

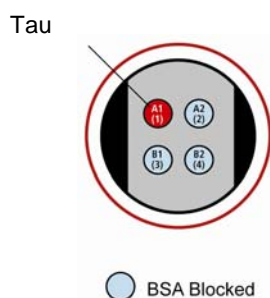
MSD[®] 96-Well MULTI-ARRAY[®] Alzheimer's Disease Assay: Phospho (Thr 231) Tau with Purified Neuronal Tau Calibrators

Tau is a microtubule-associated protein found predominantly in neurons, where it promotes the assembly and/or stability of microtubules. Phosphorylation at Thr231 of tau is involved in inhibition of the interaction between tau and microtubules, and may be more prevalent in Alzheimer's disease than in other tauopathies.

Storage

Materials Included

<input type="checkbox"/> Read Buffer T (with surfactant) (4X)	RT
<input type="checkbox"/> Blocker A	RT
<input type="checkbox"/> MULTI-SPOT [®] 96-well 4 Spot Phospho-Tau Plate(s)	2-8°C
<input type="checkbox"/> Tris Wash Buffer (10X)	2-8°C
<input type="checkbox"/> SULFO-TAG [™] Anti-Phospho-Tau Antibody	2-8°C
<input type="checkbox"/> Blocker D-B (10%)	≤-10 °C
<input type="checkbox"/> Purified Neuronal Tau Calibrator	≤-70 °C



The SECTOR[®] Imager data file will identify spots according to their well location, not by the coated capture antibody name.

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



Notes:

Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting Wash Buffer and Read Buffer
- ❑ One 250 mL bottle
- ❑ Two 50 mL tube
- ❑ One 15 mL tube
- ❑ 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

Read the entire detailed instructions before beginning work.

Protocol at a Glance

The following protocol describes the most conservative approach toward achieving highly sensitive results using MSD technology to quantify total and phosphorylated tau. The protocol takes approximately 3 hours to complete if each reagent is prepared during the preceding incubation. All reagents, with the exception of the diluted lysates, can be prepared ahead of time. This lengthens the overall time required but frees up time during the incubation steps.

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

1. Add Blocking Solution-A, incubate 1 hour, wash.
2. Add samples or Calibrators, incubate 1 hour, wash.
3. Add Detection Antibody, incubate 1 hour, wash.
4. Add Read Buffer and analyze plate.

Detailed Instructions

Prepare a stock of 1X Tris Wash Buffer:

- a) 1X Tris Wash Buffer is used throughout the assay to make other reagents as well as to wash plates. Approximately 250 mL per plate is required – more if using an automatic plate washer to account for waste.
- b) In a 250 mL bottle combine:
 - ❑ 25 mL 10X Tris Wash Buffer
 - ❑ 225 mL deionized water

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



Notes:

Prepare Blocking Solution-A:

- a) In a 50 mL tube combine (per plate):
 - ❑ 20 mL 1X Tris Wash Buffer
 - ❑ 600 mg Blocker A (30 mg/mL or 3%)

Solutions containing MSD Blocker A should be kept at 4°C and discarded after 14 days.

Prepare Antibody Dilution Buffer:

- a) Prepare 3 mL per plate.
- b) In a 15 mL tube combine:
 - ❑ 1 mL Blocking Solution-A
 - ❑ 1.97 mL 1X Tris Wash Buffer
 - ❑ 30 µL 10% Blocker D-B

Begin with a MULTI-SPOT Custom Plate. No pre-treatment is necessary.

STEP 1

Add 150µL/well of Blocking Solution-A.
Incubate at room temperature for 1 hour.
Wash plates four times with Tris Wash Buffer.

Prepare dilutions of purified neuronal tau Calibrator:

1 µg/mL: 5 µL of the 50 µg/mL stock plus 245 µL of diluent
300 ng/mL: 100 µL of the 1 µg/mL solution plus 216 µL diluent
100 ng/mL: 100 µL of the 300 ng/mL solution plus 216 µL diluent
30 ng/mL: 100 µL of the 100 ng/mL solution plus 216 µL diluent
10 ng/mL: 100 µL of the 30 ng/mL solution plus 216 µL diluent
3 ng/mL: 100 µL of the 10 ng/mL solution plus 216 µL diluent
1 ng/mL: 100 µL of the 3 ng/mL solution plus 216 µL diluent
300 pg/mL: 100 µL of the 1 ng/mL solution plus 216 µL diluent
100 pg/mL: 100 µL of the 300 pg/mL solution plus 216 µL diluent
30 pg/mL: 100 µL of the 100 pg/mL solution plus 216 µL diluent
10 pg/mL: 100 µL of the 30 pg/mL solution plus 216 µL diluent
0 pg/mL: diluent alone

The source vial of calibrator informs the user of the units of pTau(Thr231) per µg of Total Tau.

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.



Notes:

STEP 2 Dispense 25 μ L/well of samples or diluted purified neuronal tau Calibrator.

Incubate with shaking at room temperature for 1 hour. Prepare Detection Antibody solution during this time.

Prepare Detection Antibody:

- a) Dilute SULFO-TAG Anti-Phospho-Tau Antibody to a final concentration of 10 nM.
- b) Use cold Antibody Dilution Buffer. Sufficient antibody is supplied to prepare 3 mL per plate.

Wash plates four times with Wash Buffer.

Shaking a 96-well MSD MULTI-ARRAY or MULTI-SPOT plate accelerates capture at the working electrode.

STEP 3 Add 25 μ L/well of Detection Antibody.

Incubate with shaking at room temperature for 1 hour. Prepare Read Buffer during this time.

Dilute Read Buffer:

- a) Approximately 20 mL per plate is required.
- b) Dilute 4X Read Buffer T (with surfactant) to 1X with deionized water.

Wash plates four times with Wash Buffer.

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

STEP 4 Add 150 μ L/well of diluted Read Buffer T (with surfactant).

Analyze with SECTOR Imager.

Bubbles in the Read Buffer will interfere with reliable imaging of the plate if carried into the wells.

Plates can be imaged immediately following the addition of 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.

