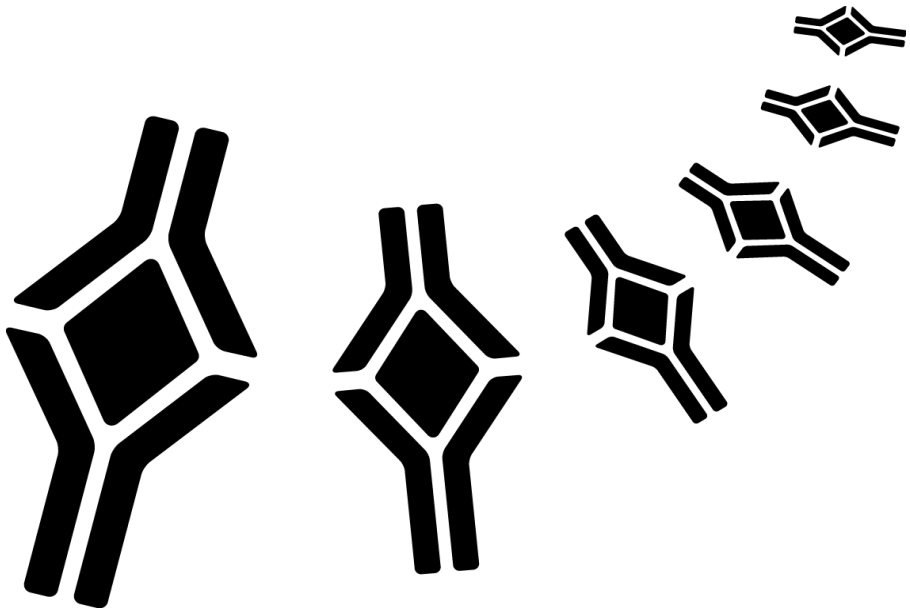
BioVendor
Research
and Diagnostic Products



Mouse Tau (Total) ELISA

Product Data Sheet

Cat. No.: RIG021R

For Research Use Only

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

Mouse Tau (Total) ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of total mouse tau in mouse brain homogenates, cell lysates, buffered solutions, or cell culture media. The assay recognizes both natural and recombinant mouse tau. 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.
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 read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

2. REAGENTS PROVIDED

1 **Antibody Coated Plate,** 96-well plate
2 vials **Mouse Tau (Total) Standard**, lyophilized. Refer to vial label for reconstitution volume
1 bottle (60 ml) **Standard Diluent Buffer;** contains 0.1% sodium azide
1 vial (0,120 ml) **Mouse Tau (Total) Biotin Conjugate (100X)**
1 vial (0, 150 ml) **Streptavidin-HRP (100X)**
1 vial (5 ml) **Assay Buffer (20X)**
1 bottle (50 ml) **Wash Buffer Concentrate (20X)**
1 vial (15 ml) **Stabilized Chromogen, Tetramethylbenzidine (TMB)**
1 vial (15 ml) **Stop Solution**
4 **Adhesive Films**

3. STORAGE INSTUCTIONS – ELISA KIT

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

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Cell Extraction Buffer

5. PRECAUCIONS FOR USE

- IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
- Review the Sample Preparation and Handling in Documents available at Biovendor.com
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

6. PREPARATION OF REAGENTS

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.

7. PREPARATION OF SAMPLES

7.1. Prepare Cell Extraction Buffer

Note: See the Sample Preparation and Handling in Documents available at Biovendor.com for detailed information on preparing Cell Extraction Buffer.

1. 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 Na4P2O7, 2 mM Na3VO4, 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 for each 1 mL of Cell Extraction Buffer.

7.2. 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.

7.3. 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. FOR EXAMPLE, 107 Neuro-2a cells can be extracted in 0.5 mL of Cell Extraction Buffer to recover ~1 mg/mL of total protein. 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).

7.4. Prepare brain homogenate

Note: See the Sample Preparation and Handling in Documents available at Biovendor.com for detailed information on preparing brain homogenates.

1. Weigh out ~100 mg (wet mass) of mouse brain sample in a microcentrifuge tube.
2. Add 8 × the brain mass of cold 5 M guanidine-HCl/50 mM Tris, pH 8.0 by 50−100 µL aliquots. Homogenize thoroughly after each addition.
3. Mix the homogenate at room temperature for 3−4 hours.
4. Dilute the sample ten-fold with cold PBS with 1X protease inhibitor cocktail.
5. Centrifuge at 16,000 × g for 20 minutes at 4°C.
6. Transfer the supernatant into clean microcentrifuge tubes and keep on ice, or store at −80°C.

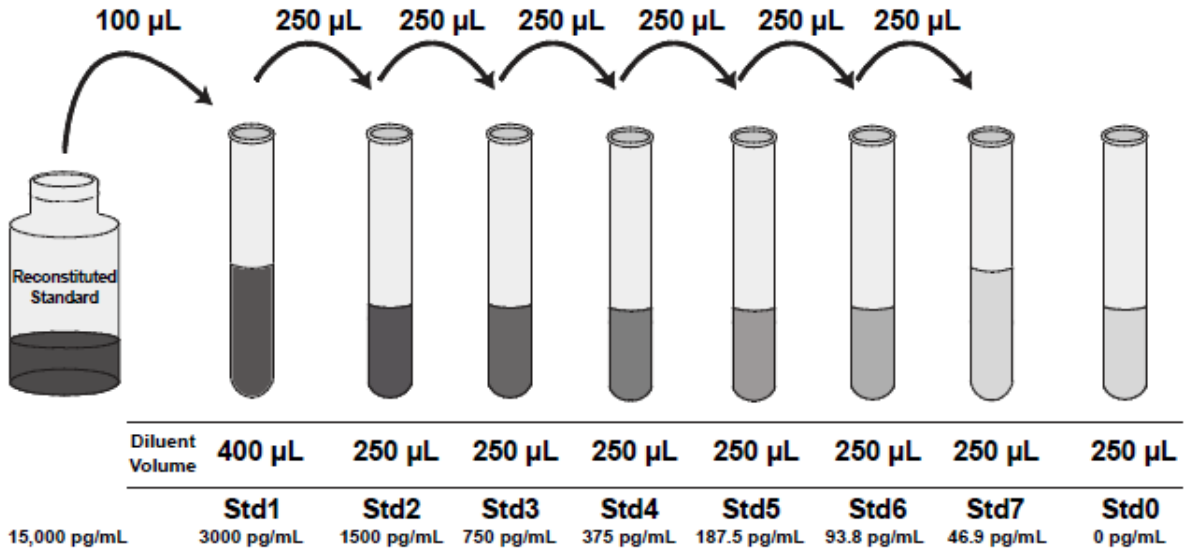
7.5. Pre-dilute samples

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.

- Perform sample dilutions with Standard Diluent Buffer.
- Dilute brain homogenate samples 1:6400 in Standard Diluent Buffer.
- Dilute samples prepared in Cell Extraction Buffer 1:320 in Standard Diluent Buffer.

7.6. Diluted Standards

Note: Use glass or plastic tubes for diluting standards.
1. Reconstitute Ms Tau (Total) Standard to 15,000 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 15,000 pg/mL total mouse tau. 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 3,000 pg/mL total mouse tau.
3. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 1,500, 750, 375, 187.5, 93.8, 46.9, and 0 pg/mL total mouse tau.
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



7.7. 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.

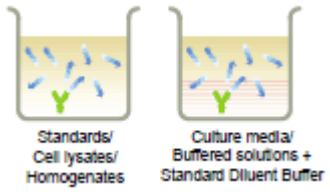
7.8. Prepare 1X Biotin Conjugate solution

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.

8. TEST PROTOCOL

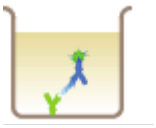
• IMPORTANT! Perform a standard curve with each assay.
• Perform all incubation steps on an orbital microplate shaker (set to approximately 400–500 rpm).
• Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
• Determine the number of 8-well strips required 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.

Bind antigen



a. Add 100 µL of standards, controls, brain homogenate, or cell lysate samples to the appropriate wells. Leave the wells for chromogen blanks empty.
b. For cell culture medium or buffered solution samples, add 50 µL of Standard Diluent Buffer to each well followed by 50 µL of sample.
c. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add Biotin-Conjugate



a. Add 100 µL 1X Ms Tau (Total) 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.

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.

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.

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.

9. CALCULATION OF RESULTS

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 and reanalyze. Multiply the concentration by the appropriate dilution factor.

10. PERFORAMNCE CHARACTERISTICS

Standard curve (example)
The following data were obtained for the various standards over the range of 0 to 3,000 pg/mL total mouse tau.

Standard Mouse Tau (Total) (pg/mL)	Optical Density (450 nm)
3,000	3.618
1,500	2.601
750	1.601
375	0.927
187.5	0.484
93.8	0.263
46.9	0.161
0	0.064

Inter-assay precision

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

Parameters	Sample 1	Sam. 2	Sam. 3	Sam. 4	Sam. 5	Sam. 6	Sam.7	Sam. 8
Mean (pg/mL)	1749.9	2644.8	768.4	201.1	3241.9	398.5	1924.2	99.8
% Coefficient of Variation	6.6	3.9	8.8	2.5	4.0	7.9	8.2	5.9

Intra-assay precision

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

Parameters	Sample 1	Sam. 2	Sam. 3	Sam. 4	Sam. 5	Sam. 6	Sam.7	Sam. 8
Mean (pg/mL)	1834.2	2669.5	761.2	202.6	3102.0	391.1	1875.9	97.1
% Coefficient of Variation	6.2	3.9	6.3	5.5	2.3	6.8	7.7	7.6

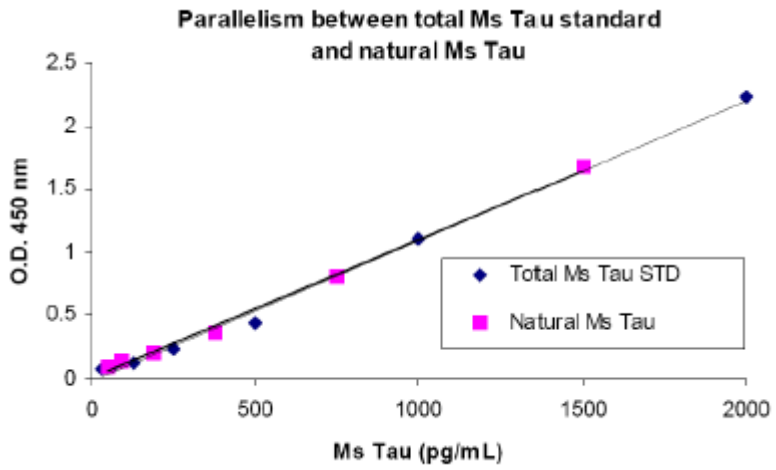
Linearity of dilution

Cell Extraction Buffer and tissue culture medium containing 10% fetal calf serum were spiked with recombinant mouse tau 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			Cell Culture		
	Measured (pg/mL)	Expected (pg/mL)	% Expected	Measured (pg/mL)	Expected (pg/mL)	% Expected
Neat	1,941	—	—	1,986	—	—
1/2	1,031	970	106	1,069	993	108
1/4	506	485	104	523	497	105
1/8	242	243	100	263	248	106
1/16	125	121	103	129	124	104

Parallelism

Endogenous Tau, from mouse brain homogenate, was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the standard curve. The standard accurately reflects natural mouse tau content in samples.



Expected values

Sample	Total protein mg/ml	Total tau (ng tau/mg total protein)
Mouse brain homogenates (1:500)	4.2	60
Mouse Neuro-2a cell line (1:200)	6.1	15

Recovery

The recovery of mouse tau added to various samples, and average recovery was determined using the Mouse Tau (Total) ELISA Kit.

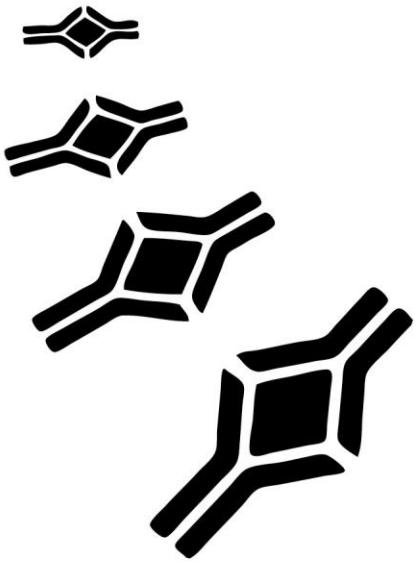
Sample	Average % Recovery
Cell Extraction Buffer	107
Homogenate Buffer (1:10 dilution)	90
Cell culture medium (1% fetal calf serum)	92
Cell culture medium (10% fetal calf serum)	110

Sensitivity

The analytical sensitivity of the assay is 6.5 pg/mL mouse tau. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed with 48 replicates.

Specificity

Buffered solutions of a panel of substances at 20,000 pg/mL were assayed with the Mouse Tau (Total) ELISA Kit. The following substances were tested and found to have no cross-reactivity: human b Amyloid 1–40, b Amyloid 1–42, a-Synuclein, b-Synuclein. Human Tau showed variable reactivity ranging from 48–72% in human brain homogenates and human SHSY-5Y neuroblastoma cell extracts. Human cerebrospinal fluid (CSF) samples showed variable reactivity ranging from 0– 87%.



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