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MSD Gold Streptavidin Antibody Preparation and Plate Run Protocol

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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 1 and MSD GOLD™ SULFO-TAG NHS-Ester Conjugation Quick Guide 2 and optimized for C9orf72 dipeptide repeat detection from human iPSC derived neurons.

MSD protocol_ClellandLab.pdf

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Buffer Exchange, Biotinylate the Antibody, Sulfo-tag the Antibody, MSD, ELISA, protein quantification, streptavidin, antibody, protein

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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

Reagents List

A	B	C
Reagents	**Manufacturer**	**Catalog Number**
MSD GOLD SULFO-TAG NHS-Ester Conjugation Pack	MSD	R31AA
Zeba Spin Desalting Columns	ThermoFisher	89882
EZ-LinkTM Sulfo-NHS-LC-Biotin, No-WeighTM 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

☒ MSD GOLD SULFO-TAG NHS-Ester Conjugation Pack **MESO SCALE DIAGNOSTICS, LLC. Catalog #R31AA**

☒ Zeba™ Spin Desalting Columns, 7K MWCO, 0.5 mL **Thermo Fisher Catalog #89882**

☒ EZ-Link™ Sulfo-NHS-LC-Biotin, No-Weigh™ Format **Thermo Fisher Catalog #A39257**

☒ MSD GOLD 96-well Small Spot Streptavidin SECTOR Plate **MESO SCALE DIAGNOSTICS, LLC. Catalog #L45SA**

☒ Blocker A **MESO SCALE DIAGNOSTICS, LLC. Catalog #R93BA**

☒ MSD GOLD Read Buffer A **MESO SCALE DIAGNOSTICS, LLC. Catalog #R92TG**

☒ PBS pH 7.4 **Thermo Fisher Scientific Catalog #10010023**

☒ Tween 20 **Sigma Catalog #P1379**

☒ Nuclease-Free Water (not DEPC-Treated) **Thermo Fisher Catalog #AM9932**

Alternative Reagents List

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A	B	C	D
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

PBS 0.05% Sodium Azide **Teknova Catalog #P0202**

Bovine Serum Albumin **Millipore Sigma Catalog #A4503**

Equipment List

A	B	C
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
HeidolphTM Titramax Vibrating Platform Shakers	Fisher Scientific	13-889-420
Centrifuge (capable of 1,500g)	Any	Any
Vortex	Any	Any

Microseal 'B' PCR Plate Sealing Film, adhesive, optical
Plate Sealing Film
Microseal **MSB1001**

Microcentrifuge Tubes with Locking Snap Cap
Microcentrifuge Tubes
Fisherbrand™ **14-666-321**

Meso Scale Discovery (MSD) Model 1250 Sector Imager 2400
multiplex assay reader
MESO SECTOR **1250**

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Heidolph™ Titramax Vibrating Platform Shakers
Shaker
Heidolph™ 13-889-420

Buffer Exchange the Antibodies 20m

1

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.

2 Chill PBS (or MSD Conjugation Buffer) and ultrapure water **On ice** .

3 Equilibrate Zeba Spin Desalting Columns, MSD Storage Buffer, and Sulfo-NHS-LC-Biotin at **Room temperature** .

3.1 Use one Zeba column per **70 µL** of antibody.

3.2 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** .

3.3 It still possible to move forward with a less concentrated sample.

3.4 Dilute the antibodies with ice-cold PBS if necessary.

4 ⚠

Remove the Zeba columns' bottom closure and loosen the cap.

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Do not remove the cap.

5 Place the column in a collection tube to remove the storage buffer.

6  1m

Spin at **1500 x g, 00:01:00** . Empty collection tube.

7  1m

Wash 1: Add **300 µL** of PBS (or MSD Conjugation Buffer) to the column. Spin at **1500 x g, 00:01:00** . Empty collection tube.

8  1m

Wash 2: Add **300 µL** of PBS (or MSD Conjugation Buffer) to the column. Spin at **1500 x g, 00:01:00** . Empty collection tube.

9  3m

Wash 3: Add **300 µL** of PBS (or MSD Conjugation Buffer) to the column. Spin at **1500 x g, 00:03:00** . Empty collection tube.

10 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.

11 Pipette **70 µL** of the antibody to the spin column.

12 

Spin at **1500 x g** for 3-4 minutes.

13 Save the eluent **On ice** .

The eluent is the buffer exchanged antibody.

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Biotinylate the Antibody 2h

14

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.

Calculate how much Sulfo-NHS-LC-Biotin you need per antibody, using the following formula:

- 1,000 × ([Concentration of antibody in mg/mL]/ 150,000 Da) × 20 × **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

15 

Add **180 µL** of ultrapure H_2O to the **1 mg** vial to Sulfo-NHS-LC-Biotin.

16 

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.

17 Add the calculated volume of diluted reconstituted Sulfo-NHS-LC-Biotin to each antibody.

18  2h

Let the antibody and biotin incubate at **Room temperature** for **02:00:00** in the dark.

Sulfo-tag the Antibody 2h

19

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.

Calculate how much SULFO-TAG NHS-Ester you need per antibody, using the following formula:

- 1,000 × ([Concentration of antibody in mg/mL]/ 150,000 Da) × 20 × **70 µL** of antibody = nmol of Sulfo-Tag reagent 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

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20 

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.

21 Vortex the solution lightly.

22 Add the calculated volume of diluted reconstituted SULFO-TAG NHS-Ester reagent to each antibody.

23  2h

Let the antibody and SULFO-TAG NHS-Ester incubate at **Room temperature** for **02:00:00** in the dark.

Clean-up the Biotinylated Antibody 15m

24 Equilibrate Zeba Spin Desalting Columns, MSD Storage Buffer at **Room temperature** .

25 Place the column in a collection tube to remove the storage buffer.

26 

Remove the columns' bottom closure and loosen the cap

> Do not remove the cap.

27  1m

Spin at **1500 x g, 00:01:00** . Empty collection tube.

28  1m

Wash 1: Add **300 µL** of MSD Conjugate Storage Buffer to the column. Spin at **1500 x g, 00:01:00** . Empty collection tube.

 1m

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29

Wash 2: Add **300 µL** of MSD Conjugate Storage Buffer to the column. Spin at **1500 x g, 00:01:00** . Empty collection tube.

30 3m

Wash 3: Add **300 µL** of MSD Conjugate Storage Buffer to the column. Spin at **1500 x g, 00:03:00** . Empty collection tube.

31 Change collection tube for sample recovery.

32 Pipette **70 µL** of the unpurified biotinylated antibody to the spin column.

33

Spin at **1500 x g** for 3-4 minutes.

34 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 15m

35 Equilibrate Zeba Spin Desalting Columns, MSD Storage Buffer at **Room temperature** .

36 Remove the columns' bottom closure and loosen the cap.

Do not remove the cap.

37 Place the column in a collection tube to remove the storage buffer.

38 1m

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Spin at **1500 x g, 00:01:00** . Empty collection tube.

39 1m

Wash 1: Add **300 µL** of MSD Conjugate Storage Buffer to the column. Spin at **1500 x g, 00:01:00** . Empty collection tube.

40 1m

Wash 2: Add **300 µL** of MSD Conjugate Storage Buffer to the column. Spin at **1500 x g, 00:01:00** . Empty collection tube.

41 3m

Wash 3: Add **300 µL** of MSD Conjugate Storage Buffer to the column. Spin at **1500 x g, 00:03:00** . Empty collection tube.

42 Change collection tube for sample recovery.

43 Pipette **70 µL** of the unpurified SULFO-TAG NHS-Ester tagged antibody to the spin column.

44

Spin at **1500 x g** for 3-4 minutes.

45 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 30m

46 Dilute biotinylated capture antibodies in 1x DPBS to your desired concentration.

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47

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.

48 Tap the plate on its edges to ensure the antibody is spread evenly across the well.

49 Seal the plate with parafilm to avoid loss of antibody due to evaporation.

50

Store in the plate at **4 °C** to incubate **Overnight** without shaking.

Day 2 of MSD 5h

51 Tap out the plate to dispose of the capture antibody.

52

Add **150 µL** /well of Blocking Solution (3% Blocker A (or BSA) + PBS) per well.

A	B
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.)	

53 1h

Seal the plate and incubate at **Room temperature** with shaking at **750 rpm, 01:00:00** .

54 Prepare the lysate samples according to plate layout, by diluting the protein samples in the lysate buffer to the desired lysate concentrations.

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55 Tap out the plate.

56 

Wash 1x with **150 µL** /well of PBS - T (0.05% Tween).

57 Discard the wash solution, without letting the plate dry.

58 

Add **25 µL** /well of the lysate to the target wells.

> Add lysate to the bottom corner of the wells.

59 

2h

Seal the plate and incubate at **Room temperature** with shaking at **750 rpm** for 1-2 hours.

60 While the lysates incubate, prepare the detection antibodies (your sulfo-tagged antibodies) in 1% MSD Blocker A in 1x DPBS.

61 Discard the lysate solution.

> Do not let the plate dry, but remove the excess solution.

62 

Wash with PBS - T (0.05% Tween).

62.1 Wash with **150 µL** /well of PBS - T (0.05% Tween). (1/3)

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62.2 Wash with **150 µL** /well of PBS - T (0.05% Tween). (2/3)

62.3 Wash with **150 µL** /well of PBS - T (0.05% Tween). (3/3)

63 Discard the wash solution, but do not let the plate dry.

64 

Add **25 µL** /well of sulfo-tag antibodies (in 1% Blocker A in DPBS) to the plate layout.

65 

1h

Seal the plate and incubate at **Room temperature** with shaking at **750 rpm, 01:00:00** .

66 Tap out the plate.

67 

Wash with PBS - T (0.05% Tween).

67.1 Wash with **150 µL** /well of PBS - T (0.05% Tween). (1/3)

67.2 Wash with **150 µL** /well of PBS - T (0.05% Tween). (2/3)

67.3 Wash with **150 µL** /well of PBS - T (0.05% Tween). (3/3)

68 Tap out the plate.

69 

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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.

70 Read the plate immediately. 5m

Sample calculations

71

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

Relevant notes, using Poly-GR 1VIABN778 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 pL is the volume of the protein solution

Sample Biotinylation Calculations

72 **Poly-GR MABN778 and Poly-PR ABN1354**

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

4.67 nmol of sulfo-NHS-LC Biotin required

73

$$\frac{4.67}{0.5nmol/\mu L} = 9.34\mu L \qquad (2)$$

9.34 µL of sulfo-NHS-LC Biotin stock solution .

74 **Poly-GA MABN889**

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

3.08 nmol of sulfo-NHS-LC Biotin required

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75
$$\frac{3.08}{0.5nmol/\mu L} = 6.16\mu L \quad (4)$$

6.16 µL of sulfo-NHS-LC Biotin stock solution.

Sample Sulfo-tag Calculations

76 **Poly-GR MABN778 and Poly-PR ABN1354**

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

4.67 nmol of sulfo-tag reagent required.

77
$$\frac{4.67}{3.0nmol/\mu L} = 1.56\mu L \quad (6)$$

1.56 µL of sulfo-tag ester solution .

78 **Poly-GA MABN889**

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

3.08 nmol of sulfo-tag reagent required.

79
$$\frac{3.08}{3.0nmol/\mu L} = 1.03\mu L \quad (8)$$

1.03 µL of sulfo-tag ester solution.

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