MESO SCALE DISCOVERY TAG-NHS-Ester

Introduction

This note describes how to label proteins of MW > 10000 using MSD® TAG-NHS-Ester. The procedure involves a 1 hour incubation step followed by a simple column separation to isolate the labeled protein.

MSD TAG-NHS-Ester, or Ruthenium (II) tris-bipyridine, N-hydroxysuccinimide is an amine-reactive label often used for detection in assays formatted with MSD technology (Figure 1). N-hydroxysuccinimide esters readily couple to primary amine groups of proteins to form a stable amide bond. The reaction is favored at slightly alkaline pH in which a nucleophilic attack by an unprotonated amine on an ester results in amide bond formation. The reaction is rapid and occurs under mild conditions.

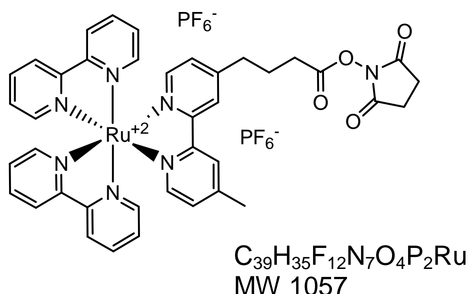
MSD TAG-labeled conjugates are stable and may be used at low concentrations. These features minimize time, costs, and labor since large, stable batches of conjugate can be prepared, validated, and used for long periods of time. MSD TAG-NHS-Ester is a popular conjugate for labeling molecules that contain primary amines (e.g., lysines in proteins) since the method is fast and easy.

MSD Technology

MSD's products are based on MULTI-ARRAY® technology, a proprietary combination of patterned arrays and electrochemiluminescence detection. Patterned arrays enable large numbers of measurements through miniaturization, organization and parallel processing of biological assays. Electrochemiluminescence detection offers a unique combination of sensitivity, dynamic range and convenience.

FIGURE 1

Ruthenium (II) tris-ipyridine, N-hydroxysuccinimide



$C_{39}H_{35}F_{12}N_7O_4P_2Ru$
MW 1057

Preparation of MSD TAG-Protein Conjugates

General Considerations

To minimize the competition of water (hydrolysis of the NHS ester) with the protein, dissolve the MSD TAG-NHS-Ester in an anhydrous solvent such as dimethyl sulfoxide (DMSO) just prior to addition of the NHS ester to the reaction mixture. The reconstituted solution is unstable and any unused material should be discarded. Consider labeling more than one protein at the same time to maximize the use of the MSD TAG-NHS-Ester.

The optimal MSD TAG:protein labeling ratio should be determined empirically. For immunoassay applications, typical MSD TAG:antibody (IgG) molar challenge ratios are 4:1, 8:1 and 12:1. If reagents are limited, an 8:1 molar challenge ratio is recommended. The best challenge ratio to use with other proteins may differ and will depend on various factors including the size of the protein and the number of lysines available for coupling. Under typical labeling conditions using a 1 mg/mL IgG solution, approximately 30-40% of MSD TAG label is incorporated into the protein. Optimal assay performance is often obtained with MSD TAG:protein molar incorporation ratios between 2:1 and 6:1. Excessively high labeling ratios can increase non-specific binding and/or cause precipitation of the protein. In general, labeling with high protein concentrations (>1 mg/mL) and slightly alkaline (i.e., pH 7.8) PBS solutions containing no preservatives yields the best labeling efficiency. When preparing multiple batches of labeled protein, it is important to maintain conditions (protein concentration, buffer type, label concentration, incubation time, shaking, temperature, etc.) to achieve consistent assay results.

The procedure outlined below describes the labeling method for proteins of MW >10000. If the protein/polypeptide to be labeled has a lysine and a MW <10000, you may still be able to use this conjugate but conditions for labeling and separation must be modified. For example, a gel filtration resin with a smaller MW cutoff or an alternative separation method independent of molecular size may be needed. MSD offers a variety of services for the custom labeling of proteins, peptides and non-proteinaceous molecules.

This labeling method uses the following materials:

1. Phosphate buffered saline (PBS), pH 7.8, without preservatives
2. PBS Buffer, pH 7.2, containing 0.05-1% (w/v) NaN_3
3. 2 M glycine
4. Polypropylene microcentrifuge tubes
5. Shaker (optional)
6. Sephadex® G-25 size exclusion columns, such as PD-10 or NAP®-5 (Amersham Biosciences)
7. Spectrophotometer capable of an OD_{455} measurement
8. A protein quantitation assay, such as BCA, Bradford or Lowry

Recommended Procedure

1. Prepare a 1 mg/mL solution of a *purified* protein in PBS, pH 7.8. Other buffers can be used, but they should be free of amines (tris- and glycine-containing buffers cannot be used) and preservatives. Affinity-purified IgG's are often eluted using high molarity glycine solutions so it is very important that they are properly desalted prior to labeling. Samples containing sodium azide or EDTA should be desalted or dialyzed prior to labeling. Dilute proteins should be concentrated to >0.2 mg/mL. Ultra-filtration concentrating devices can be used to achieve both buffer exchange and concentration of the protein. Protein concentrations should be confirmed prior to labeling. To calculate protein recovery following labeling and purification, MSD recommends a colorimetric protein assay kit (e.g., Bradford or BCA method) for both the pre- and post-labeling determinations. The MSD TAG label will affect OD_{280} absorbance readings so this method cannot be used for calculation of the labeled protein.

2. Use the formulas on the worksheet provided (also shown below) to calculate the amount of MSD TAG-NHS-Ester stock solution needed for the labeling reaction. An example calculation for the labeling of 1000 µL of a 1 mg/mL solution of IgG (MW 150000) with an 8:1 molar excess of MSD TAG-NHS-Ester (MW 1057) is also shown.

Formula

First, determine the mass of MSD TAG label required for labeling:

$$\frac{\text{Protein Conc. (mg/mL)}}{\text{MW protein}} \times (\text{MW of MSD TAG}) \times (\text{Challenge ratio}) \times (\text{Vol. to label in µL}) = \textbf{µg MSD TAG}$$

Then, using this value, determine the volume of MSD TAG stock solution required:

µg MSD TAG ÷ Conc. MSD TAG stock solution (µg/µL) **= µL MSD TAG stock solution required**

For Example

$$\frac{\text{(1 mg/mL Prot. Conc.)}}{\text{(150000 MW IgG)}} \times (\text{1057 MW MSD TAG}) \times (\text{8 ratio}) \times (\text{1000 µL IgG solution}) = \textbf{56.4 µg MSD TAG}$$

Then,

56.4 µg MSD TAG ÷ 1.5 (µg/µL) Conc. MSD TAG stock solution = **37.6 µL MSD TAG stock solution required**

Note that the % (v/v) of DMSO in the reaction should never exceed 15% (v/v) or the reaction will be progressively inhibited. If high concentrations are needed, a more concentrated labeling stock should be prepared so that less organic solvent is added to the protein solution.

3. Immediately before use, prepare the MSD TAG-NHS-Ester stock solution by adding 50 µL of DMSO to the vial. Twirl the vial gently to wet the bottom and lower sides of the vial with the DMSO. This volume of DMSO easily dissolves the available sizes of MSD TAG-NHS-Ester (0.075 mg, 0.150 mg and 0.500 mg), yielding stocks of 1.5, 3 and 10 µg/µL respectively.

4. Add the calculated volume of MSD TAG-NHS-Ester solution to the protein solution and vortex the tube. Discard the remaining MSD TAG-NHS-Ester.

5. Incubate the tube contents at room temperature for 60 minutes, with or without shaking. Shaking the solution will improve the incorporation efficiency.

6. Stop the reaction by adding 20 µL of 2 M glycine and incubate at room temperature for 10 minutes.

7. To remove the uncoupled MSD TAG label, load the mixture onto a size exclusion purification column that is equilibrated with PBS containing 0.05-0.1% (w/v) sodium azide. Pre-packed Sephadex G-25 columns such as PD-10 columns for 1 mL volumes or NAP-5 columns for volumes up to 0.5 mL (Amersham Biosciences) are very convenient to use. MSD does not recommend dialysis of MSD TAG conjugates or the use of membrane-based spin columns to separate labeled protein from free MSD TAG. For larger sample volumes, a longer column with a larger bed volume may be needed. Two yellow bands will be formed as the separation of bound from free MSD TAG proceeds. The labeled protein will elute first, the second yellow band corresponds to the unreacted MSD TAG. When using a PD-10 column to purify the conjugate, eight 0.5 mL fractions are typically collected after the sample volume has entered the PD-10 resin bed. A protein such as IgG is usually in the fourth fraction.

8. Determine the protein concentration using a standard protein assay (e.g., Bradford, Lowry, or a Pierce BCA Protein Assay kit). As mentioned above, an absorbance reading at OD_{280} is not recommended since MSD TAG will absorb light at this wavelength. Collect and pool the appropriate protein-containing fractions and determine the final pooled protein concentration and molarity. The percent protein recovered is dependent upon the separation technique but typically ranges from 70-90%.

9. Measure the absorbance of the MSD TAG-IgG conjugate at 455 nm using a 1 cm path cuvette. Divide the value by 13700 to obtain the MSD TAG concentration in moles per liter.

10. To calculate the MSD TAG:IgG ratio, divide the value obtained in step 9 by the value determined for step 8. The formula is also provided on the attached worksheet

11. Stabilize dilute protein solutions (<0.1 mg/mL) by adding additional proteins such as 1-3% (w/v) bovine serum albumin or other appropriate serum proteins. Antibody conjugates are usually stable for at least 12 months at 4°C, but the stability of other proteins should be assessed. Many proteins require storage at < -20°C. For long term storage, labeled conjugates should be stored in amber or opaque vials. Aliquots of the conjugate can usually be stored frozen as long as the protein is stable to freeze-thaw cycles.

Storage, Stability and Handling

MSD TAG-NHS-Ester is supplied as a dry orange to dark red solid (appears as a film on the vial) which is stable for at least 24 months from date of manufacture when stored frozen (-10 to -30°C) and desiccated. After reconstitution of the reagent with anhydrous solvent any unused material should be discarded.

Note: MSD TAG-NHS-Ester is light sensitive, therefore, MSD TAG-protein conjugates should be stored in light shielded containers such as amber polypropylene vials.

Labeling Worksheet for MSD TAG-NHS-Ester

This worksheet is intended to be used as a guide while following the MSD Labeling Method. Refer to the MSD Labeling Method text for instructions and examples.

Protein to be Labeled: ______________________ Concentration: __________

____

Vendor: ______________________ Catalog No. __________

____

Lot No. ______________________ Date: __________

____

Labeling Buffer: ______________________ Vendor: __________

____

Lot No. ____

____

Storage Buffer: ______________________ Vendor: __________

____

Lot No. ____

____

2 M Glycine Lot No. ____

____

DMSO Source: ______________________ Lot No. __________ Date __________

MSD TAG-NHS-Ester Reconstitution and Use

Vial Size: ____

____

Lot No. ____

____

Volume of DMSO Added: ____

Concentration of MSD TAG Solution: ____

Calculation of MSD TAG-NHS-Ester Required for Labeling

This worksheet is intended to be used as a guide while following the MSD Labeling Method. Refer to the MSD Labeling Method text for instructions and examples.

(____ mg/mL Protein Conc.) x (1057 MW MSD TAG) x (___ ratio) x (_____ µL) = _____ **µg MSD TAG**
MW (IgG = 150000)

Next, divide the micrograms of label determined above by the concentration of your MSD TAG stock solution. This will give you the volume of MSD TAG-NHS-Ester stock solution to be added to the antibody for labeling.

_____ µg MSD TAG ÷ µg/µL MSD TAG stock solution = ____ **µL MSD TAG stock solution required**

________ µL of MSD TAG Solution Added and Incubation Started at: ____________________________

____

With or Without Shaking: ____

____

Time of Addition of 20 µL of 2 M Glycine: _______________________________________

____

Calculation and Work Performed By: ________________________ Date: _________________

____

Desalting Method: ______________________________________ Date: _________________

____

Protein Concentration Post-Labeling: ________________________ Assay Method: ____________

____

OD_{455} Reading. ____

____

Formula for Calculation of MSD Incorporation

Concentration of Protein ______ (mg/mL), ÷ Molecular Weight of Protein (IgG = 150000) = (A) ______ M

OD_{455} Results _______________________ , ÷ 13700 (Extinction Coefficient) = (B) _________________ M

Ratio of MSD TAG to Protein = (B) ___________________, ÷ (A)___________________ = ____________

Protein Added to Storage Buffer? Yes (type) ____________________________ No _________________

____

Storage Temperature and Location: __________________________________ Date _______________

____

Work Performed By: ____________________________________ Reviewed By / Date _______________

Catalog Numbers

Product	Size	Catalog Number
MSD TAG-NHS-Ester	150 nmol	R91BN-1
	500 nmol	R91BN-2
	Larger sizes	Please Inquire

Company Address

MESO SCALE DISCOVERY®
A division of Meso Scale Diagnostics, LLC.
9238 Gaither Road
Gaithersburg, MD 20877 USA
www.mesoscale.com

Ordering Information

MSD Customer Service

Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support

Phone: 1-301-947-2025
Fax: 1-240-632-2219 attn: Scientific Support
Email: ScientificSupport@mesoscale.com

For Research Use Only. Not for use in diagnostic procedures.

17875-v2-2011Jan

MESO SCALE DISCOVERY, MESO SCALE DIAGNOSTICS, WWW.MESOSCALE.COM, MSD, MSD (DESIGN), MULTI-ARRAY and SPOT THE DIFFERENCE are trademarks and/or service marks of Meso Scale Diagnostics, LLC.
Sephadex and NAP are registered trademarks of Amersham Biosciences.
© 2011 Meso Scale Diagnostics, LLC. All rights reserved