BioVendor
Research
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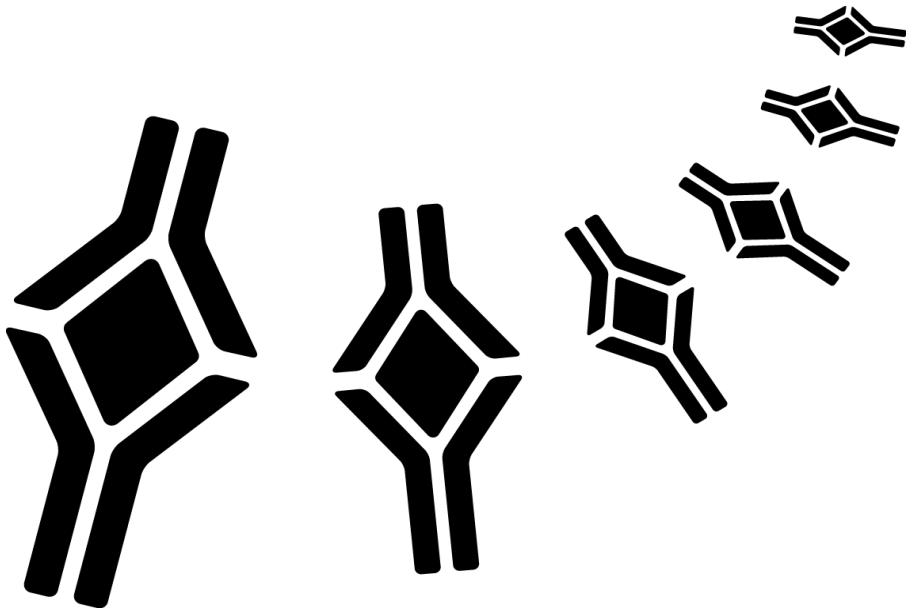


Human Tau (pS396) ELISA

Product Data Sheet

Cat. No.: RIG014R

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

Human Tau (pS396) 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 (pS396) in human cerebrospinal fluid (CSF), buffered solution, or cell culture medium. The assay recognizes both natural and recombinant human tau (pS396).

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. The hyperphosphorylated form of tau (including serine 396 and serine 404) 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 (pS396) **Detection Antibody**; contains 0.1% sodium azide
1 vial (0.125 ml) **Anti-Rabbit IgG HRP (100X)**
2 **Human Tau (pS396) 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)

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.

7. PREPARATION OF SAMPLES

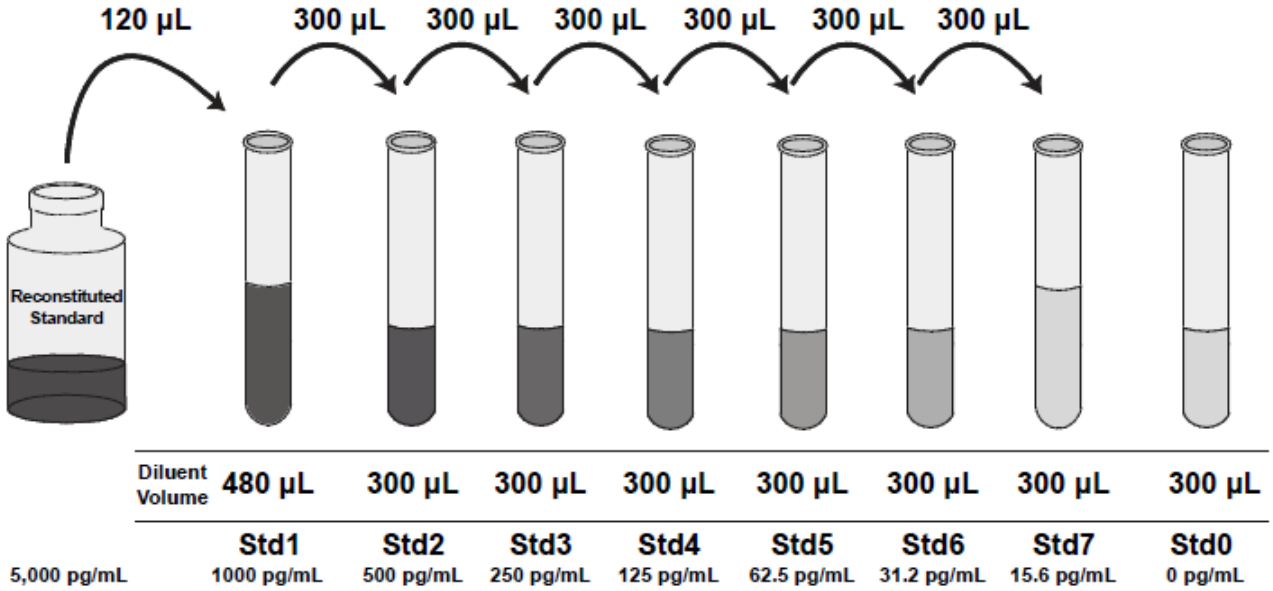
Sample preparation

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-diluted 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.

Diluted Standards

Note: Use glass or plastic tubes for diluting standards.
Note: This Hu Tau (pS396) Standard was calibrated against the mass of ligand-affinity purified GSK-3b-phosphorylated, recombinant Hu Tau-441 protein expressed in E. coli.
1. Reconstitute Hu Tau (pS396) Standard to 5,000 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 5,000 pg/mL human tau (pS396). **Use the standard within 1 hour of reconstitution.**
2. Add 120 µL Reconstituted Standard to a tube containing 480 µL Standard Diluent Buffer and mix. Label as 1,000 pg/mL human tau (pS396).
3. 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 (pS396).
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. 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.
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.

Add detector antibody

a) Add 100 µL of Hu Tau (pS396) Detection Antibody solution into each well except the chromogen blanks.
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 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 1,000 pg/mL human tau (pS396).

Standard Human Tau (pS396) (pg/mL)	Optical Density (450 nm)
1,000	2.64
500	1.49
250	0.71
125	0.37
62.5	0.20
31.2	0.11
15.6	0.07
0	0.03

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)	52	204	597
Standard Deviation	4.0	13.2	27.1
% Coefficient of Variation	7.7	6.5	4.5

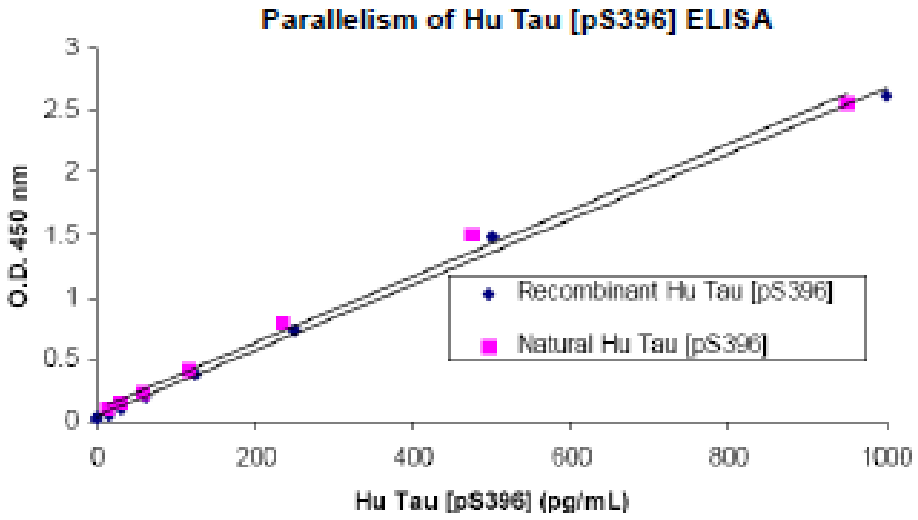
Intra-assay precision
Samples of known human tau (pS396) concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	52	192	612
Standard Deviation	3.0	5.9	23.5
% Coefficient of Variation	5.8	3.1	3.8

Linearity of dilution
Human CSF and cell culture medium were spiked with human tau (pS396) 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		Measured (pg/mL)	Expected	
		(pg/mL)	%		(pg/mL)	%
Neat	823	—	—	846	—	—
1/2	444	412	108	424	423	100
1/4	207	206	100	214	212	101
1/8	108	103	105	113	106	107
1/16	59	52	113	56	53	106

Parallelism
Natural human tau (pS396) from SHSY-5Y cell lysate was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the human tau (pS396) standard curve. The standard accurately reflects the human tau (pS396) content in samples.



Recovery

The recovery of human tau (pS396) added to human cerebrospinal fluid (CSF) or tissue culture medium containing fetal calf serum (FCS) was measured with the Human Tau (pS396) ELISA Kit.

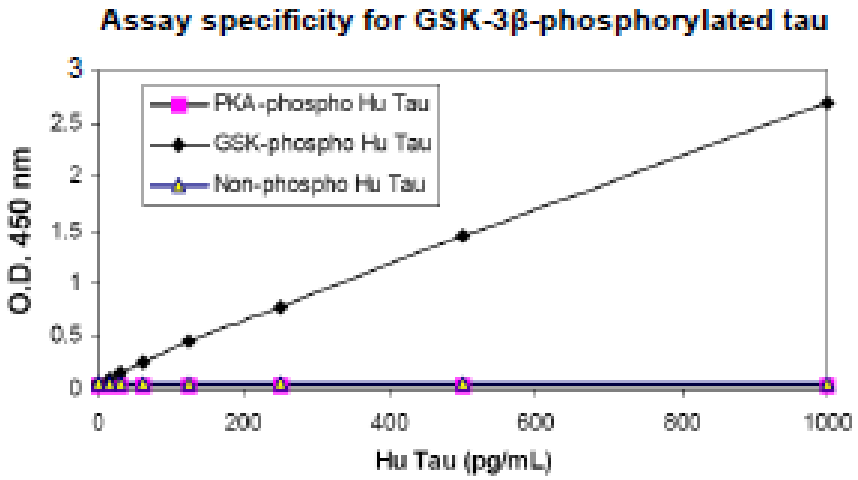
Sample	Average % Recovery
Human CSF	99
Tissue culture medium + 1% FCS	90
Tissue culture medium + 10%FCS	100

Sensitivity

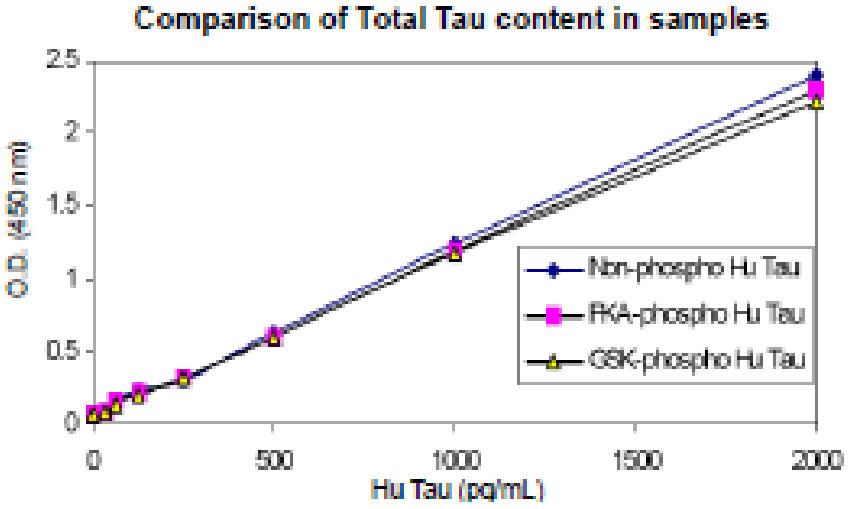
The analytical sensitivity of the assay is <2 pg/mL human tau (pS396).
This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

Specificity

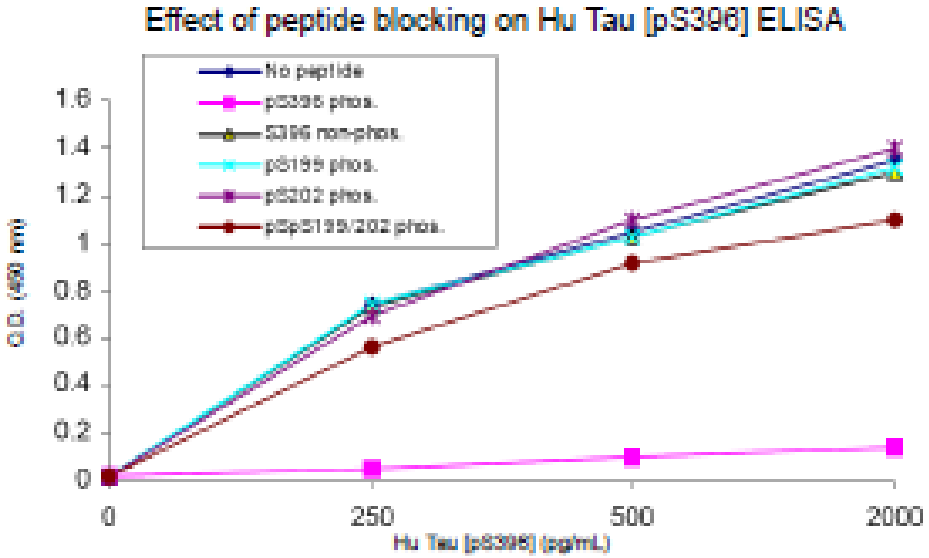
Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Human Tau (pS396) ELISA Kit. The following substances were found to have no cross-reactivity: human b-amyloid 1-40, b-amyloid 1-42, a-synuclein, b-synuclein, PKA-phosphorylated tau, and non-phosphorylated tau. The following data shows specificity of the assay for GSK-3b-phosphorylated tau.

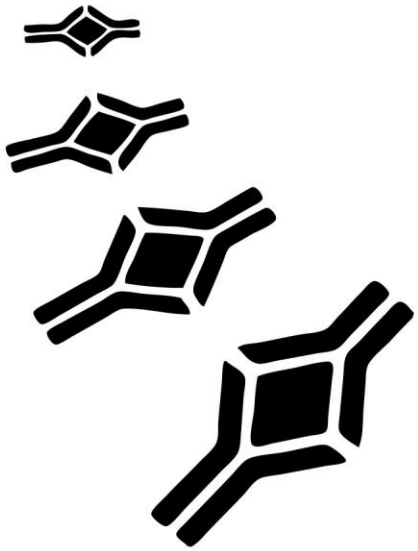


The Tau (Total) ELISA Kit (Cat. No. RIG011R) was used to verify that the total amount of tau in all samples was similar regardless of phosphorylation status.



Specificity of the assay for human tau (pS396) was confirmed by peptide competition. The data show that only the phosphopeptide containing the phosphorylated serine blocks the ELISA signal.


Effect of peptide blocking on Hu Tau [pS396] ELISA
No peptide
pS396 phos.
S396 non-phos.
pS199 phos.
pS202 phos.
pSpS199/202 phos.
O.D. (450 nm)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
250
500
2000
Hu Tau [pS396] (pg/mL)



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