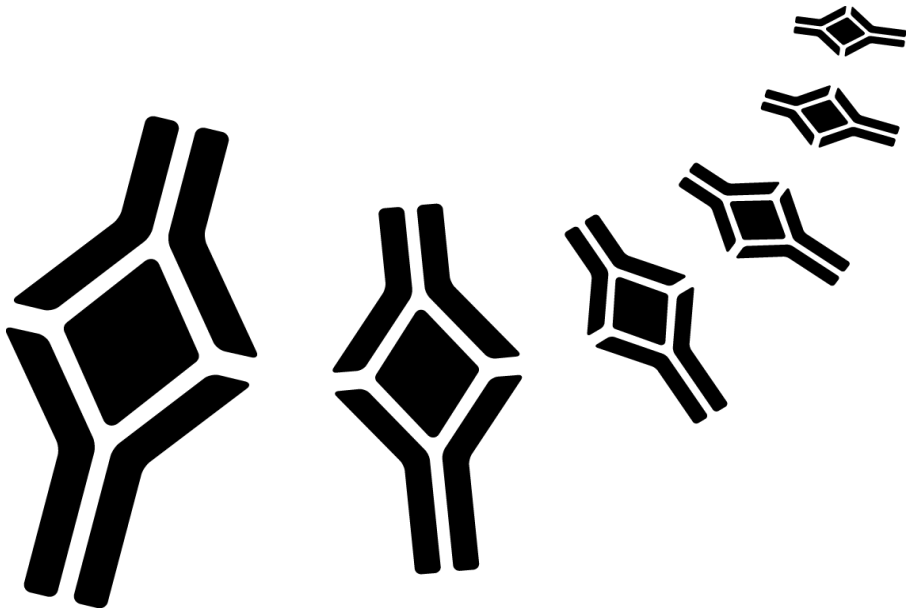
BioVendor
Research
and Diagnostic Products



Human Tau (Total) ELISA

Product Data Sheet

Cat. No.: RIG011R

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 (Total) 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 (total) in human cerebrospinal fluid (CSF), buffered solution, or cell culture medium. The assay will recognize both natural and recombinant human tau (total).
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 **Human Tau Antibody Coated Plate,** 96-well plate
1 vial (11 ml) Human Tau **Biotin Conjugate**; contains 0.1% sodium azide
1 vial (0.15 ml) **Streptavidin-HRP (100X)**
2 vials **Human Tau (Total) Standard**; recombinant Human Tau-352 expressed in E. coli; contains 0.1% sodium azide.; lyophized; Refer to the Quality Control Sheet for reconstitution volume
1 vial (25 ml) **Streptavidin-HRP Diluent; contains 3.3 mM thymol**
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 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)

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

6.1 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.

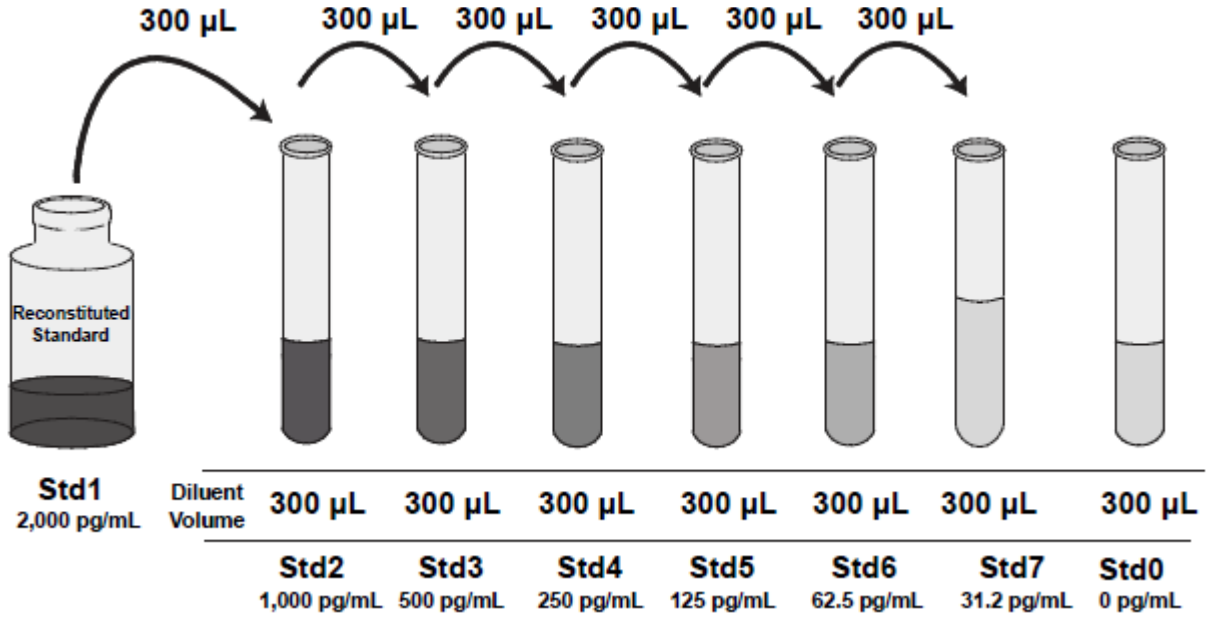
7. PREPARATION OF SAMPLES

Sample preparation

- Refer to the Sample Preparation and Handling in Documents available at Biovendor.com for detailed sample preparation procedures on homogenization of human or transgenic mouse brains.
- 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.

Diluted Standards

Note: Use glass or plastic tubes for diluting standards.
1. Reconstitute Hu Tau (Total) Standard to 2,000 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 2,000 pg/mL human tau (total). Use the standard within 1 hour of reconstitution.
2. Add 300 µL Reconstituted Standard to one tube containing 300 µL Standard Diluent Buffer and mix. Label as 1,000 pg/mL human tau (total).
3. Add 300 µL Standard Diluent Buffer to each of 6 tubes labeled as follows: 500, 250, 125, 62.5, 31.2, and 0 pg/mL human tau (total).
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.



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, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

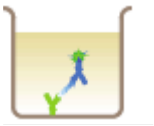
8. TEST PROTOCOL

• 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 Standard Diluent Buffer to zero wells except the chromogen blanks.
b) Add 100 µL of standards to the appropriate wells. For all samples (CSF, buffered solution, cell culture medium, and controls), 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 Hu 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 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. PERFORAMNCE CHARACTERISTICS

Standard curve (example)

The following data were obtained for the various standards over the range of 0 to 2,000 pg/mL human tau (total).

Standard Human Tau (Total) (pg/mL)	Optical Density (450 nm)
2,000	2.96
1,000	1.65
500	0.93
250	0.54
125	0.35
62.5	0.26
31.2	0.20
0	0.15

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)	60	243	994
Standard Deviation	5.9	12.1	44.8
% Coefficient of Variation	9.9	5.0	4.5

Intra-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	58	236	931
Standard Deviation	3.4	7.9	40.8
% Coefficient of Variation	5.9	3.4	4.4

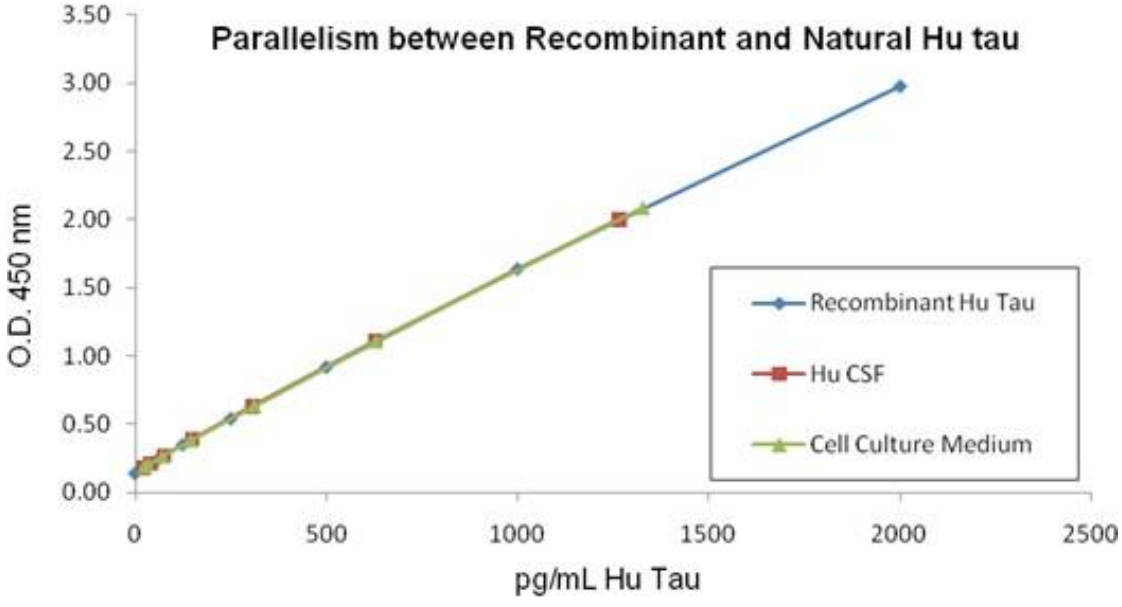
Linearity of dilution

Human CSF and tissue culture medium containing 10% fetal bovine serum were spiked with Human 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 for the spiked Human CSF and 0.96 for the spiked culture medium containing 10% fetal bovine serum.

Dilution	CSF			Cell Culture		
	Measured (pg/mL)	Expected (pg/mL)	% Expected	Measured (pg/mL)	Expected (pg/mL)	% Expected
1/2	1265	—	—	1327	—	—
1/4	629	633	99	630	664	95
1/8	305	314	97	311	315	99
1/16	150	153	98	146	156	94
1/32	75	75	100	71	73	98
1/64	39	38	104	33	36	93

Paralelism

Human CSF and tissue culture medium containing 10% fetal bovine serum were spiked with recombinant human tau (total) and serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the standard curve. The standard accurately reflects Tau content in samples…



Recovery
The recovery of human tau (total) was tested in various sample types.

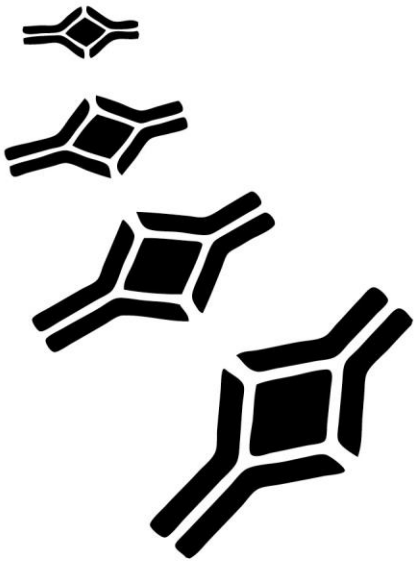
Sample	Average % Recovery
Human cerebrospiral fluid (CSF)	101
Cell culture medium (1% fetal bovine serum)	97
Cell culture medium (10% fetal calf serum)	96

Sensitivity
The analytical sensitivity of the assay is <10 pg/mL human tau (total).
This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 40 times, and calculating the corresponding concentration.

Specificity
Buffered solutions of a panel of substances at 20,000 pg/mL were assayed with the Human Tau (Total) 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.

Cross-reactivity
Human β-Synuclein and Mouse tau measured in mouse brain homogenate showed less than 1.3% cross-reactivity.

Notes



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