

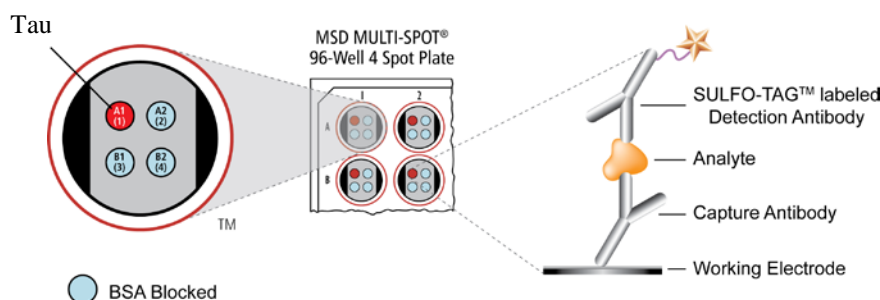
MSD 96-Well MULTI-ARRAY[®] Alzheimer's Disease Assay: Mouse Total Tau with Purified Neuronal Tau Calibrators

Tau is a microtubule-associated protein with multiple spliceforms, found primarily in neurons. Hyperphosphorylated tau is the major component of paired helical filaments and other large intracellular aggregates in the brains of patients with Alzheimer's disease and some other neurodegenerative disorders. The antibodies used in this assay cross-react with human, mouse, and bovine samples.

Storage

Materials Included

❑ MULTI-SPOT [®] 96-well 4-Spot Mouse Total Tau Plate(s)	2-8°C
❑ SULFO-TAG [™] Anti-Total Tau Antibody ¹	2-8°C
❑ Purified Neuronal Tau Calibrator	≤-70 °C
❑ Read Buffer T (4X)	RT
❑ Blocker A	RT
❑ Tris Wash Buffer (10X)	2-8°C
❑ Blocker D-B (10%) ²	≤-10 °C



The SECTOR[®] Imager data file will identify spots according to their well location, not by the capture antibody name. EGFR is located on spot A1.

¹ SULFO-TAG conjugated detection antibodies should be stored in the dark.

² Blocker D-B can tolerate at least 5 freeze-thaw cycles. Alternatively, an aliquot of the blocker can be stored at 2-8°C up to 1 month.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.



Notes:

Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting concentrated buffers
- ❑ One 250 mL bottle
- ❑ Two 50 mL tubes
- ❑ Three 15 mL tubes
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Various microcentrifuge tubes for making serial dilutions of lysates (if desired)
- ❑ Automated plate washer or other efficient multi-channel pipetting equipment for washing 96-well plates
- ❑ Appropriate liquid handling equipment for desired throughput that must accurately dispense 25 μ L and 150 μ L into a 96-well micro plate

Protocol at a Glance

1. Add blocking solution, incubate 1 hour, wash.
2. Add sample or calibrator; incubate 1 hour at room temperature, wash.
3. Add detection antibody, incubate 1 hour, wash.
4. Add Read Buffer T and analyze plate.

Read the entire detailed instructions before beginning work.

The full protocol that follows describes the most conservative approach to achieving highly sensitive results using MSD technology to quantify total and phosphorylated tau. The protocol can be completed in approximately 3 to 3 1/2 hours if reagent for each step is prepared during the preceding incubation.

Alternatively, all reagents can be prepared ahead of time. This lengthens the overall time required for the assay but frees up time during incubation steps.

Once desired results are achieved, the protocol can be streamlined to eliminate multiple incubation and wash steps and increase throughput.

Detailed Instructions

Prepare a stock of 1X Tris Wash Buffer. 1X Tris Wash Buffer is used throughout the assay to dilute other reagents and wash plates. Approximately 350 mL per plate is required—more if using an automatic plate washer.

In a 500 mL bottle, combine:

- ❑ 35 mL 10X Tris Wash Buffer
- ❑ 315 mL deionized water

A larger amount of Tris Wash Buffer may be prepared and stored at room temperature for later use.



Prepare Blocker A Solution. You will need 20 mL per plate.

In a 50 mL tube, combine:

- 20 mL 1X Tris Wash Buffer
- 600 mg Blocker A (3% w/v)

Prepare Antibody Dilution Buffer. You will need 3 mL per plate.

In a 15 mL tube, combine:

- 1 mL Blocker A Solution
- 1.97 mL 1X Tris Wash Buffer
- 30 µL 10% Blocker D-B

Solutions containing Blocker A should be kept at 2–8°C and discarded after 14 days.

STEP 1

Begin with a 96-well 4-spot custom Mouse Total Tau plate.

No pre-treatment is necessary.

Add 150 µL/well of Blocker A solution.

Incubate with shaking at room temperature for 1 hour. During this time, prepare complete lysis buffer and dilute cell lysates.

Prepare dilutions of purified neuronal tau calibrator.

1 µg/mL: 5 µL of the 50 µg/mL stock plus 245 µL of diluent

316 ng/mL: 100 µL of the 1 µg/mL solution plus 216 µL diluent

100 ng/mL: 100 µL of the 316 ng/mL solution plus 216 µL diluent

32 ng/mL: 100 µL of the 100 ng/mL solution plus 216 µL diluent

10 ng/mL: 100 µL of the 32 ng/mL solution plus 216 µL diluent

3.2 ng/mL: 100 µL of the 10 ng/mL solution plus 216 µL diluent

1 ng/mL: 100 µL of the 3.2 ng/mL solution plus 216 µL diluent

318 pg/mL: 100 µL of the 1 ng/mL solution plus 216 µL diluent

100 pg/mL: 100 µL of the 318 pg/mL solution plus 216 µL diluent

32 pg/mL: 100 µL of the 100 pg/mL solution plus 216 µL diluent

10 pg/mL: 100 µL of the 32 pg/mL solution plus 216 µL diluent

0 pg/mL: diluent alone

The purified neuronal tau Calibrator and all diluted samples should be prepared in a diluent that mimics the sample matrix as closely as possible (i.e., cell culture medium, lysis buffer, immunodepleted CSF, etc...).

The diluent used must contain sufficient protein to prevent non-specific sticking of tau to the assay well. In the absence of an optimized diluent, 10% Blocker A in 1X MSD Wash buffer is recommended.

Samples derived from biological fluids and/or tissues may require independent manipulations not described here.

This dilution series will cover the entire linear range of the neuronal tau Calibrators for both pTau231 and total Tau. The number of concentrations used may be able to be reduced, depending on the samples being tested.

STEP 2

Wash plate(s) three times with 300 µL/well of 1X Tris Wash Buffer.

Dispense 25 µL/well of samples or diluted purified neuronal tau calibrator prepared during Step 1 incubation.

Incubate with shaking for 1 hour at room temperature. During this time, prepare detection antibody solution.

Prepare Detection Antibody Solution. You will need 3.0 mL per plate at a 10 nM final concentration.

Shaking the plate accelerates analyte capture.



In a 15 mL tube, combine:

- 2.91 mL cold antibody dilution buffer
- 91 µL 0.33µM Anti-Total Tau Antibody

STEP 3

Wash plate(s) three times with 300 µL/well of 1X Tris Wash Buffer.

Add 25 µL/well of detection antibody solution.

Incubate with shaking at room temperature for 1 hour. During this time, prepare Read Buffer T.

Prepare Read Buffer T. You will need 20 mL per plate at a final 1X concentration.

In a 50 mL tube, combine:

- 5 mL 4X Read Buffer T
- 15 mL deionized water

STEP 4

Wash plate(s) three times with 300 µL/well of 1X Tris Wash Buffer.

Add 150 µL/well of diluted Read Buffer T.

Analyze with SECTOR Imager.

Diluted read buffer may be kept in a tightly sealed container at room temperature for later use.

Bubbles in the read buffer will interfere with reliable imaging of the plate if carried into the wells.

Read plate(s) immediately after adding read buffer. Most biological interactions tolerate incubation in read buffer; however, each unique assay should be tested for stability in read buffer before being left to sit for extended periods.