Mouse Tau [pS199] ELISA Kit

Catalog Number KMB7041 (96 tests)

Pub. No. MAN0014945 **Rev.** 2.0 (30)

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Mouse Tau [pS199] ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of mouse tau [pS199] in buffered solution, cell culture medium, cell extract, or brain homogenate. The assay will recognize both natural and recombinant mouse tau [pS199]. **Read the instructions carefully before starting this assay, as the kit has been redeveloped**.

Tau exists as six different isoforms that result from alternative splicing of a single transcript. The molecular weight of the tau isoforms range from 48 kDa to 68 kDa. Regulation of tau is controlled though phosphorylation by numerous serine/threonine kinases. The hyperphosphorylated form of Tau, is the major component of paired helical filaments (PHFs). Tau protein is highly soluble and normally attached to axonal microtubules, but circulating tau can be detected in cerebrospinal fluid (CSF) under certain conditions.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KMB7041 (96 tests)
Ms Tau [pS199] Standard, lyophilized. Refer to vial label for reconstitution volume	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide	27 mL
Antibody Coated Plate, 96-well plate	1 plate
Ms Tau [pS199] Biotin Conjugate (100X)	0.120 mL
Streptavidin-HRP (100X)	0.150 mL
Assay Buffer (20X)	5 mL
Wash Buffer Concentrate (20X)	50 mL
Substrate Solution, Tetramethylbenzidine (TMB)	15 mL
Stop Solution	15 mL
Plate Covers, adhesive strips	4

Required materials not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Cell Extraction Buffer (Cat. No. FNN0011, or see "Prepare Cell Extraction Buffer")

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 25 mL of Wash Buffer Concentrate (20X) with 475 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Prepare Cell Extraction Buffer

Note: See the *ELISA Technical Guide* for detailed information on preparing Cell Extraction Buffer.

1. Prepare Cell Extraction Buffer.

   Cell Extraction Buffer consists of 10 mM Tris (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 20 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 1% Triton™ X-100, 10% glycerol, 0.1% SDS, and 0.5% deoxycholate.
2. Immediately before use, add PMSF (0.3 M stock in DMSO) to 1 mM and 50 µL protease inhibitor cocktail (e.g., Sigma Cat. No. P-2714) for each 1 mL of Cell Extraction Buffer.

Prepare 1X Assay Buffer

1. Dilute 5 mL of Assay Buffer (20X) with 95 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store 1X Assay Buffer at 2–8°C. The diluted buffer is stable for 30 days.

Prepare cell lysate

1. Collect cells by centrifugation (non-adherent cells) or scraping from culture flasks (adherent cells), then wash cells twice with cold PBS.
2. Remove and discard the supernatant and collect the cell pellet. The pellet can be stored at −80°C and lysed at a later date if desired.
3. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes, on ice. Vortex at 10-minute intervals.

   Note: The volume of Cell Extraction Buffer used depends on the number of cells in the cell pellet, and expression levels of mouse tau [pS199]. FOR EXAMPLE, 10^7 Neuro-2a can be extracted in 0.5 mL of Cell Extraction Buffer. Researchers must optimize the extraction procedures for their own applications.
4. Transfer the lysate into microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C.
5. Transfer the supernatant into clean microcentrifuge tubes. Samples can be stored at −80°C (avoid multiple freeze-thaw cycles).

Pre-dilute samples

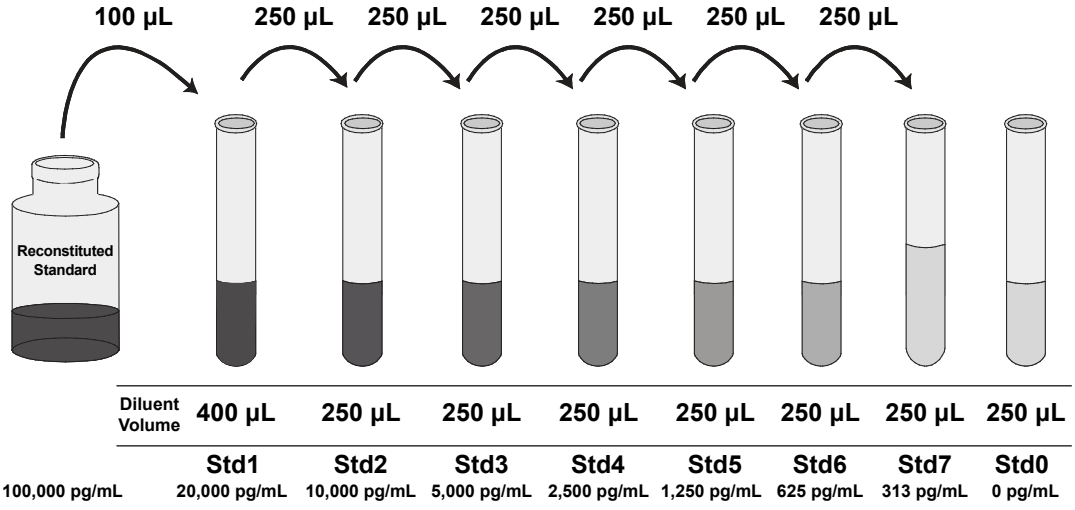
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Dilute brain homogenate samples 1:200 in Standard Diluent Buffer.
- Dilute samples prepared in Cell Extraction Buffer 1:80 in Standard Diluent Buffer
- Dilute samples in cell culture medium or buffer solutions at least 1:2 with Standard Diluent Buffer (e.g., 50 µL sample into 50 µL buffer).
- Perform sample dilutions with Standard Diluent Buffer (serum/plasma) or with the corresponding cell culture medium (cell culture supernatant).

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Ms Tau [pS199] Standard to 100,000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 100,000 pg/mL mouse tau [pS199]. **Use the standard within 1 hour of reconstitution.**
2. Add 100 µL Reconstituted Standard to one tube containing 400 µL Standard Diluent Buffer and mix. Label as 20,000 pg/mL mouse tau [pS199].
3. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 10,000, 5,000, 2,500, 1,250, 625, 313, and 0 pg/mL mouse tau [pS199].
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Remaining reconstituted standard should be discarded or frozen in aliquots at –80C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of 1X Assay Buffer. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Prepare 1X Biotin Conjugate solution

Note: Prepare 100 µL 1X Biotin Conjugate solution for each well used in the assay. Use the 1X Biotin Conjugate within 15 minutes of preparation.

1. Dilute appropriate volume of Biotin Conjugate (100X) by 1:100 in 1X Assay Buffer.
2. Return unused Biotin Conjugate (100X) to the refrigerator. Discard 1X Biotin Conjugate solution after use.

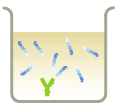
Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

IMPORTANT! Perform all incubation steps on an orbital microplate shaker (set to approximately 400–500 rpm).

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen



a. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. For cell culture medium or buffered solutions, add 50 µL of Standard Diluent Buffer followed by 50 µL of sample (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate 2 hours at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

a. Add 100 µL 1X Ms Tau [pS199] Biotin Conjugate solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 1 hour at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 Add Streptavidin-HRP

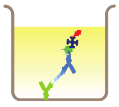
a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

4 Add Substrate Solution

a. Add 100 µL Substrate Solution to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution



Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer (serum/plasma) or with the corresponding cell culture medium (cell culture supernatant) and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Inter-assay precision

Samples were assayed in 8 replicates of 3 independent experiments to determine precision between assays.

Parameters	Sample							
	1	2	3	4	5	6	7	8
Mean (pg/mL)	4144.2	502.1	5357.1	308.4	5277.1	1897.7	320.6	732.4
% Coefficient of Variation	7.6	4.7	1.7	2.8	3.7	3.3	1.3	5.3

Intra-assay precision

Samples of known mouse tau [pS199] concentration were assayed in replicates of 6 to determine precision within an assay.

Parameters	Sample							
	1	2	3	4	5	6	7	8
Mean (pg/mL)	3925.4	474.8	5459.8	304.0	5485.7	1935.6	316.4	709.0
% Coefficient of Variation	6.3	6.4	3.3	6.8	7.3	6.8	3.3	4.6

Standard curve example

The following data were obtained for the various standards over the range of 0 to 20,000 pg/mL mouse tau [pS199].

Standard Mouse Tau [pS199] (pg/mL)	Optical Density (450 nm)
20,000	3.354
10,000	2.172
5,000	1.192
2,500	0.595
1,250	0.298
625	0.156
313	0.080
0	0.063

Sensitivity

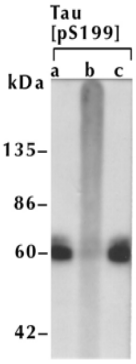
The analytical sensitivity of the assay is 58.5 pg/mL mouse tau [pS199]. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed with 48 replicates.

Specificity

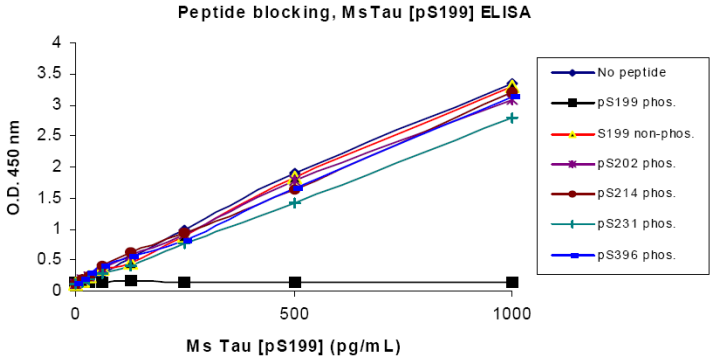
Antibody Specificity

The specificity of the antibody for mouse tau [pS199] is demonstrated by western blot of cell lysates from African green monkey kidney (CV-1) cells, stably expressing human four repeat tau. After protein transfer to nitrocellulose, membranes were either incubated in buffer without the peptide immunogen (a), with the peptide immunogen (b), or the non-phosphopeptide corresponding to the tau phosphopeptide (c). After incubation, the membranes were incubated with 0.50 µg/mL anti-phospho mouse tau [pS199], then visualized using a chemiluminescent detection method.

Only the phosphopeptide corresponding to the site blocks the antibody signal, showing the specificity of the anti-phospho mouse tau [pS199] antibody against the phosphorylation site.



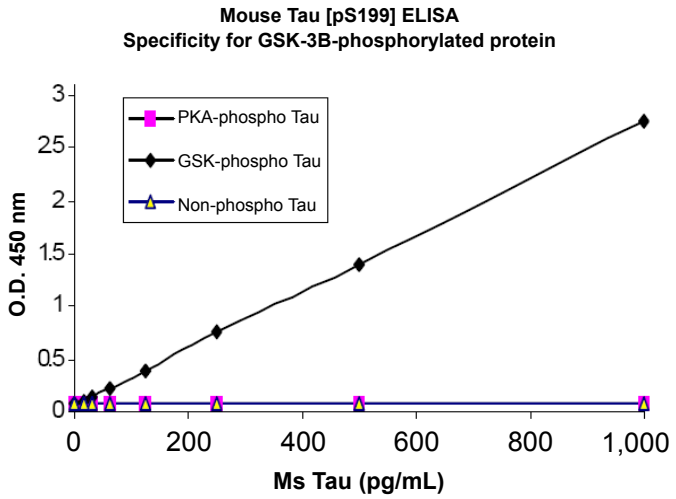
The specificity of this assay for mouse tau [pS199] was confirmed by peptide competition. Phospho-tau was serially diluted and quantitated in the assay as described in the protocol, except the detection antibody was preincubated with 1 µg/mL of pS199 phosphopeptide. The data shows that only the peptide corresponding to the region surrounding serine 199 blocks the ELISA signal.

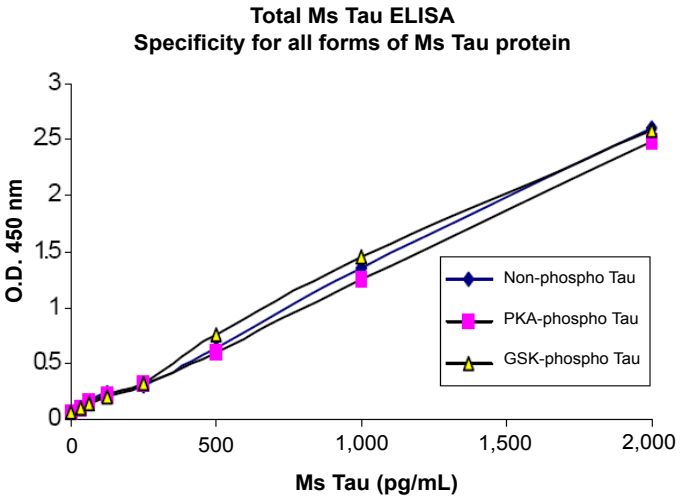


Cross-reactivity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Mouse Tau [pS199] ELISA Kit. The following substances were tested and found to have no cross-reactivity: human β amyloid 1-40, β amyloid 1-42, α-synuclein, β-synuclein, PKA-phosphorylated tau, and non-phosphorylated tau.

Results of assays of the last two proteins are shown in the following graphs. Tau protein was measured using the Mouse Tau [pS199] ELISA Kit (Catalog No. KMB7011) as a control.





Results of the assay with human brain extracts and human SHSY-5Y neuroblastoma cell lysates showed variable reactivity (20–90%) using the Mouse Tau [pS199] ELISA Kit. The kit did not detect tau pS199 in human cerebrospinal fluid (CSF).

Expected values

Sample	Total protein (mg/mL)	Total mouse tau (ng tau/mg protein)	Mouse tau [pS199] (ng [pS199] tau/ng total tau)
Mouse brain homogenates	4.2	60	0.31
Mouse Neuro-2a cell extracts	6.1	15	0.52

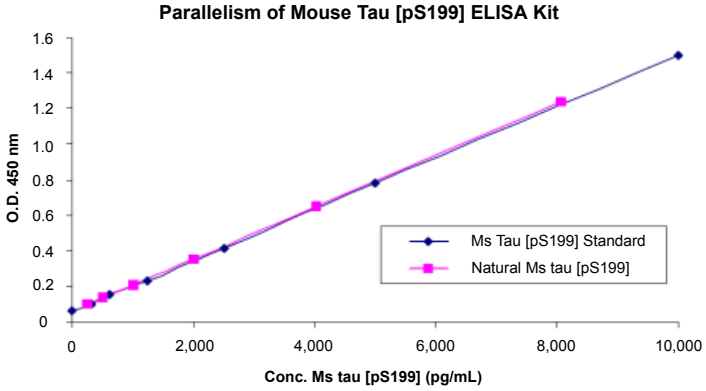
Linearity of dilution

Cell Extraction Buffer and cell culture medium containing 10% fetal calf serum were spiked with recombinant mouse tau [pS199] and serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

Dilution	Cell Extraction Buffer			Culture medium		
	Measured (pg/mL)	Expected		Measured (pg/mL)	Expected	
		(pg/mL)	%		(pg/mL)	%
Neat	19920	—	—	17740	—	—
1/2	9760	9960	98	8540	8860	96
1/4	4780	4980	96	4120	4440	93
1/8	2280	2480	92	2240	2220	101
1/16	1160	1240	93	1160	1100	105

Parallelism

Natural mouse tau [pS199] from Neuro-2a cell lysate was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the standard curve. Parallelism demonstrates that the standard accurately reflects mouse tau [pS199] content in samples.



Recovery

The recovery of mouse tau [pS199] added to various samples was determined using the Mouse Tau [pS199] ELISA Kit.

Sample	Average % recovery
Tissue homogenate buffer (1:10 dilution)	90
Cell Extraction Buffer (1:10 dilution)	107
Cell culture media (with 1% or 10% fetal calf serum)	92

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.