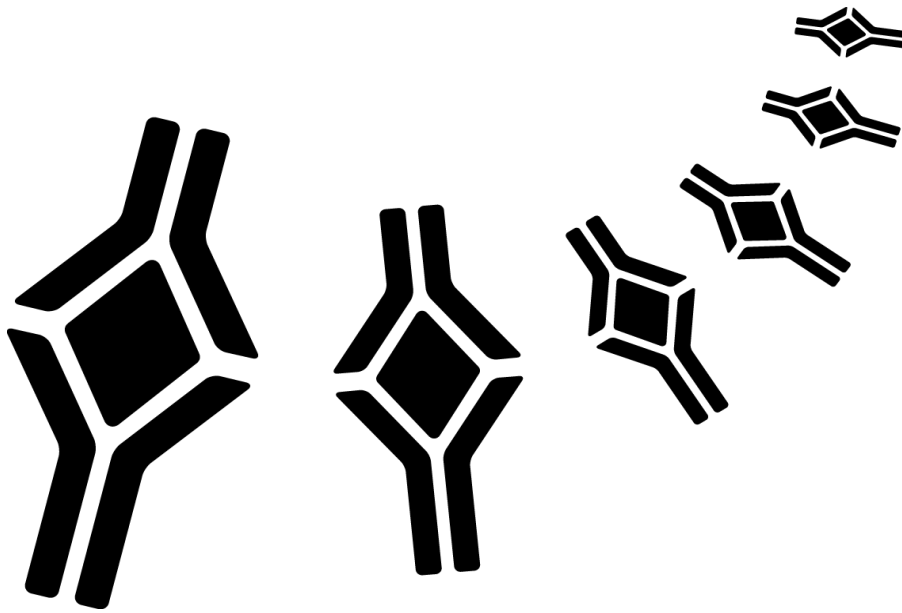


**BioVendor**

Research  
and Diagnostic Products



## Human Tau (pT231) ELISA

Product Data Sheet

Cat. No.: RIG016R

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

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Human Tau (pT231) 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 (pT231) in human cerebrospinal fluid (CSF), buffered solution, or cell culture medium. The assay will recognize both natural and recombinant human tau (pT231).

Human Tau (pT231) is a microtubule-associated protein of considerable importance to neuronal axons of vertebrate brain. Human Tau (pT231) exists as six different isoforms that result from alternative splicing of a single transcript derived from a gene located on chromosome 17. The molecular weights of the tau isoforms range from 48–68 kDa. Human Tau (pT231) protein is highly soluble and normally attached to axonal microtubules. Tau stabilizes the microtubules and makes them rigid. Human Tau (pT231) interacts with actin in the cytoskeleton and neuronal outgrowth, anchors enzymes such as protein kinases and phosphatases, and regulates intracellular vesicle transport.

**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

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- 1 **Antibody-Coated Wells;** 96-well plate
- 1 vial (11 ml) Hu Tau (pT231) **Detection Antibody;** contains 0.1% sodium azide; blue dye[1]
- 1 vial (0.125 ml) **Anti-Rabbit IgG HRP (100X)**
- 2 vials **Human Tau (pT231) Standard,** lyophilized; contains 0.1% sodium azide. Refer to Quality Control Sheet for reconstitution volume
- 1 vial (25 ml) **HRP Diluent;** contains 3.3 mM thymol; yellow dye[1]
- 1 vial (25 ml) **Standard Diluent Buffer;** contains 0.1% sodium azide; red dye[1]
- 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**

[1] To help monitor the addition of reagents to the reaction wells and avoid any pipetting errors, we provide colored Standard Diluent Buffer, Detection Antibody, and HRP Diluent. The colored dye does not interfere with the test results.

### 3. STORAGE INSTRUCTIONS – ELISA KIT

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

### 4. MATERIALS REQUIRED BUT NOT PROVIDED

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- 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 “Sample Preparation and Handling”)

### 5. PRECAUTIONS FOR USE

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- **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

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#### **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 the Sample Preparation and Handling

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<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 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.

## 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  $-80^{\circ}\text{C}$  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 (pT231).

[FOR EXAMPLE, 107 SH-SY5Y cells can be extracted in 0.5 mL of Cell Extraction Buffer to recover 1 mg/mL of total protein. Under these conditions, cell extract dilutions from 1:10 to 1:100 with Standard Diluent Buffer are sufficient for Tau (pT231) detection. 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  $4^{\circ}\text{C}$ .
5. Transfer the supernatant into clean microcentrifuge tubes. Samples can be stored at  $-80^{\circ}\text{C}$  (avoid multiple freeze-thaw cycles).

## 7. PREPARATION OF SAMPLES

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### Pre-diluted samples

Because conditions may vary, we recommend that each investigator determine the optimal dilution for each application.

- Perform sample dilutions with Standard Diluent Buffer.
- Dilute samples prepared in Cell Extraction Buffer 1:50 or greater in Standard Diluent Buffer (e.g., 2  $\mu\text{L}$  sample into 98  $\mu\text{L}$  buffer). This dilution is necessary to avoid SDS interference with the assay.

### Diluted Standards

**Note:** Use glass or plastic tubes for diluting standards.

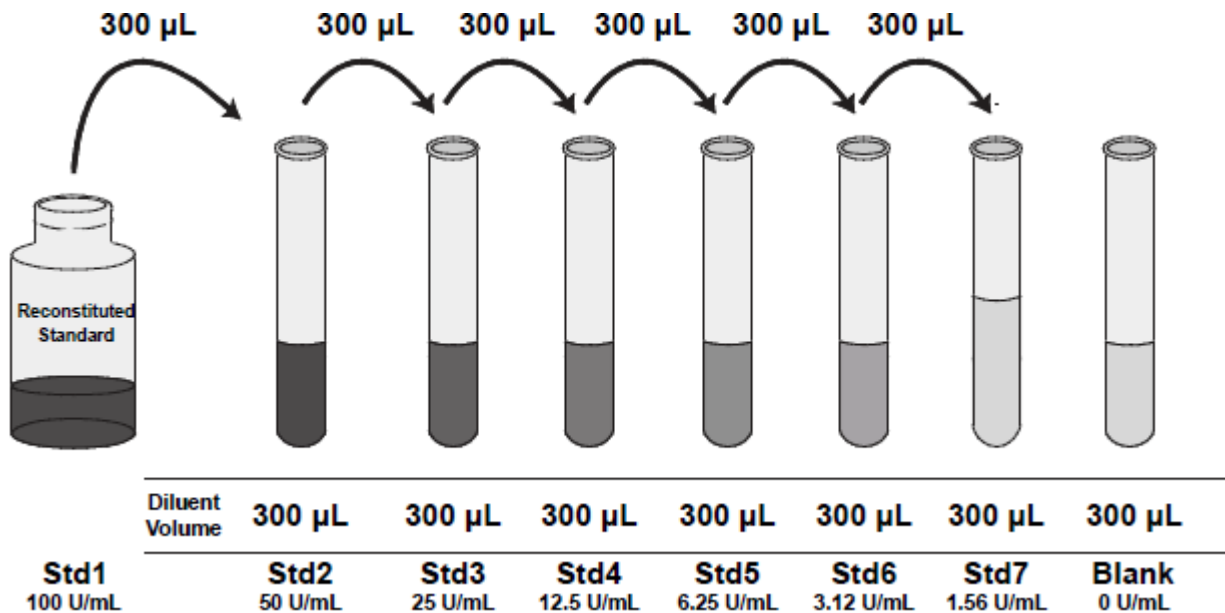
**Note:** The Hu Tau (pT231) was calibrated using recombinant Hu Tau-441 protein expressed in *E. coli* conjugated to phospho-peptide T231.

1. Reconstitute Human Tau (pT231) Standard to 100 U/mL with Standard Diluent Buffer. Refer to the Quality Control Sheet for instructions.

Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 100 U/mL human tau (pT231). Use the standard within 1 hour of reconstitution.

2. Add 300  $\mu\text{L}$  Standard Diluent Buffer to each of 7 tubes labeled as follows: 50, 25, 12.5, 6.25, 3.12, 1.56, and 0 U/mL human tau (pT231).

3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. Remaining reconstituted standard should be discarded or frozen in aliquots at  $-80^{\circ}\text{C}$  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  $\mu\text{L}$  Streptavidin-HRP (100X) 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.

## 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



- Add 100  $\mu\text{L}$  of standards, controls, or samples (see Sample Preparation and Handling) to the appropriate wells. Leave the wells for chromogen blanks empty.
- Cover the plate with a plate cover and incubate 2 hours at room temperature.
- Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

### Add detector antibody



- Add 100  $\mu\text{L}$  of Human Tau (pT231) Detection Antibody solution into each well except the chromogen blanks.
- Cover the plate with a plate cover and incubate 1 hour at room temperature.
- Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

### Add IgG-HRP



- Add 100  $\mu\text{L}$  Anti-Rabbit IgG HRP solution (see page 10) into each well except the chromogen blanks.
- Cover the plate with plate cover and incubate for 30 minutes at room temperature.
- Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

### Add Stabilized Chromogen



- Add 100  $\mu\text{L}$  Stabilized Chromogen to each well. The substrate solution begins to turn blue.
  - 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  $\mu\text{L}$  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 100 U/mL human tau (pT231).

| Standard Human Tau (pT231) (U/ml) | Optical Density (450 nm) |
|-----------------------------------|--------------------------|
| 100                               | 3.46                     |
| 50                                | 2.31                     |
| 25                                | 1.33                     |
| 12.5                              | 0.74                     |
| 6.25                              | 0.43                     |
| 3.12                              | 0.25                     |
| 1.56                              | 0.17                     |
| 0                                 | 0.07                     |

### Inter-assay precision

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

| Parameters                 | Sample 1 | Sample 2 | Sample 3 |
|----------------------------|----------|----------|----------|
| Mean (U/mL)                | 53.92    | 11.73    | 3.29     |
| Standard Deviation         | 4.60     | 0.61     | 0.32     |
| % Coefficient of Variation | 8.53     | 5.22     | 9.60     |

### Intra-assay precision

Samples of known human tau (pT231) concentration were assayed in replicates of 16 to determine precision within an assay.



| Parameters                 | Sample 1 | Sample 2 | Sample 3 |
|----------------------------|----------|----------|----------|
| Mean (U/mL)                | 50.21    | 11.51    | 3.26     |
| Standard Deviation         | 2.38     | 0.69     | 0.17     |
| % Coefficient of Variation | 4.74     | 5.99     | 5.33     |

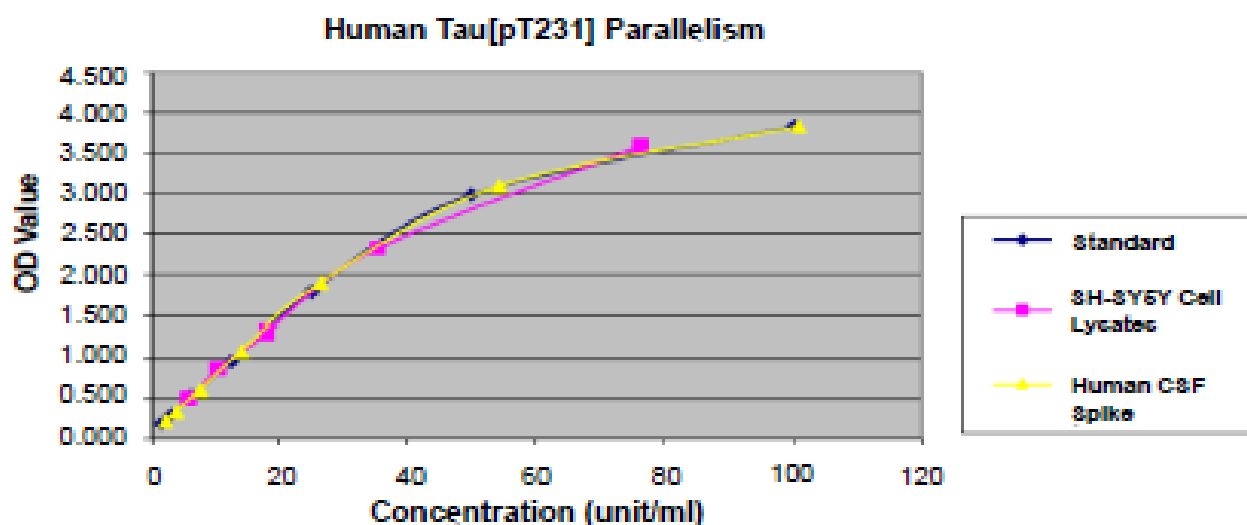
### Linearity of dilution

Human CSF samples (spiked with recombinant human tau (pT231) and human tau (pT231) from SH-SY5Y cell extracts were 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 0.999 in CSF spiked with recombinant human tau (pT231) and 0.997 in SH-SY5Y cell lysate.

| Dilution | CSF             |          |       | SH-SY5Y Cell Lysate |                 |          |       |
|----------|-----------------|----------|-------|---------------------|-----------------|----------|-------|
|          | Measured (U/mL) | Expected |       | Dilution            | Measured (U/mL) | Expected |       |
|          |                 | (U/mL)   | %     |                     |                 | (U/mL)   | %     |
| Neat     | 100.74          | 100.74   | 100   | 1/80                | 76.55           | 76.55    | 100   |
| 1/2      | 54.30           | 50.37    | 107.8 | 1/160               | 35.3            | 38.3     | 92.2  |
| 1/4      | 26.42           | 25.19    | 104.9 | 1/320               | 17.3            | 19.1     | 93.7  |
| 1/8      | 14.07           | 12.59    | 111.7 | 1/640               | 10.3            | 9.6      | 107.7 |
| 1/16     | 7.51            | 6.30     | 119.2 | 1/1280              | 5.8             | 4.8      | 120.6 |
| 1/32     | 3.85            | 3.15     | 122.3 | —                   |                 |          |       |

### Parallelism

Human CSF samples (spiked with recombinant human tau (pT231) and human tau (pT231) from SH-SY5Y cell extracts were serially diluted in Standard Diluent Buffer over the range of the assay. The optical density of each dilution was plotted against the standard curve. The standard accurately reflects the full human tau (pT231) content in samples.



## Recovery

The recoveries of human tau (pT231) added to human CSF and Cell Extraction Buffer were measured on the Human Tau (pT231) ELISA Kit.

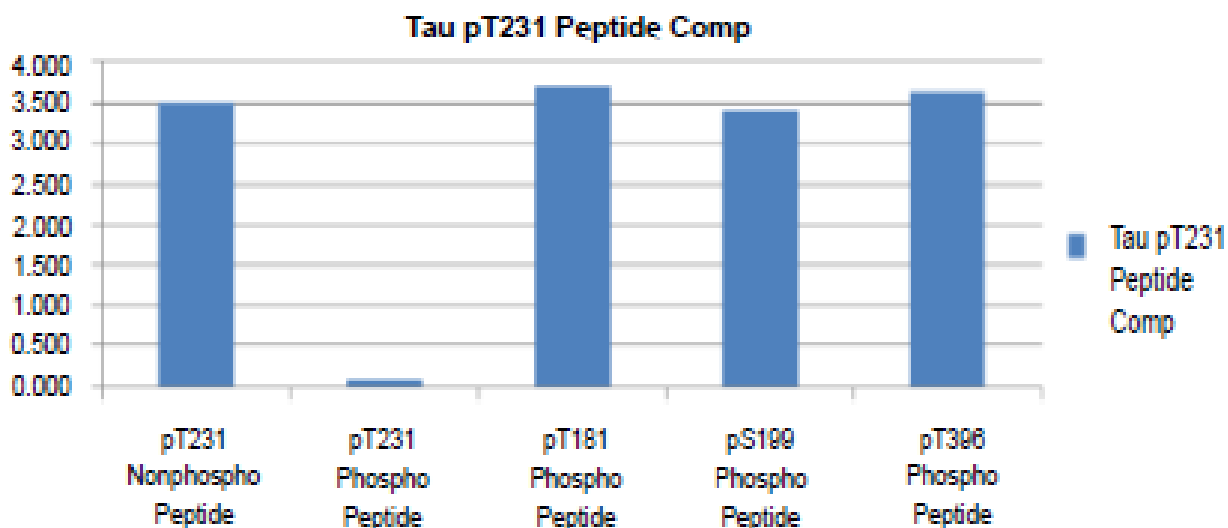
| Sample                 | Average % Recovery |
|------------------------|--------------------|
| Human CSF              | 110                |
| Cell Extraction Buffer | 104                |

## Sensitivity

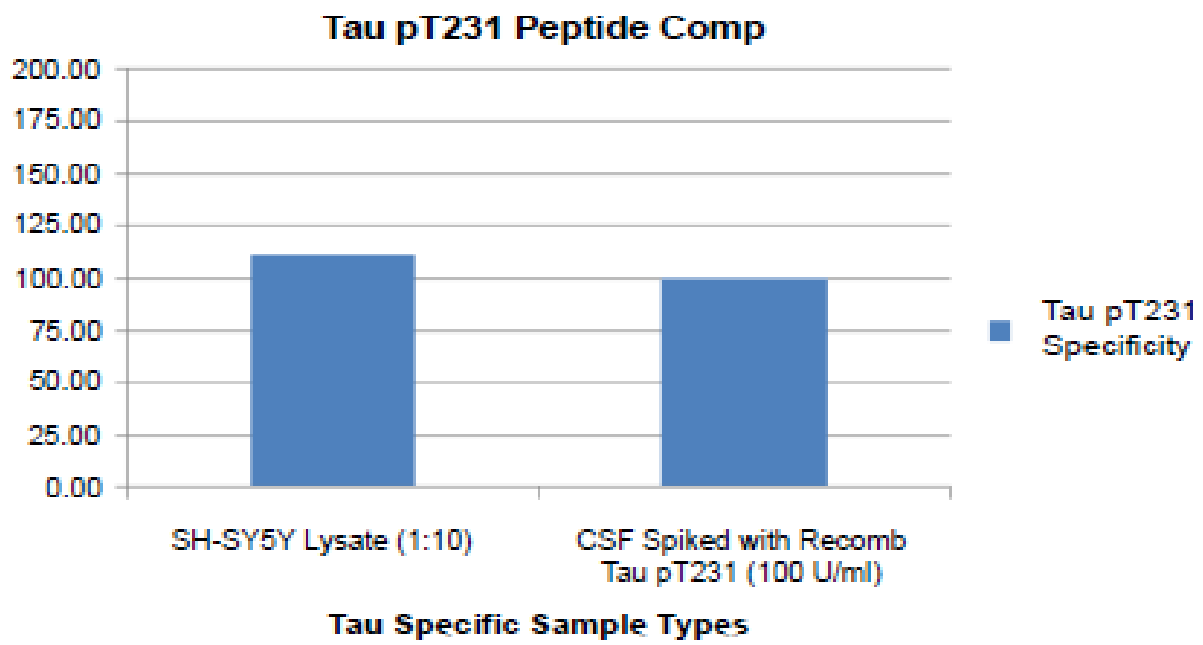
The analytical sensitivity of human tau (pT231) is <1 U/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 48 times over two separate assays.

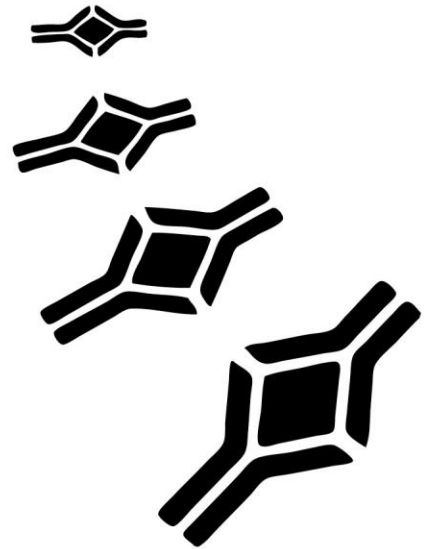
## Specificity

The peptide blocking competition data presented shows that only the phosphopeptide containing phosphorylated threonine 231 can block the ELISA signal. The non-phosphorylated peptide sequence or other phosphopeptides from the Tau sequence did not block the signal.



The Human Tau (pT231) ELISA Kit is suitable for the measurement of human tau (pT231) in different sample matrices. Human CSF and cell extract from neuroblastoma, were analyzed. Human CSF samples were spiked at various concentrations prior to performing assay. The data presented show that the kit detects various concentrations of human tau (pT231) in different sample types.





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