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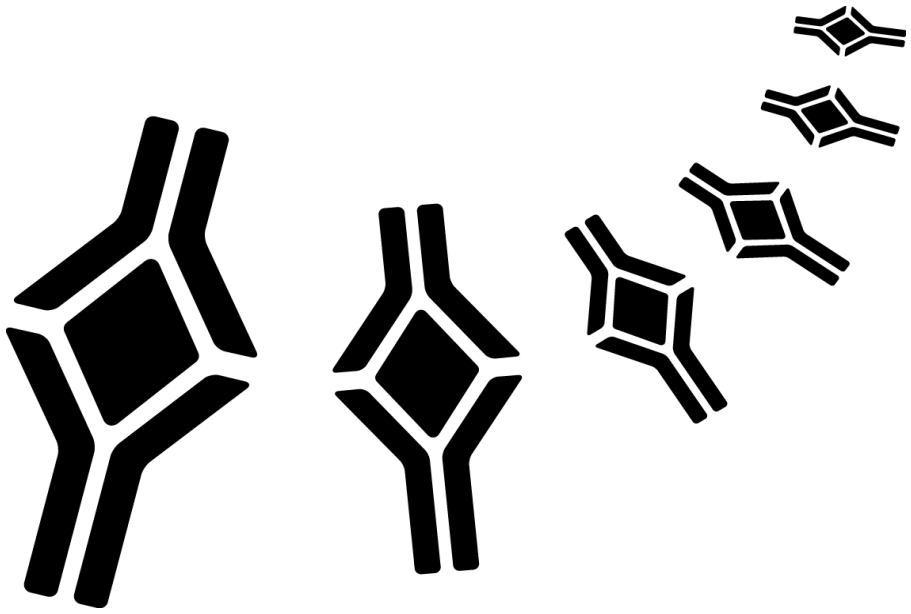

Human Tau (pS199) ELISA

Product Data Sheet

Cat. No.: RIG015R

For Research Use Only

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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

Human 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 human tau (pS199)in human cerebrospinal fluid (CSF), cell lysates, brain homogenates, buffered solution, or cell culture medium. The assay recognizes both natural and recombinant human tau [pS199].
Human tau exists as six different isoforms that result from alternative splicing of a single transcript. The molecular weights of the tau isoforms range from 48 kDa to 68 kDa. Tau protein is highly soluble and normally attached to axonal microtubules, but circulating tau can be detected in cerebrospinal fluid (CSF) under certain conditions.
Tau is regulated though phosphorylation by numerous serine/threonine kinases at sites including serine 199. The hyperphosphorylated form of tau is the major component of paired helical filaments (PHFs).
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 Wells;** 96-well plate
1 vial (11 ml) Human Tau (pS199) **Detection Antibody**. Rabbit anti-Tau [pS199]; contains 0.1% sodium azide
1 vial (0.125 ml) **Anti-Rabbit IgG HRP (100X)**
2 **Human Tau (pS199) Standard**, lyophilized; contains 0.1% sodium azide
1 vial (25 ml) **HRP Diluent;** contains 0.1% Kathon™ CG/ICP
1 vial (25 ml) **Standard Diluent Buffer;** contains 0.1% sodium azide
1 bottle (100 ml) **Wash Buffer Concentrate (25X)**
1 vial (25 ml) **Stabilized Chromogen, Tetramethylbenzidine (TMB)**
1 vial (25 ml) **Stop Solution**
3 **Adhesive Films**

3. STORAGE INSTRUCTIONS – 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 (see "Prepare Cell Extraction Buffer")

5. PRECAUTIONS FOR USE

- IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

6. PREPARATION OF REAGENTS

Preparation of 1x Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 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.

Preparation of Cell Extraction Buffer

Note: See Samples Preparation and Handling 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 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 (e.g., Sigma Cat. No. P-2714) for each 1 mL of Cell Extraction Buffer.

Preparation of 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 human tau [pS199]. 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).

Preparation of Brain homogenate

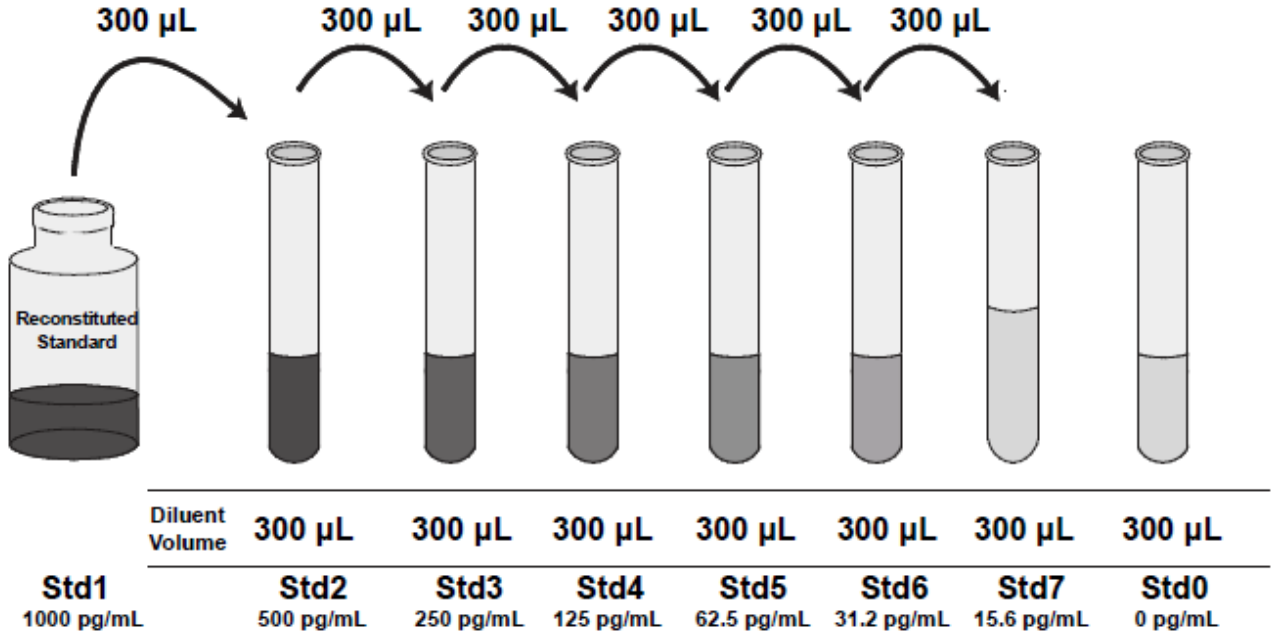
Note: See Samples Preparation and Handling for detailed information on preparing Brain homogenate
1. Weigh out ~100 mg (wet mass) of human brain sample in a microcentrifuge tube.
2. Add 8 × the brain mass of cold 5 M guanidine-HCl/50 mM Tris, pH 8.0 by 50−100 µL aliquots. Homogenize thoroughly after 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 (e.g., Sigma Cat. No. P-2714).
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. Carefully remove the supernatant and keep on ice. Brain tissue extract should be diluted an additional 1:10 - 1:100 with Standard Diluent prior to application in the ELISA.

7. PREPARATION OF SAMPLES

Dilute Standards

Note: Use glass or plastic tubes for diluting standards.
Note: This Human Tau (pS199)Standard was calibrated against the mass of ligand-affinity purified GSK-3b-phosphorylated, recombinant Human Tau-441 protein expressed in E. coli.
1. Reconstitute Human Tau [pS199]Standard to 1000 pg/mL with Standard Dilution 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 1000 pg/mL Human tau [pS199]. Use the standard within 1 hour of reconstitution.
2. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 500, 250, 125, 62.5, 31.2, 15.6, and 0 pg/mL human tau [pS199].
3. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.

4. Discard all remaining diluted standards after completing assay. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Anti-Rabbit IgG HRP solution

Note: Prepare 1X Anti-Rabbit IgG HRP solution within 15 minutes of usage.
1. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
2. Return the unused Anti-Rabbit IgG HRP (100X) solution to the refrigerator.

8. TEST PROTOCOL

- IMPORTANT! Perform a standard curve with each assay.
- 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 to the appropriate wells. For samples and controls, add 50 µL of Standard Diluent Buffer to each well followed by 50 µL of sample. Well(s) reserved for chromogen blanks should be left empty. Tap gently on side of plate to mix.

b) 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.

Add detector antibody

a) Add 100 µL of Human Tau (pS199)Detection Antibody solution into each well except the chromogen blanks. Tap gently on side of plate to mix.
b) Cover the plate with a plate cover and incubate 1 hour at room temperature.
c) Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add IgG-HRP

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

Add Stabilized Chromogen

a) Add 100 µL Stabilized Chromogen 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. PERFORMANCE CHARACTERISTIC

Standard curve (example)
The following data were obtained for the various standards over the range of 0 to 1000 pg/mL Human Tau [pS199].

Standard Human Tau (pS199)(pg/mL)	Optical Density (450 nm)
1000	2.69
500	1.60
250	1.03
125	0.66
62.5	0.44
31.2	0.31
15.6	0.27
0	0.17

Intra-assay precision
Samples of known Human Tau (pS199)concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	45	332	663
Standard Deviation	2.8	8.3	19.6
% Coefficient of Variation	6.1	2.5	3.0

Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	48	333	651.0
Standard Deviation	5.3	12.0	24.1
% Coefficient of Variation	10.2	3.6	3.7

Linearity of dilution

Human CSF and cell culture medium with 10% fetal bovine serum were spiked with human tau (pS199)and serially diluted in Standard

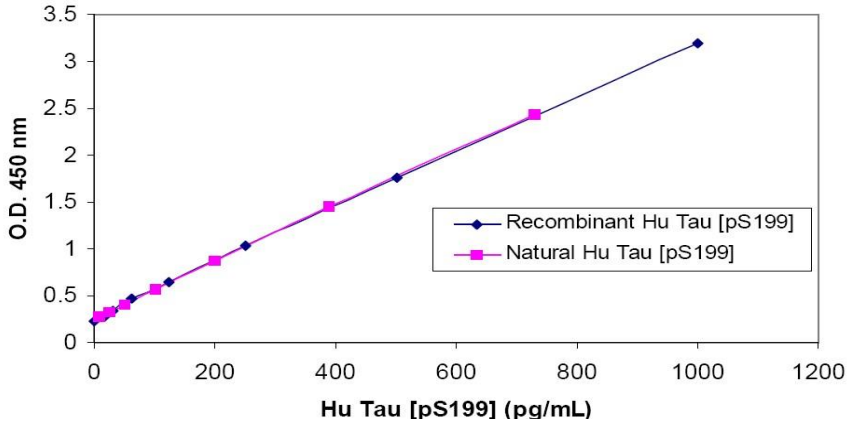
Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

Dilution	CSF			Cell culture medium		
	Measured (pg/mL)	Expected (pg/mL)	%	Measured (pg/mL)	Expected (pg/mL)	%
Neat	721	—	—	795	—	—
1/2	340	360	94	381	398	96
1/4	165	180	92	180	199	91
1/8	83	90	92	95	99	95
1/16	44	45	97	51	50	102

Paralelism

Natural human tau (pS199)from SHSY-5Y cell extract was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the standard curve. The standard accurately reflects the human tau (pS199)content in samples.

Parallelism of Human Tau (pS199)ELISA



Recovery

The recovery of human tau (pS199)added to human cerebrospinal fluid (CSF) or tissue culture medium containing fetal bovine serum (FBS) was measured with the Human Tau (pS199)ELISA Kit.

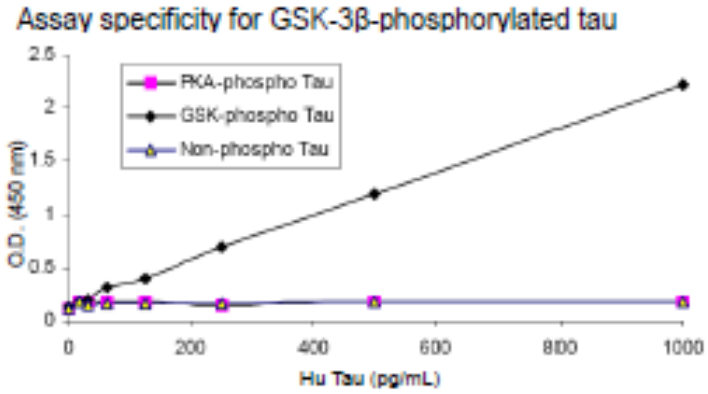
Sample	Avg % Recovery
Human CSF	97
Tissue culture medium + 1% FBS	98
Tissue culture medium + 10% FBS	99.4

Sensitivity

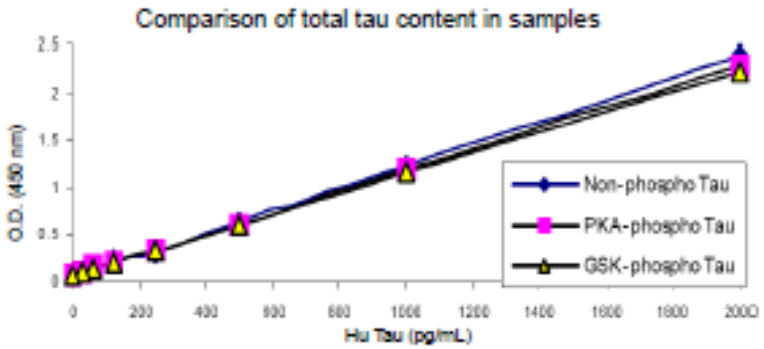
The analytical sensitivity of Hu Tau (pS199)is <2 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

Specificity

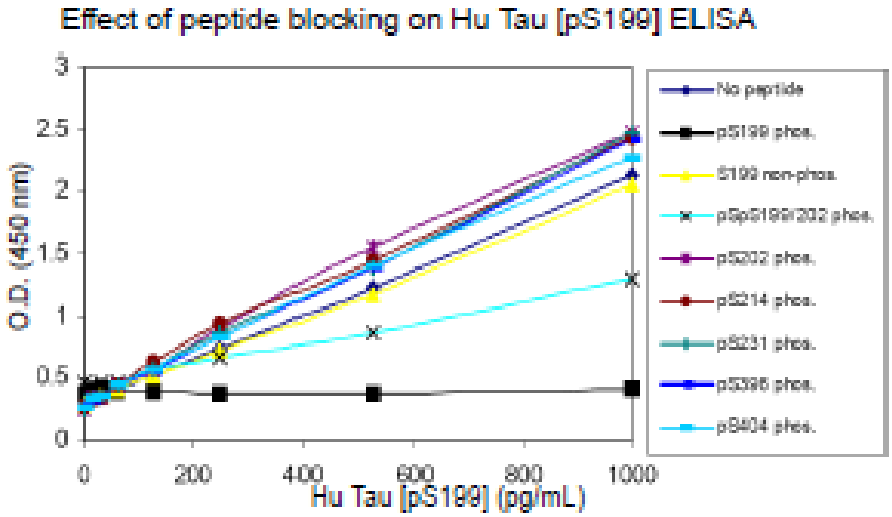
Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Human Tau (pS199)ELISA Kit. The following substances were tested and found to have no cross-reactivity: human b-amyloid 1-40, b-amyloid 1-42, a-synuclein, b-synuclein, PKA-phosphorylated tau, and non-phosphorylated tau. The following data shows specificity of the assay for GSK-3b-phosphorylated tau.



The Tau (Total) ELISA Kit (Cat. No.RIG011R) was used to verify that the total amount of tau in all samples was similar regardless of phosphorylation status.

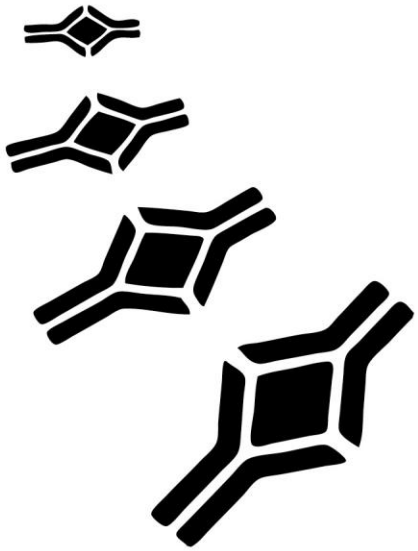


Specificity of the assay for human tau (pS199)was confirmed by peptide competition. The data show that only the phosphopeptide containing the phosphorylated serine blocks the ELISA signal



Expected Values

Sample	Total Protein	Total Tau	Tau (pS199)
CSF (Range)	-	174-1052 pg/ml	-
CSF (Average)	-	579 pg/ml	<2 pg/ml
Brain homogenate (Range)	1.6-7.7 mg/ml	0.5–2.4 ng/µg protein	2.8–176.1 ng/µg tau
Brain homogenate (Average)	2.9 mg/mL	1.3 ng/µg protein	45.7 ng/µg tau
SH5Y lysate (Range)	1.0–1.2 mg/mL	0.059–0.061 ng/µg protein	64.7–78.2 ng/µg tau
SH5Y lysate (Average)	1.1 mg/mL	0.059 ng/µg protein	81.4 ng/µg tau



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