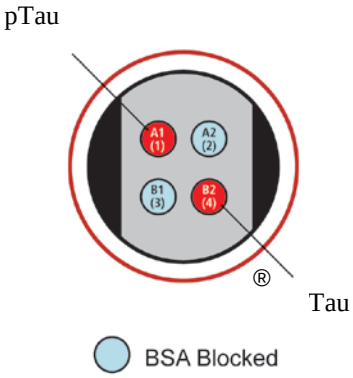
MSD® MULTI-SPOT Phospho (Thr 231)/Total Tau Assay

	Storage

Materials Included

❑ MULTI-SPOT® 96-Well 4-Spot Phospho (Thr231)/Total Tau Plate(s)	2–8ºC
❑ SULFO-TAG™ Anti-Total Tau Antibody[1]	2–8ºC
❑ Tau441 Calibrator (phosphorylated)	≤-70ºC
❑ Tris Wash Buffer (10X)	2–8ºC
❑ Blocker D–B (10%)[2]	≤-10ºC
❑ Blocker A (dry powder)	RT
❑ Read Buffer T (4X)	RT



Note: A spot map identifying the location of the assay can be found on the plate packaging. This information will be needed for data analysis.

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines. Additional safety information is available in the product safety data sheet, which can be obtained from MSD Customer Service.

[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.

Other Materials & Equipment (not supplied)

Notes:

- ❑ Deionized water for diluting concentrated buffers
- ❑ 500 mL bottle
- ❑ 50 mL tubes
- ❑ 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 µL and 150 µL into a 96-well microplate

Protocol at a Glance

Read the entire detailed instructions before beginning work.

1. Add Blocker A solution; incubate 1 hour; wash.
2. Add samples or calibrators; incubate 1 hour; wash.
3. Add detection antibody solution; incubate 1 hour; wash.
4. Add Read Buffer T and analyze plate.

The protocol can be completed in approximately 3 to 3 1/2 hours if each reagent is prepared during the preceding incubation. Alternatively, all reagents with the exception of diluted lysates can be prepared ahead of time. This lengthens the overall time required for the assay but frees up time during incubation steps.

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.

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

In a 500 mL bottle, combine:

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

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

Solutions containing Blocker A should be kept at 2–8ºC and discarded after 5 weeks.

Prepare Antibody Dilution Buffer. You will need 3.0 mL per plate.

In a 15 mL tube, combine:

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

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

In a 15 mL tube, combine:

- ❑ 91 µL Anti-Total Tau Antibody
- ❑ 2909 µL cold Antibody Dilution Buffer

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

Prepare Dilutions of Tau441 Calibrator (phosphorylated).

Note: The source vial of calibrator specifies the units of pTau (Thr231) per µg of Total Tau.

To prepare 7 calibrator solutions plus a zero calibrator for up to 4 replicates:

a) Thaw the stock calibrator and mix well by vortexing.

b) Prepare the highest calibrator by diluting the supplied calibrator with your selected assay diluent.
   a. Add 10 µL of Tau441 Calibrator to 490 µL of assay diluent.
   b. Mix well by vortexing.

c) Prepare the next calibrator by transferring 100 µL of the highest calibrator to 300 µL of assay diluent. Mix well by vortexing. Repeat 4-fold dilutions 5 additional times to generate 7 calibrators.

d) Use assay diluent as the zero calibrator.

Notes:

Excess diluted read buffer may be stored in a tightly sealed container at room temperature.

To avoid the possibility of aggregation and/or sticking to the dilution tubes, the dilutions should be prepared immediately before use.

The calibrator and all diluted samples should be prepared in a diluent that mimics the sample matrix as closely as possible (e.g., 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% MSD Blocker A in 1X Tris Wash buffer is recommended.

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

This yields the following calibrator concentrations:

Calibrator	Phospho-Tau (Thr231) (units/well)	Tau (ng/mL)	Dilution Factor
Calibrator-01	50	1 000	
Calibrator-02	13	250	4
Calibrator-03	3.1	63	4
Calibrator-04	0.78	16	4
Calibrator-05	0.20	3.9	4
Calibrator-06	0.049	0.98	4
Calibrator-07	0.012	0.24	4
Calibrator-08	0	0	n/a

Begin with a MULTI-SPOT 96-Well 4-Spot Phospho (Thr231)/Total Tau Plate. No pre-treatment is necessary.

Notes:

STEP 1

Add 150 µL/well of Blocker A Solution.

Incubate with shaking at room temperature for 1 hour. During this time, prepare calibration curve and samples.

Shaking the plate accelerates analyte capture.

STEP 2

Wash plate(s) four times with at least 150 µL/well of 1X Tris Wash Buffer.

Add 25 µL/well of calibrator or samples prepared during Step 1 incubation.

Incubate with shaking for 1 hour at room temperature.

STEP 3

Wash plate(s) four times with at least 150 µL/well of 1X Tris Wash Buffer

Add 25 µL/well of detection antibody solution.

Incubate with shaking for 1 hour at room temperature.

STEP 4

Wash plate(s) four times with at least 150 µL/well of 1X Tris Wash Buffer.

Add 150 µL/well of diluted Read Buffer T.

Analyze with MSD instrument.

Add read buffer carefully using reverse pipetting technique. 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.