MSD Gold Streptavidin Antibody Preparation and Plate Run Protocol

Kamaljot Gill*[1,2], Maria Sckaff*[1,2], Claire Clelland[1,2]**

[1]Gladstone Institutes, San Francisco, CA, United States
[2]University of California, San Francisco, Weill Institute for Neurosciences, San Francisco, CA, United States
*These authors contributed equally
**Contact: claire.clelland@ucsf.edu

This protocol describes how to conjugate antibodies and run the Meso Scale Discovery (MSD) Sandwich enzyme-linked immunosorbent assay (ELISA) on MSD GOLD 96-well Small Spot Streptavidin SECTOR Plates. This protocol is adapted from MSD GOLD™ Streptavidin Plate and Avidin Plates Quick Guide, the MSD® Biotin Conjugation Quick Guide, and the MSD GOLD™ SULFO-TAG NHS-Ester Conjugation Quick Guide, and it was optimized for C9orf72 dipeptide repeat detection from human iPSC derived neurons.

Buffer Exchange the Antibodies
It is only needed to buffer exchange your antibodies if they are in buffers with preservatives such as sodium azide or EDTA or in buffers that contain primary amines or glycerol.

1. Chill PBS (or MSD Conjugation Buffer) and ultrapure water on ice
2. Equilibrate Zeba Spin Desalting Columns, MSD Storage Buffer, and Sulfo-NHS-LC-Biotin at RT
    a. Need one Zeba column per 70 µL of antibody
    b. In order to both biotinylate and sulfo-tag the antibody, you need at least 140 µL of antibody at an optimal concentration of 1.0 mg/mL
    c. It is still possible to move forward with a less concentrated sample
    d. Dilute the antibodies with ice-cold PBS if necessary
3. Remove the Zeba columns' bottom closure and loosen the cap (DO NOT REMOVE THE CAP)
4. Place the column in a collection tube to remove the storage buffer
5. Spin at 1,500 x g for 1 minute. Empty collection tube
6. Wash 1: Add 300 µL of PBS (or MSD Conjugation Buffer) to the column. Spin at 1,500 x g for 1 minute. Empty collection tube
7. Wash 2: Add 300 µL of PBS (or MSD Conjugation Buffer) to the column. Spin at 1,500 x g for 1 minute. Empty collection tube
8. Wash 3: Add 300 µL of PBS (or MSD Conjugation Buffer) to the column. Spin at 1,500 x g for 3 minutes. Empty collection tube
9. Change collection tube to a clean Eppendorf tube for sample recovery
   Label one Eppendorf tube/sample for each sample to be biotinylated or sulfo-tagged
10. Pipette 70 µL of the antibody to the spin column
11. Spin at 1,500 x g for 3-4 minutes
12. Save the eluent on ice
    The eluent is the buffer exchanged antibody

Biotinylate the Antibody

Note on planning when to biotinylate your antibodies: Before performing this step, plan to tag multiple antibodies at the same time to save both money and reagents.

1. Calculate how much Sulfo-NHS-LC-Biotin you need per antibody, using the following formula:
   1,000 X ([Concentration of antibody in mg/mL]/ 150,000 Da) X 20 X 70 µL of antibody = nmol of Biotin needed
   This nmol of Biotin needed divided by 0.5 nmol/µL Biotin reagent = µL of Sulfo-NHS-LC Biotin needed
   See attached examples at the end of this document
2. Add 180 µL of ultrapure H2O to the 1 mg vial to Sulfo-NHS-LC-Biotin
3. Dilute the Sulfo-NHS-LC-Biotin by adding 10 µL of the stock to 190 µL of cold water
   Once formed, this is highly unstable and should be used immediately
4. Add the calculated volume of diluted reconstituted Sulfo-NHS-LC-Biotin to each antibody
5. Let the antibody and biotin incubate at RT for 2 hours in the dark

Sulfo-tag the Antibody

Note on planning when to sulfo-tag: Before performing this step, plan to tag multiple antibodies at the same time to save both money and reagents

1. Calculate how much SULFO-TAG NHS-Ester you need per antibody, using the following formula:
   1,000 X ([Concentration of antibody in mg/mL]/ 150,000 Da) X 20 X 70 µL of antibody = nmol of Sulfo-Tag needed
   This nmol of Sulfo-Tag needed divided by 3.0 nmol/µL = µL of sulfo-tag ester solution needed
   See attached examples at the end of this document
2. Add 50 µL of ice-cold ultrapure H_2O to the 150 nmol SULFO-TAG NHS-Ester Vial to make a 3 nmol/µL solution
   Once formed, this is highly unstable and should be disposed
3. Vortex the solution lightly
4. Add the calculated volume of diluted reconstituted SULFO-TAG NHS-Ester reagent to each antibody
5. Let the antibody and SULFO-TAG NHS-Ester incubate at RT for 2 hours in the dark

Clean-up the Biotinylated Antibody

1. Equilibrate Zeba Spin Desalting Columns, MSD Storage Buffer at RT
2. Place the column in a collection tube to remove the storage buffer
3. Remove the columns' bottom closure and loosen the cap (DO NOT REMOVE THE CAP)
4. Spin at 1,500 x g for 1 minute. Empty collection tube
5. Wash 1: Add 300 µL of MSD Conjugate Storage Buffer to the column. Spin at 1,500 x g for 1 minute. Empty collection tube
6. Wash 2: Add 300 µL of MSD Conjugate Storage Buffer to the column. Spin at 1,500 x g for 1 minute. Empty collection tube
7. Wash 3: Add 300 µL of MSD Conjugate Storage Buffer to the column. Spin at 1,500 x g for 3 minutes. Empty collection tube

8. Change collection tube for sample recovery
9. Pipette 70 µL of each unpurified biotinylated antibody to the spin column
10. Spin at 1,500 x g for 3-4 minutes
11. Save the eluent on ice
    The eluent is the biotinylated antibody. It is stable at 4°C for 1 year

Cleanup the Sulfo-Tagged Antibody

1. Equilibrate Zeba Spin Desalting Columns, MSD Storage Buffer at RT
2. Remove the columns' bottom closure and loosen the cap (do not remove the cap)
3. Place the column in a collection tube to remove the storage buffer
4. Spin at 1,500 x g for 1 minute. Empty collection tube
5. Wash 1: Add 300 µL of MSD Conjugate Storage Buffer to the column. Spin at 1,500 x g for 1 minute. Empty collection tube
6. Wash 2: Add 300 µL of MSD Conjugate Storage Buffer to the column. Spin at 1,500 x g for 1 minute. Empty collection tube
7. Wash 3: Add 300 µL of MSD Conjugate Storage Buffer to the column. Spin at 1,500 x g for 3 minutes. Empty collection tube
8. Change collection tube for sample recovery
9. Pipette 70 µL of each unpurified SULFO-TAG NHS-Ester tagged antibody to the spin column
10. Spin at 1,500 x g for 3-4 minutes
11. Save the eluent on ice
    This is the SULFO-TAG NHS-Ester tagged antibody. It is stable at 4°C for 1 year

Day 1 of MSD: Coating the Plate with Capture Antibody

1. Dilute biotinylated capture antibodies in 1x DPBS to your desired concentration
2. Add 30 µL of the diluted antibody to the corner of each well of the MSD 96 Streptavidin Small Spot Plate according to the plate format
   IMPORTANT: From this point forward, never let the plate dry
3. Tap the plate on its edges to ensure the antibody is spread evenly across the well
4. Seal the plate with parafilm or an adhesive plate cover to avoid loss of antibody due to evaporation overnight
5. Store in the plate at 4°C to incubate overnight without shaking

Day 2 of MSD

1. Tap out the plate to dispose of the capture antibody
2. Add 150 µL/well of Blocking Solution (3% Blocker A (or BSA) + PBS) per well

3% Blocker A in 1x PBS (Store at 4˚C)	
For 100 mL	
Blocker A	**3 g**
1X PBS	**100 mL**
(Stir or shake overnight at 4°C to make sure it is dissolved completely.)	

3. Seal the plate and incubate at RT with shaking at 750 rpm for 1 hour
4. Prepare the lysate samples according to plate layout, by diluting the protein samples in the lysate buffer to the desired lysate concentrations
5. Tap out the plate
6. Wash 1x with 150 µL/well of PBS - T (0.05% Tween)
7. Discard the wash solution, without letting the plate dry
8. Add 25 µL/well of the lysate to the target wells
    a. Add lysate to the bottom corner of the wells
9. Seal the plate and incubate at RT with shaking at 750 rpm for 1-2 hours
10. While the lysates incubate, prepare the detection antibodies (your sulfo-tagged antibodies) in 1% MSD Blocker A in 1x DPBS
11. Discard the lysate solution
    a. Do not let the plate dry, but remove the excess solution
12. Wash 3x with 150 µL/well of PBS - T (0.05% Tween).
13. Discard the wash solution, but do not let the plate dry
14. Add 25 µL/well of sulfo-tag antibodies (in 1% Blocker A in PBS) to the plate layout
15. Seal the plate and incubate at RT with shaking at 750 rpm for 1 hour
16. Tap out the plate
17. Wash 3x with 150 µL/well of PBS - T (0.05% Tween)
18. Tap out the plate
19. Add 150 µL/well of Read Buffer A using reverse pipetting to avoid making bubbles
    IMPORTANT: Ensure there is no plastic wrap, tape, or parafilm on the plate
20. Read the plate immediately

Reagents	**Manufacturer**	**Catalog Number**
MSD GOLD SULFO-TAG NHS-Ester Conjugation Pack	MSD	R31AA
Zeba Spin Desalting Columns	ThermoFisher	89882
EZ-Link™ Sulfo-NHS-LC-Biotin, No-Weigh™ Format	ThermoFisher	A39257
MSD GOLD 96-well Small Spot Streptavidin SECTOR Plate	MSD	L45SA
Blocker A	MSD	R93BA
MSD GOLD Read Buffer A	MSD	R92TG
PBS, pH 7.4	ThermoFisher	10010023
Tween-20	Sigma	P1379
Nuclease-Free Water (not DEPC-Treated)	ThermoFisher	AM9932

Reagents	**Alternative Reagents**	**Manufacturer**	**Catalog Number**
MSD Conjugation Buffer	PBS, pH 7.4	ThermoFisher	10010023
MSD Conjugation Storage Buffer	PBS, 0.05% Sodium Azide	Teknova	P0202
Blocker A	BSA	Sigma	A4503

Equipment	Manufacturer	Catalog Number
Microseal 'B' PCR Plate Sealing Film, adhesive, optical	BioRad	MSB1001
1.5 mL Eppendorf tubes	Fisher Scientific	14-666-321
Meso Scale Discovery (MSD) Model 1250 Sector Imager 2400	MSD	1250
Heidolph™ Titramax Vibrating Platform Shakers	Fisher Scientific	13-889-420
Centrifuge (capable of 1,500g)	Any	Any
Vortex	Any	Any

References

[1]MSD GOLD™ SULFO-TAG NHS-Ester Conjugation Quick Guide
https://www.mesoscale.com/~/media/files/handout/msd%20gold%20sulfo-tag%20conjugation%20quick%20guide.pdf

[2]MSD® Biotin Conjugation Quick Guide
https://www.mesoscale.com/~/media/files/handout/biotin%20conjugation%20quick%20guide%20v2.pdf

[3]MSD® GOLD Streptavidin and Avidin Plates Quick Guide
https://www.mesoscale.com/~/media/files/handout/msd%20gold%20strep_avidin%20plates%20quick%20guide.pdf

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Clelland Lab

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This document outlines sample calculations for the biotinylation and sulfo-tagging of antibodies for the MSD assay, as described in the protocol above.

Relevant notes, using Poly-GR MABN778 and Poly-PR ABN1354 antibodies as references:

- 0.5mg/mL is the concentration of the antibody
- 150,000Da is the protein weight for IgG protein
- 20:1 is the challenge ratio
- 70 µL is the volume of the protein solution

1 Biotinylation Calculations

Poly-GR MABN778 and Poly-PR ABN1354

$$1000 \times \frac{0.5mg/mL}{150,000Da} \times \frac{20}{1} \times 70\mu L = 4.67nmol \tag{1}$$

4.67 nmol of sulfo-NHS-LC Biotin required

$$\frac{4.67}{0.5nmol/\mu L} = 9.34\mu L \tag{2}$$

9.34 µL of sulfo-NHS-LC Biotin stock solution

Poly-GA MABN889

$$1000 \times \frac{0.33mg/mL}{150,000Da} \times \frac{20}{1} \times 70\mu L = 3.08nmol \tag{3}$$

3.08 nmol of sulfo-NHS-LC Biotin required

$$\frac{3.08}{0.5nmol/\mu L} = 6.16\mu L \tag{4}$$

6.16 µL of sulfo-NHS-LC Biotin stock solution

2 Sulfo-tag Calculations

Poly-GR MABN778 and Poly-PR ABN1354

$$1000 \times \frac{0.5mg/mL}{150,000Da} \times \frac{20}{1} \times 70\mu L = 4.67nmol \tag{5}$$

4.67 nmol of sulfo-tag reagent required

$$\frac{4.67}{3.0nmol/\mu L} = 1.56\mu L \tag{6}$$

1.56 µL of sulfo-tag ester solution

Poly-GA MABN889

$$1000 \times \frac{0.33mg/mL}{150,000Da} \times \frac{20}{1} \times 70\mu L = 3.08nmol \tag{7}$$

3.08 nmol of sulfo-tag reagent required

$$\frac{3.08}{3.0nmol/\mu L} = 1.03\mu L \tag{8}$$

1.03 µL of sulfo-tag ester solution