

## Product Information

### Phospho-Tau [pSer<sup>199</sup>] ELISA, Mouse

Product Number CS0440

Storage Temperature 2-8 °C

#### Technical Bulletin

##### Product Description

Phospho-Tau [pSer<sup>199</sup>] ELISA, Mouse is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) for the *in vitro* quantitative determination of mouse Tau [pSer<sup>199</sup>] in buffered solution, cell culture medium, cell extract, or brain homogenate. The assay will recognize both natural and recombinant mouse Tau phosphorylated at [pSer<sup>199</sup>].

A monoclonal antibody specific for Tau, regardless of phosphorylation state, has been coated onto the wells of the multiwell strips provided. Samples, including standards of known mouse Tau content, control specimens, and unknowns, are pipetted into these wells. During the first incubation, the mouse Tau antigen binds to the immobilized (capture) antibody on one site. After washing, a rabbit polyclonal antibody specific for Tau [pSer<sup>199</sup>] is added. During the second incubation, this antibody binds to the immobilized mouse Tau [pSer<sup>199</sup>] captured during the first incubation. After removal of excess second antibody, a horseradish peroxidase-labeled anti-rabbit antibody is added. This binds to the rabbit polyclonal antibody to complete the four-member sandwich. After a third incubation and washing to remove all the excess anti-rabbit HRP, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of mouse Tau [pSer<sup>199</sup>] present in the original specimen.

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

transport. Tau is phosphorylated by numerous serine/threonine kinases, including GSK-3 $\beta$ , PKA, PKC, CDK5, MARK, JNK, p38MAPK and casein kinase II. Tau phosphorylation regulates both normal and pathological functions of this protein. Tau, in its hyperphosphorylated form, is the major component of paired helical filaments (PHFs), the building block of neurofibrillary lesions in Alzheimer's disease (AD). Hyperphosphorylation impairs the microtubule binding function of tau, resulting in the destabilization of microtubules in AD brains, ultimately leading to neuronal degeneration. Deposition of filamentous tau is implicated in other neurodegenerative diseases including cortical basal degeneration (CBD), progressive supranuclear palsy (PSP), Pick's disease, and certain forms of Parkinson's disease.

Phosphorylation of serine 199 is regulated by a number of enzymes. Phosphopeptide mapping shows that serine 199 is phosphorylated by GSK-3 $\beta$  *in vitro* and *in vivo*. Phosphorylated serine 199 of tau can be dephosphorylated by some protein phosphatases including protein phosphatase 2A and 2B. Tau phosphorylation regulates both normal and pathological functions of this protein. For example, phosphorylation of tau by GSK-3 $\beta$  affects the ability of tau to promote microtubule self-assembly. Although the role of the phosphorylation of serine 199 on tau in neurodegenerative diseases remains to be investigated, an over-representation of certain phosphorylated residues on tau, such as serine 396 and 404, have been reported in neurodegenerative disorders including Alzheimer's disease (AD), cortical basal degeneration (CBD), progressive supranuclear palsy (PSP), Pick's disease, and certain forms of Parkinson's disease. Levels of tau and phosphorylated tau are reportedly increased in Alzheimer's disease and other neurodegenerative diseases.

The Phospho-Tau [pSer<sup>199</sup>] ELISA, Mouse is designed for research use only and provides a sensitive method to measure levels of mouse tau phosphorylated on serine 199 in samples of tissue culture supernatants, brain homogenates, or cell extracts.

## Reagents

- **Phospho-Mouse Tau [pSer<sup>199</sup>] Standard, 2 vials, Product No. T 3700** - full-length recombinant mouseTau, expressed in *E. coli*. and phosphorylated *in vitro* with GSK-3 $\beta$  enzyme. Refer to vial label for quantity and reconstitution volume.
- **Standard Diluent Buffer, 25 mL, Product No. S5694** - contains 0.095% sodium azide.
- **Monoclonal Anti-Tau-Coated 96-Well Plate, Product No. T 3075** - A plate using break-apart strips coated with monoclonal antibody specific for full-length mouse Tau (regardless of phosphorylation state).
- **Anti-phospho-Tau [pSer<sup>199</sup>], 11 mL, Product No. T 3325** - A detection antibody, produced in rabbit. Contains sodium azide. Ready to use.
- **Anti-Rabbit IgG- HRP concentrate (100X), 1 vial Product No. I 9158** - contains 3.3 mM thymol and 50% glycerol, viscous. See *Reagent Preparation for handling, dilution and storage instructions*
- **HRP Diluent, 25 mL, Product No. H 5538** - Contains 0.1% Kathon<sup>®</sup> CG/ICP as preservative. Ready to use.
- **Wash Buffer Concentrate (25X), 100 mL, Product No. W 2639** - See *Reagent Preparation for handling, dilution and storage instructions*
- **Stabilized Chromogen, Tetramethylbenzidine (TMB), 25 mL, Product No. S 3318** -Avoid prolonged exposure to light. Avoid exposure to metal. Ready to use.
- **Stop Solution, 25 mL, Product No. S 2818** - Ready to use.
- **Plate Covers, Adhesive strips, 3 each, Product No. P 4870**

## Reagents and Equipment required but not provided

- Multiwell plate reader capable of readings at 450 nm
- Calibrated adjustable precision pipettes for volumes between 5  $\mu$ L and 1,000  $\mu$ L. (A manifold multi-channel pipette is desirable for large assays.)
- Deionized or distilled water.
- Plate washer (optional), use squirt bottle, manifold dispenser, etc.
- Graph paper: linear, log-log, or semi-log, as desired.
- Glass or plastic 1.0 – 1.5 mL tubes for diluting and aliquoting standard.
- Absorbent paper towels to blot the plate.

- Calibrated beakers and graduated cylinders in various sizes.
- Vortex mixer.

## Precautions and Disclaimer

The kit is for R&D use only, not for drug, household or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

## Preparation Instructions

### Sample Preparation

- Samples used in Phospho-Tau [pSer<sup>199</sup>] ELISA, Mouse include mouse brain homogenates, cell extracts, buffered solutions, or cell culture supernatants.
- Samples should be collected in pyrogen/endotoxin-free tubes.
- Samples should be frozen if not analyzed shortly after collection.
- Avoid multiple freeze-thaw cycles of frozen samples.
- Thaw completely and mix well prior to analysis.
- If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
- All samples, standards and controls should be run in duplicate.

### Extraction of proteins from cells

- Cell Extraction Buffer
    - 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<sup>®</sup> X-100
    - 10% glycerol
    - 0.1% SDS
    - 0.5% deoxycholate
    - 1 mM PMSF (stock is 0.3 M in DMSO)  
*PMSF is very unstable and must be added prior to use, even if added previously.*
- Protease inhibitor cocktail (Product. No. P 2714) Add 250  $\mu$ L of reconstituted cocktail per 5 mL of Cell Extraction Buffer.

Stable for 2-3 weeks at 4 °C or for up to 6 months when aliquoted (without protease inhibitors and PMSF added) and stored at -20 °C. *Thaw on ice. Add the protease inhibitors just before use.*

### Extraction Protocol

The recommended Cell Extraction Buffer and protocol are optimized to achieve effective protein phosphorylation. Researchers may use the procedures that work best in their hands. They will have to assay their lysates for the satisfactory extraction and/or phosphorylation

1. Collect cells in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent).
2. Wash twice with cold PBS.
3. Remove and discard the supernatant and collect the cell pellet. (At this point the cell pellet can be frozen at  $-70^{\circ}\text{C}$  and lysed at a later date).
4. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes on ice with vortexing at 10-minute intervals. The volume of Cell Extraction Buffer depends on the cell number in cell pellet and expression level. For example,  $10^7$  mouse Neuro-2a cells can be extracted in 0.5 mL of Cell Extraction Buffer to recover approximately 1 mg/mL of total protein.
5. Transfer extracts to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at  $4^{\circ}\text{C}$ .
6. Aliquot the clear lysate to clean microcentrifuge tubes. Lysates can be stored at  $-70^{\circ}\text{C}$ .
7. Under these conditions, cell extract dilutions from 1:10 to 1:400 in Standard Diluent Buffer are sufficient for detection of Tau in this ELISA.

### Homogenization of brain tissue

#### Buffers:

Buffer A: 5 M guanidine HCl/50 mM Tris-HCl, pH 8.0

Buffer B: 1X PBS Buffer supplemented with 1X protease inhibitor cocktail (Prod. No. P 2714).

#### Protocol for homogenization

1. Determine the wet mass of the mouse brain sample (~ 100 mg) in an Eppendorf tube.
2. Add 8X mass of cold Buffer A (5 M guanidine HCl/50 mM Tris-HCl) to the tube in 50 - 100  $\mu\text{L}$  aliquots and grind thoroughly with a hand-held motor (Fisher: K749540-0000) after each addition.
3. (Optional: transfer the homogenate from above to 1 mL Dounce homogenizer and homogenize thoroughly.)
4. Mix the homogenate at room temperature for three to four hours. The sample is stable and can be freeze-thawed many times at this stage.
5. Dilute the sample ten fold with Buffer B (cold PBS with 1X protease inhibitor cocktail). Centrifuge at 16,000 x g for 20 minutes at  $4^{\circ}\text{C}$ .

6. Carefully remove the supernatant and keep on ice.
7. Brain tissue extract should be diluted 1:10 to 1:1000 with Standard Diluent Buffer prior to application in the ELISA.
8. Prepare the standard curve in a diluent matrix that contains the same concentration of extraction buffer.

### Optional protocol

Homogenization can be performed with cold 4X volume of PBS supplemented with the 1X protease inhibitor cocktail, followed by the addition of guanidine HCl to make the final concentration 5 M with 8.2 M guanidine HCl/82 mM Tris-HCl, pH 8.0.

### Reagents Preparation

#### **Phospho Mouse Tau [pSer<sup>199</sup>] Standard**

1. The standard is a full-length recombinant mouseTau, calibrated against the mass of a highly purified GSK-3 $\beta$  phosphorylated, recombinant Tau protein expressed in *E. coil*.
2. Reconstitute one vial of Standard to 5,000 pg/mL with Standard Diluent Buffer. Refer to Standard vial label for reconstitution volume.
3. Mix gently and wait 10 minutes to ensure complete reconstitution.
4. Label as 5,000 pg/mL
5. Prepare serial standard dilutions as follows:

#### **Dilution of Phospho Mouse Tau [pSer<sup>199</sup>] Standard**

Tube#	Standard Diluent Buffer	Standard from tube #:	Final Phospho-Mouse Tau [pSer <sup>199</sup> ] pg/mL
1	Reconstitute according to label instructions		5,000 pg/mL
2	0.480 mL	0.120 mL (1)	1000
3	0.300 mL	0.300 mL (2)	500
4	0.300 mL	0.300 mL (3)	250
5	0.300 mL	0.300 mL (4)	125
6	0.300 mL	0.300 mL (5)	62.5
7	0.300 mL	0.300 mL (6)	31.2
8	0.300 mL	0.300 mL (7)	15.6
9	0.300 mL	-	0

Mix thoroughly between steps.

6. Use within 1 hour of reconstitution
7. Discard all remaining standard dilutions.

### Anti-Rabbit IgG-HRP Concentrate (100X)

Contains 50% glycerol and is very viscous. To ensure accurate dilution follow instructions below:

1. Equilibrate to room temperature, mix gently, pipette slowly.
2. Remove excess concentrate solution from pipette tip with clean absorbent paper.
3. Mix: 10  $\mu$ L IgG-HRP concentrate + 1 mL HRP Diluent (sufficient for one 8-well strip, prepare more as needed)
4. Label as **Anti-Rabbit IgG-HRP Working Solution**

# of 8-Well Strips	Volume of Anti-Rabbit IgG-HRP Concentrate	Volume of Diluent
2	20 $\mu$ L	2 mL
4	40 $\mu$ L	4 mL
6	60 $\mu$ L	6 mL
8	80 $\mu$ L	8 mL
10	100 $\mu$ L	10 mL
12	120 $\mu$ L	12 mL

Return the unused Anti-IgG-HRP concentrate to the refrigerator.

### Wash Buffer

1. Equilibrate to room temperature and mix to redissolve any precipitated salts.
2. Mix 1 volume Wash Buffer Concentrate 25X + 24 volumes of deionized water
3. Label as **Working Wash Buffer**.
4. Store both the concentrate and the Working Wash Buffer in the refrigerator. Use within 14 days.

### Storage/Stability

All components of this kit are stable at 2 to 8 °C. Any unused reconstituted standard should be discarded or frozen at -70 °C. Standard can be frozen and thawed one time only without loss of immunoreactivity.

Refer to the Certificate of Analysis for kit shelf life. To obtain C of A go to [www.sigma-aldrich.com](http://www.sigma-aldrich.com)

### Procedure

#### Precautions

- 20-30 minutes before use equilibrate kit and all reagents to room temperature (15-30 °C).
- Use only the coated 96 well capture plate provided with the kit

- Multiwell plate: equilibrate to room temperature in unopened foil bag. Remove desired number of strips, reseal the bag and refrigerate at 2 – 8 °C to maintain plate integrity.
- When not in use all kit components should be refrigerated.
- Assay all standards, controls and samples in duplicate.
- If particulate matter is present, centrifuge or filter prior to analysis.
- A standard curve must be run with each assay
- Maintain a consistent order of component and reagent addition from well to well. This ensures equal incubation times for all wells.
- Run in-house controls with every assay. If control values fall outside pre-established ranges, the accuracy of the assay may be suspect.
- All reagents are lot-specific. Do not mix reagents from different kit lots.
- Do not use reagents after the kit expiration date.
- Standards and samples can be made up in either glass or plastic tubes.
- Pre-rinse the pipette tip with the reagent and use fresh pipette tips for each sample, standard or reagent.
- Read absorbances within 2 hours of assay completion.

#### Washing directions

- The purpose of washing is to remove unbound proteins and other non-specific parts of lysate.
- Incomplete washing will adversely affect the assay and render false results.
- Use only Wash Buffer provided in kit.
- Washing may be performed using automated washer, manifold pipette or squirt bottle.
- Wash cycle four times, blotting as dry as possible after the 4<sup>th</sup> wash.
- When washing manually, fill wells with Wash Buffer, aspirate thoroughly and tap dry on absorbent tissue.

Note: It is recommended to use laboratory tape to hold plate strips to the plate frame while performing the plate washing and drying procedure to avoid strips coming free of the frame.

## Assay Procedure

### **Phospho-Tau [pSer<sup>199</sup>] ELISA, Mouse Assay Summary**

- 1) 100 mL of Standards or Samples (samples diluted 1:10 in Standard Diluent Buffer)**

**Incubate 2 hours at RT  
aspirate and wash 4x**

- 2) Add 100 mL Anti-phospho-Tau [pSer<sup>199</sup>]  
Incubate 1 hour at RT.  
aspirate and wash 4x**

- 3) Add 100 mL Anti-Rabbit IgG-HRP  
Incubate 30 min at RT.  
aspirate and wash 4x**

- 4) Add 100 mL Stabilized Chromogen  
Incubate 30 minutes at RT  
(in the dark).**

- 5) Add 100 mL of Stop Solution  
Read at 450nm.**

#### **Total Assay Time - 4 hours**

- Determine the number of wells for the assay run, including 2 zero wells, 2 chromogen blank wells, 14 standard dilution wells and 2 wells for each sample to be assayed.
- Remove appropriate number of multiwell strips and return the unused strips to the pouch. Reseal pouch

#### 1<sup>st</sup> incubation

- Add 100 µL Standard Diluent to zero wells.
- Add 100 µL standards, samples or controls to the appropriate wells.
- Samples prepared in cell extraction buffer or brain homogenate buffer must be diluted 1:10 or greater in *Standard Diluent Buffer* (for example, 10 µL sample plus 90 µL buffer). *The dilutions should be optimized for each assay.*
- Cell culture supernatants or buffered solutions; dilute 1:2 in *Standard Diluent Buffer* (50 µL buffer + 50 µL sample).
- Tap gently on the plate to mix, cover with Plate Cover and incubate 2 hours at room temperature
- Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells for a total of 4 times following washing instructions.

#### 2<sup>nd</sup> incubation

- Add 100 µL Anti-Phospho-Tau [pSer<sup>199</sup>] detection antibody to all wells except chromogen blanks.
- Tap gently on the plate to mix, cover with Plate Cover and incubate 1 hour at room temperature.
- Wash wells for a total of 4 times following washing instructions.
- After the final wash blot dry on a lint free paper towel to remove any remaining wash buffer.

#### 3<sup>rd</sup> incubation

- Add 100 µL Anti-Rabbit IgG-HRP Working Solution to each well except the chromogen blanks.
- Cover with Plate Cover and incubate 30 minutes at room temperature.
- Wash wells for a total of 4 times following washing instructions.
- After the final wash blot dry on a lint free paper towel to remove any remaining wash buffer.

#### Substrate incubation

- Add 100 µL of Stabilized Chromogen into all wells. *The liquid in the wells will begin to turn blue.*
- Do not cover the plate
- Incubate approximately 30 minutes at room temperature in the dark (*place plate in a drawer or cabinet*).

*Note: If your multiwell plate reader does not register optical density (OD) above 2.0, incubate only 20-25 minutes.*

#### Stop reaction

- Add 100 µL of Stop Solution to each well. This stops the reaction
- Tap gently to mix. *The solution will turn yellow.*

#### Absorbance reading

- Any commercially available multiwell plate reader capable of reading at OD 450 nm may be used.
- Blank the plate reader against the Chromogen Blank wells (containing Chromogen and Stop Solution).
- Read the absorbance of the entire plate at 450 nm within 2 hours after addition of Stop Solution.

#### **Results**

- The results may be calculated using any immunoassay software package.
- The four-parameter algorithm provides the best curve fit.
- If the software program is not readily available, the concentrations of Tau may be calculated manually.

4. Calculate the Average Net OD (average reading of 2 wells) for each standard dilution and samples as follows:
5. Average Net OD = Average Bound OD – Average Chromogen Blank OD
6. On graph paper plot the Average Net OD of standard dilutions against the standard concentration (pg/mL) of Tau. Draw the best curve through these points to construct the standard curve.
7. The Tau concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
8. Multiply the values obtained for the samples by dilution factor of each sample.
9. Samples producing signals higher than the 1000 pg/mL standard should be further diluted and assayed again.

- The immunoassay literature contains frequent references to aberrant signals, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

### Performance characteristics

#### Sensitivity

The minimum detectable dose of Phospho-Tau [pSer<sup>199</sup>] is <7.4 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

#### Precision

##### 1. Intra-Assay Precision

Samples of known Phospho-Tau [pSer<sup>199</sup>] concentration were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	47	160	560
Standard Deviation (SD)	2.6	9.6	28.1
% Coefficient of Variation	5.6	6.0	5.0

##### 2. Inter-Assay Precision

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

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	45	169	560
Standard Deviation (SD)	3.4	12.2	27.9
Coefficient of Variation %	7.4	7.2	5.1

#### Linearity of Dilution

Cell Extraction Buffer and tissue culture medium containing 10% fetal calf serum were spiked with recombinant mTau [pSer<sup>199</sup>] 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.

### Product Profile

#### Typical Results

The standard curve below is for illustration only and **should not be used** to calculate results in your assay. Run standard curve in each assay.

Standard Phospho-Tau [pSer <sup>199</sup> ] pg/mL	Optical Density 450 nm
0	0.064
15.6	0.096
31.2	0.120
62.5	0.198
125	0.328
250	0.619
500	1.270
1000	2.540

#### Limitations:

- Do not extrapolate the standard curve beyond the 1000 pg/mL standard point.
- The dose response is non-linear in this region and accuracy will be compromised.
- Other buffers and matrices have not been investigated.
- The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of cell extract and brain homogenate samples have not been thoroughly investigated.
- The rate of degradation of native Tau in various matrices has not been investigated.

Dilution	Cell Extraction Buffer			Cell Culture		
	Measured pg/mL	Expected pg/mL	% Expected	Measured pg/mL	Expected pg/mL	% Expected
Neat	996			887		
1:2	488	498	98	427	443	96
1:4	239	249	96	206	222	93
1:8	114	124	92	112	111	101
1:16	58	62	93	58	55	105

### Recovery

- The recovery of mTau [pSer<sup>199</sup>] homogenate buffer (1:10 dilution) averaged 90%.
- The recovery of mouseTau [pSer<sup>199</sup>] added to cell extraction buffer (1:10 dilution) averaged 107%.
- The recovery of mouse Tau [pSer<sup>199</sup>] added to tissue culture medium containing 1% fetal calf serum averaged 92%.

### Parallelism

Natural mouseTau [pSer<sup>199</sup>], from mouse Neuro 2a cells, was serially diluted in *Standard Diluent Buffer*. The optical density of each dilution was plotted against the standard curve. Parallelism between the natural and recombinant protein was demonstrated by the figure below and indicated that the standard accurately reflects natural mouse Tau [pSer<sup>199</sup>] content in samples.

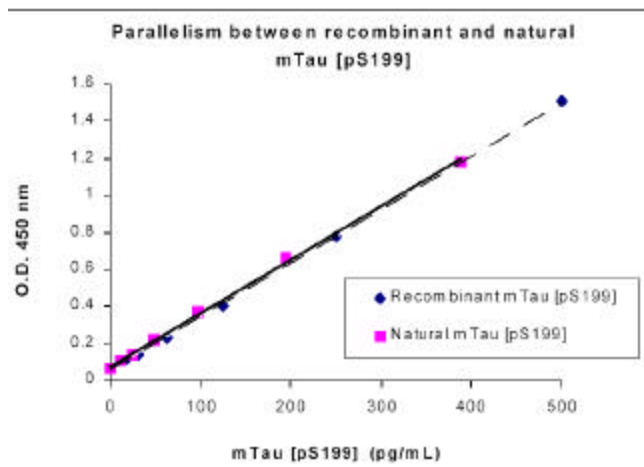


Figure 1

### Antibody Specificity

- Cell extracts from African green monkey kidney (CV-1) cells, stably expressing human four repeat tau, were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF.
- Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
- After blocking, membranes were preincubated with different peptides as follow:  
Lane a no peptide  
Lane b immunogen  
Lane c non phosphorylated peptide corresponding to the immunogen.

- After preincubation membranes were incubated with 0.50 µg/mL anti-phospho Tau [pSer<sup>199</sup>], for two hours at room temperature in a 3% BSA-TBST buffer.
- After washing, membranes were incubated with goat F(ab')<sub>2</sub> anti-rabbit IgG alkaline phosphatase and signals were detected.

The data in Figure 2 show that only phosphopeptide corresponding to this site blocks the antibody signal, illustrating the specificity of the anti-Tau [pSer<sup>199</sup>] antibody for this phosphorylation site.

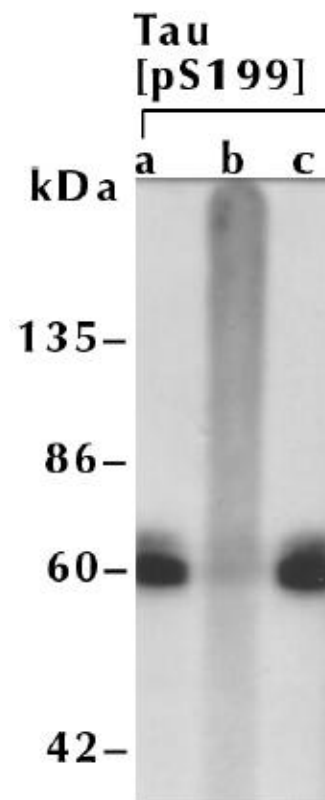


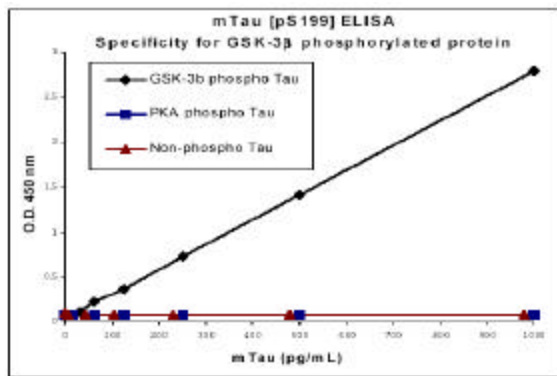
Figure 2

**Kit specificity and Application**

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Phospho-Tau [pSer<sup>199</sup>] ELISA, Mouse. The following substances were tested and found to have no cross-reactivity:

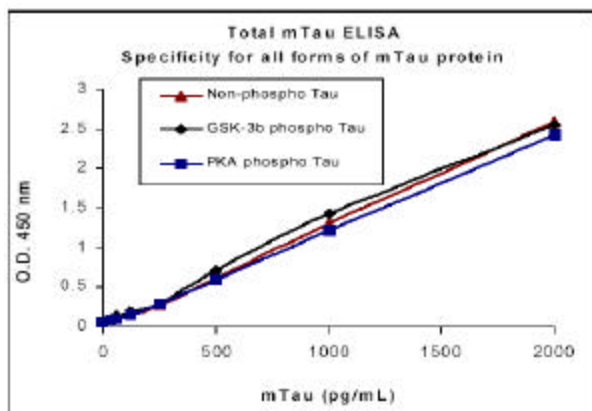
- Human β amyloid 1-40
- β amyloid 1-42,
- α-synuclein
- β-synuclein
- PKA-phosphorylated Tau,
- non-phosphorylated Tau.

These last two proteins are depicted graphically below (Figure 3).



**Figure 3**

As control, each of the Tau proteins was readily measured by the Mouse Tau ELISA (Figure 4).



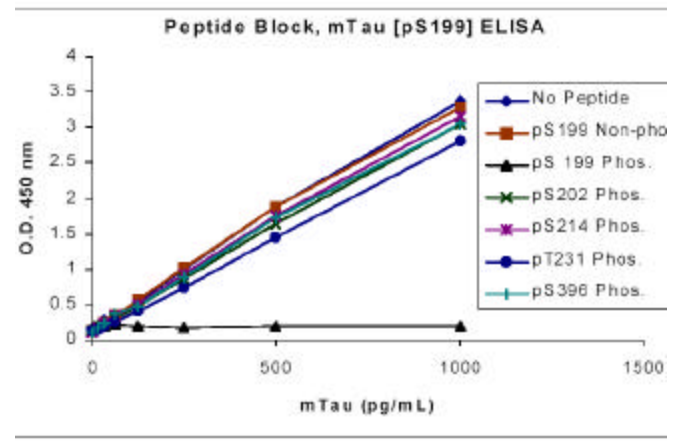
**Figure 4**

Human Phospho- Tau [pSer<sup>199</sup>] from human brain extracts and human SHSY-5Y neuroblastoma cell extracts showed variable reactivity (20-90%) in the Mouse Tau [pSer<sup>199</sup>] ELISA. The Mouse Tau [pSer<sup>199</sup>] ELISA did not measure Tau [pSer<sup>199</sup>] in human cerebrospinal fluid (CSF).

**Peptide competition**

The specificity of this assay for Phospho-Tau [pSer<sup>199</sup>] was confirmed by peptide competition. Phospho-Tau was serially diluted and quantitated in the assay as usual except that the detection antibody was preincubated with phospho-peptide at a concentration of 1 μg/mL.

The data presented in Figure 5 show that only the peptide corresponding to the region surrounding serine 199, containing the phospho-serine, could block the ELISA signal.



**Figure 5 Peptide Competition**

**Expected values**

Samples Mean Range	Total Protein mg/mL	Total Mouse Tau	Mouse Tau [pSer <sup>199</sup> ]
Mouse brain Homogenates	4.2	60 ng Tau/mg protein	0.33 ng Tau [pSer <sup>199</sup> ] per ng Tau
Mouse Neuro 2a cell extracts	6.1	15 ng Tau/mg protein	0.52 ng Tau [pSer <sup>199</sup> ] per ng Tau

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