes, and discard the columns.

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

### Table 5: Specifications for Zeba Spin Desalting Columns, 40K MWCO

<table>
<thead>
<tr>
<th>Size of Column (mL)</th>
<th>0.5</th>
<th>2</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Volume Range (µL)</td>
<td>70–100</td>
<td>200–450</td>
<td>300–1,000</td>
<td>1,000–2,000</td>
</tr>
<tr>
<td>Wash Buffer Volume</td>
<td>300 µL</td>
<td>1 mL</td>
<td>2.5 mL</td>
<td>5 mL</td>
</tr>
<tr>
<td>Sample Volumes to use a stacker</td>
<td>N/A</td>
<td>&lt;350 µL</td>
<td>&lt;750 µL</td>
<td>&lt;1,500 µL</td>
</tr>
<tr>
<td>Optional Stacker Volume</td>
<td>N/A</td>
<td>40 µL</td>
<td>100 µL</td>
<td>200 µL</td>
</tr>
<tr>
<td>Centrifugation Speed</td>
<td>1,500 x g</td>
<td>1,000 x g</td>
<td>1,000 x g</td>
<td>1,000 x g</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Centrifugation Time (Min)</th>
<th>Storage Solution Removal</th>
<th>1</th>
<th>2</th>
<th>2</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash 1</td>
<td></td>
<td>1</td>
<td>4–8</td>
<td>4–8</td>
<td>4–8</td>
</tr>
<tr>
<td>Wash 2</td>
<td></td>
<td>1</td>
<td>2–8</td>
<td>2–8</td>
<td>2–8</td>
</tr>
<tr>
<td>Wash 3 (1)</td>
<td></td>
<td>3</td>
<td>5–8</td>
<td>5–8</td>
<td>5–8</td>
</tr>
<tr>
<td>Sample Recovery (2)</td>
<td></td>
<td>3–4</td>
<td>6–8</td>
<td>6–8</td>
<td>6–8</td>
</tr>
</tbody>
</table>

1. 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.

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

3. 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.

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 7 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.

   **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.

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 $\leq$-20°C or $\leq$-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 the dark or in 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 $\leq$-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 Scientific Zeba Spin Desalting 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 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) **What conjugation ratio is recommended for an IgM?**

A challenge ratio range from 8:1 to 12:1 may be used for conjugating IgM antibodies. The molecular weight of an IgM is in the order of 900,000 Da. IgMs can be unstable at higher pHs, therefore conjugation at pH 7.0 to 7.2 may be better than the standard labeling buffer of pH 7.9 used for IgG.

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

For best results, it is recommended to use 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 prior to conjugation. Using alternative conjugation buffers may result in lower incorporation efficiencies.

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

The desalting 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.

10) **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.

11) **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.

12) **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. Please contact MSD Scientific Support (Phone: 1-240-314-2798, Email: ScientificSupport@mesoscale.com) or your local MSD Application Scientist for details.
13) 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. It is recommended to repeat the desalting step into PBS to remove any trace quantities of Tris-glycine that can hinder conjugation with MSD GOLD SULFO-TAG.

14) 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. It is recommended to use a lower challenge ratio for conjugation to compensate for the increased efficiency.

15) 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.

16) 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 columns.

17) 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.

18) 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.

19) 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 conjugation efficiency of the protein. Very low concentrations of the starting material (below 0.5 mg/mL) may also reduce conjugation ratios. It has also been observed that some IgGs label more efficiently than others.

20) 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.

21) Who should I contact if I have any questions on MSD GOLD SULFO-TAG conjugation?
Please contact MSD Scientific Support (Phone: 1-240-314-2798, Email: ScientificSupport@mesoscale.com) or your local MSD Application Scientist for details.
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)}} = \mu\text{L of SULFO-TAG stock solution required for 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

\[
\text{Protein conc. (mg/mL)} = \frac{\text{Protein MW (Da)}}{A} \\
\text{OD}_{455} = \frac{15,400 \times \text{optical path length (cm)}}{B} \\
\text{Labeling incorporation ratio (MSD GOLD SULFO-TAG label:Protein)} = \frac{B}{A}
\]

Storage Information

Aliquot size: ______________________ Storage temperature: _______________________
Location: __________________________ Date: ____________________
Notes: _______________________________________

____________________ __________________________