ELISA Kit
Catalog #KMB7041

Mouse
Tau [pS199]

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INTRODUCTION

Tau is a microtubule-associated protein of considerable importance to neuronal axons of vertebrate brain. Tau 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 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-3β, PKA, PKC, CDK5, MARK, JNK, p38/MAPK and casein kinase II. Phosphorylation of serine 199 is regulated by a number of enzymes. Phosphopeptide mapping shows that serine 199 is phosphorylated by GSK-3β *in vitro* and *in viv*o. 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-3β affects the ability of tau to promote microtubule self-assembly. The Mouse Tau [pS199] ELISA kit is designed to measure levels of Ms Tau phosphorylated at serine 199 in tissue culture supernatants, or cell and tissue extracts, or extracts of brain homogenates.

PURPOSE

The Invitrogen Mouse (Ms) Tau [pS199] ELISA is to be used for the quantitative determination of Ms Tau [pS199] in buffered solution, cell culture medium, cell extract, or brain homogenate. The assay will recognize both natural and recombinant Ms Tau [pS199].

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Read entire protocol before use.

PRINCIPLE OF THE METHOD

The Invitrogen Mouse Tau [pS199] kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Tau (whether phosphorylated or non-phosphorylated) has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Ms Tau [pS199] content, control specimens, and unknowns, are pipetted into these wells.

During the first incubation, the Ms Tau [pS199] antigen binds to the immobilized (capture) antibody on one site. After washing, a rabbit polyclonal antibody specific for Tau [pS199] is added. During the second incubation, this antibody binds to the immobilized Ms Tau [pS199] captured during the first incubation.

After removal of excess second antibody, a horseradish peroxidase-labeled Anti-Rabbit IgG antibody is added. This binds to the rabbit polyclonal antibody to complete the four-member sandwich. After a third incubation and washing to remove excess Anti-Rabbit IgG 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 Ms Tau [pS199] present in the original specimen.

REAGENTS PROVIDED

Note: *Store all reagents at 2 to 8°C.*

Reagent	***96 Test Kit***
Ms Tau [pS199] Standard, recombinant Tau expressed in *E. coli* and phosphorylated *in vitro* with GSK-3β enzyme. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
Standard Diluent Buffer. Contains 0.1% sodium azide; 25 mL per bottle.	1 bottle
Antibody Coated Wells. 12 x 8 Well Strips.	1 plate
Ms Tau [pS199] Detection Antibody. Rabbit anti-Tau [pS199]. Contains 0.1% sodium azide; 11 mL per bottle.	1 bottle
Anti-Rabbit IgG HRP (100X). Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial
HRP Diluent. Contains 0.1% Kathon® CG/ICP; 25 mL per bottle.	1 bottle
Wash Buffer Concentrate (25X); 100 mL per bottle.	1 bottle
Stabilized Chromogen, Tetramethylbenzidine (TMB); 25 mL per bottle.	1 bottle
Stop Solution; 25 mL per bottle.	1 bottle
Plate Covers, adhesive strips.	3

SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
6. Glass or plastic tubes for diluting and aliquoting standard.
7. Absorbent paper towels.
8. Calibrated beakers and graduated cylinders in various sizes.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. 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.
5. If particulate matter is present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.

7. Samples that are >1000 pg/mL should be diluted with *Standard Diluent Buffer*. Cell extract and brain homogenate samples containing Tau [pS199] protein should be diluted at least 1:10 with *Standard Diluent Buffer*.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Cover or cap all reagents when not in use.
10. **Do not mix or interchange different reagent lots from various kit lots.**
11. Read absorbances within 2 hours of assay completion.
12. Do not use reagents after the kit expiration date.
13. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
14. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
15. Because *Stabilized Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.

SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with *Wash Buffer Concentrate (25X)* provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed. If your automated washer allows, 30 second soak cycles should be programmed into the wash cycle.

PROCEDURE FOR EXTRACTION OF PROTEINS FROM CELLS

A. Recommended formulation of Cell Extraction Buffer:

10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM Na_3VO_4
1% Triton X-100
10% glycerol
0.1% SDS
0.5% deoxycholate
1 mM PMSF (stock is 0.3 M in DMSO)
Protease inhibitor cocktail (e.g., Sigma Cat # P-2714; reconstituted according to manufacturer's guideline). Add 250 µL per 5 mL Cell Extraction Buffer.

This buffer is stable for 2 to 3 weeks at 4°C or for up to 6 months when aliquoted (without protease inhibitors and PMSF added) and stored at -20°C. When stored frozen, the Cell Extraction Buffer should be thawed on ice. Important: add the protease inhibitors just before using. The protease inhibitor supplemented Cell Extraction Buffer is stable for 24 hours at 4°C. PMSF is very unstable and must be added prior to use, even if added previously.

B. Protocol for Cell Extraction

This protocol has been applied to several cell lines with the Cell Extraction Buffer above. Researchers may optimize the cell extraction procedures that work best in their hands.

1. Collect cells in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent).
2. Wash cells 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 -80^{o}C and lysed at a later date).
4. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes on ice with vortexing every 10 minutes. The volume of Cell Extraction Buffer depends on the cell number in cell pellet and expression and phosphorylation levels of the protein. For example, 10^{7} mouse Neuro-2a cells can be extracted in 0.5 mL of Extraction Buffer to recover 1 mg/mL of total protein.
5. Transfer extracts to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4^{o}C.
6. Aliquot the clear lysate into clean microfuge tubes. These samples are ready for assay. Lysates can be stored at -80^{o}C. Avoid multiple freeze-thaws. Under these conditions, cell extract dilutions from 1:10 – 1:400 with Standard Diluent Buffer are sufficient for detection of Tau [pS199] in ELISA. Prepare the standard curve in a diluent matrix that contains the same concentration of extraction buffer.

HOMOGENIZATION OF BRAIN TISSUE

Recommendation for Buffers:

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

B. 1x PBS Buffer supplemented with 1x protease inhibitor cocktail from Sigma (P-2714).

Protocol

1. Determine the wet mass of the mouse brain sample (~100 mg) in an Eppendorf tube.
2. Add 8 x mass of cold 5 M guanidine-HCl/50 mM Tris to the tube by 50 - 100 µL aliquots and grind thoroughly with a hand-held motor (Fisher: K749540-0000) after each addition.
   (Optional: transfer the homogenate from above to 1 mL Dounce homogenizer and homogenize thoroughly.)
3. 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.
4. Dilute the sample ten-fold with cold PBS with 1x protease inhibitor cocktail. Centrifuge at 16,000 x g for 20 minutes at 4°C.
5. Carefully remove the supernatant and keep on ice. Brain tissue extract should be diluted an additional 1:10 – 1:1000 with Standard Diluent prior to application in the ELISA. Prepare the standard curve in a diluent matrix that contains the same concentration of extraction buffer.

Optional:

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

REAGENT PREPARATION AND STORAGE

This *Ms Tau [pS199] Standard* was calibrated against the mass of ligand-affinity purified GSK-3β-phosphorylated, recombinant Tau protein expressed in *E. coli*.

A. Reconstitution and Dilution of Ms Tau [pS199] Standard

Note: Either glass or plastic tubes may be used for standard dilutions.

1. Reconstitute standard to 5,000 pg/mL with *Standard Diluent Buffer*. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use standard within 1 hour of reconstitution.
2. Add 0.120 mL of the reconstituted standard to a tube containing 0.480 mL *Standard Diluent Buffer*. Label as 1000 pg/mL Ms Tau [pS199]. Mix.
3. Add 0.300 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 500, 250, 125, 62.5, 31.2, and 15.6 pg/mL Ms Tau [pS199].
4. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of Ms Tau [pS199] Standard

Standard:	Add:	Into:
1000 pg/mL	Prepare as described in Step 2.	
500 pg/mL	0.300 mL of the 1000 pg/mL std.	0.300 mL of the Diluent Buffer
250 pg/mL	0.300 mL of the 500 pg/mL std.	0.300 mL of the Diluent Buffer
125 pg/mL	0.300 mL of the 250 pg/mL std.	0.300 mL of the Diluent Buffer
62.5 pg/mL	0.300 mL of the 125 pg/mL std.	0.300 mL of the Diluent Buffer
31.2 pg/mL	0.300 mL of the 62.5 pg/mL std.	0.300 mL of the Diluent Buffer
15.6 pg/mL	0.300 mL of the 31.2 pg/mL std.	0.300 mL of the Diluent Buffer
0 pg/mL	0.300 mL of the Diluent Buffer	An empty tube

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

C. Storage and Final Dilution of Anti-Rabbit IgG HRP (100X)

Please Note: The *Anti-Rabbit IgG HRP (100X)* is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow *Anti-Rabbit IgG HRP (100X)* to reach room temperature. Gently mix. Pipette *Anti-Rabbit IgG HRP (100X)* slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µL of this 100X concentrated solution with 1 mL of *HRP Diluent* for each 8-well strip used in the assay. Label as Anti-Rabbit IgG HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of Anti-Rabbit IgG HRP (100X)	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

2. Return the unused *Anti-Rabbit IgG HRP (100X)* to the refrigerator.

D. Dilution of Wash Buffer

Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the *Procedural Notes/Lab Quality Control* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 100 µL of the *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 100 µL of standards to the appropriate microtiter 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 in 90 µL buffer). For samples such as cell culture medium or buffer solutions, add 50 µL of *Standard Diluent Buffer* to each well followed by 50 µL of sample. Tap

gently on side of plate to thoroughly mix. (See **REAGENT PREPARATION AND STORAGE**, Section B.)

4. Cover plate with *plate cover* and incubate for **2 hours at room temperature**.
5. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
6. Pipette 100 µL of *Ms Tau [pS199] Detection Antibody* solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
7. Cover plate with *plate cover* and incubate for **1 hour at room temperature**.
8. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
9. Add 100 µL Anti-Rabbit IgG HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in **REAGENT PREPARATION AND STORAGE**, Section C.)
10. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.
11. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
12. Add 100 µL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.

13. Incubate for **30 minutes at room temperature and in the dark**. ***Please Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
14. Add 100 µL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
15. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
16. Plot on graph paper the absorbance of the standards against the standard concentration. (The background absorbance may be subtracted from *all* data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
17. Read the Ms Tau [pS199] concentrations for unknown samples and controls from the standard curve plotted in step 16. **Multiply value(s) obtained for sample(s) by 2 to correct for the 1:2 dilution in step 3.** If cell/brain homogenates were diluted further, multiply the concentration by the appropriate dilution factor.

Samples producing signals greater than that of the highest standard (1000 pg/mL) should be diluted in *Standard Diluent Buffer* and reanalyzed, multiplying the concentration found by the appropriate dilution factor.

TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 1000 pg/mL Ms Tau [pS199].

Standard Ms Tau [pS199] (pg/mL)	Optical Density (450 nm)
0	0.065
	0.064
15.6	0.096
	0.097
31.2	0.122
	0.119
62.5	0.199
	0.198
125	0.325
	0.333
250	0.625
	0.613
500	1.252
	1.290
1000	2.492
	2.581

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 1000 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >1000 pg/mL with *Standard Diluent Buffer*; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs and the use of biological fluids in place of cell extract and brain homogenate samples have not been thoroughly investigated. The rate of degradation and dephosphorylation of native Ms Tau [pS199] in various matrices has not been investigated. 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.

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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The minimum detectable dose of Ms Tau [pS199] 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 Ms Tau [pS199] 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
SD	2.6	9.6	28.1
%CV	5.6	6.0	5.0

SD = Standard Deviation
CV = Coefficient of Variation

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	550
SD	3.4	12.2	27.9
%CV	7.4	7.2	5.1

SD = Standard Deviation
CV = Coefficient of Variation

LINEARITY OF DILUTION

Cell extract buffer and tissue culture medium containing 10% fetal calf serum were spiked with recombinant Ms 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	Cell Extract 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

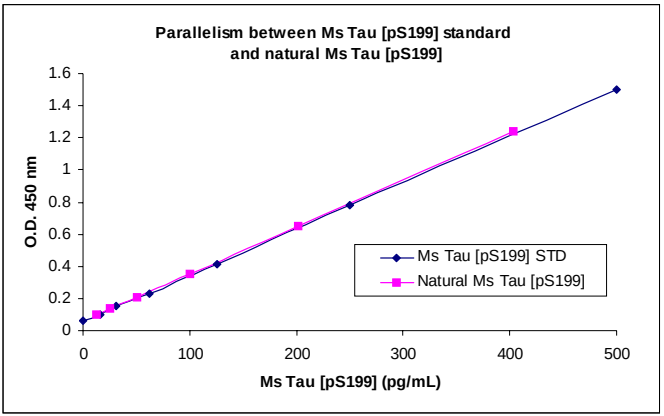
RECOVERY

The recovery of Ms Tau [pS199] added to tissue homogenate buffer (1:10 dilution) averaged 90%. The recovery of Ms Tau [pS199] added to cell extraction buffer (1:10 dilution) averaged 107%. The recovery of Ms Tau [pS199] added to tissue culture media containing 1% or 10% fetal calf serum averaged 92%.

PARALLELISM

Natural Ms Tau [pS199] extracted 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 is demonstrated in Figure 1 and indicates that the standard accurately reflects natural Ms Tau [pS199] content in samples.

Figure 1

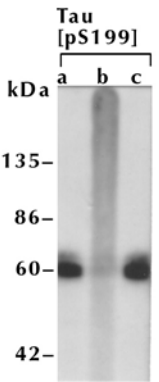


ANTIBODY SPECIFICITY

Figure 2 shows cell extracts from African green monkey kidney (CV-1) cells, stably expressing human four repeat tau. Samples were resolved by SDS PAGE on a 10% Tris-glycine gel. The proteins were transferred to nitrocellulose. Membranes were incubated with 0.50 µg/mL anti-phospho Tau [pS199], following prior incubation in the absence (a) or presence of the peptide immunogen (b), or the non-phosphopeptide corresponding to the Tau phosphopeptide (c). After washing, membranes were incubated with goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase (Cat. # ALI4405) and bands were detected using the Tropix WesternStar™ detection method.

The data show that only the phosphopeptide corresponding to this site blocks the antibody signal, illustrating the specificity of the anti-Tau [pS199] 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 Invitrogen Mouse Tau [pS199] kit. 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, and non-phosphorylated Tau. These last two proteins are depicted graphically below (Figure 3). As control, each of the Tau proteins was readily measured by the Mouse Tau (Total) ELISA (Catalog # KMB7011) shown in Figure 4. Human Tau [pS199] from human brain extracts and human SHSY-5Y neuroblastoma cell extracts. showed variable reactivity (20-90%) in the Ms Tau [pS199] kit. The Ms Tau [pS199] ELISA did not measure Tau [pS199] in human cerebrospinal fluid (CSF).

Figure 3

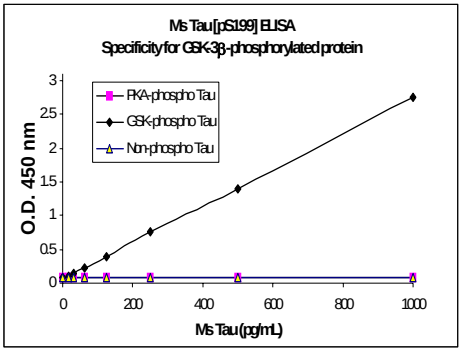
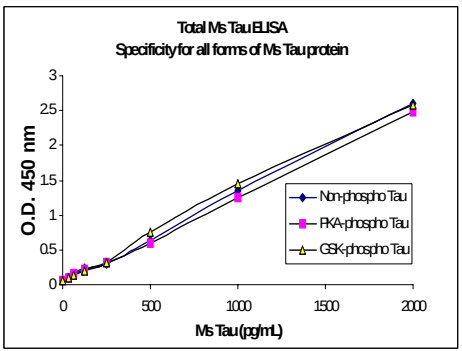
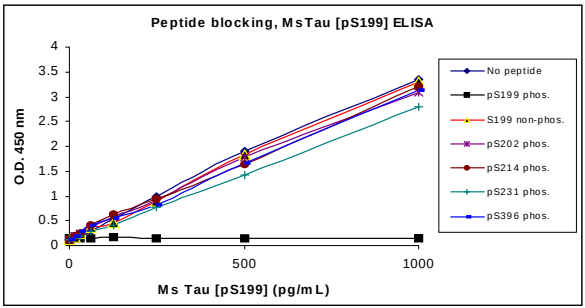


Figure 4



The specificity of this assay for Ms Tau [pS199] 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



EXPECTED VALUES

Samples Mean (range)	Total protein (mg/mL)	Total mouse Tau	Mouse Tau [pS199]
Mouse brain homogenates	4.2	60 ng Tau/mg protein	0.31 ng [pS199] Tau/ ng Total Tau
Mouse Neuro-2a cell extracts	6.1	15 ng Tau/mg protein	0.52 ng Tau [pS199]/ng Total Tau

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Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

Mouse Tau [pS199] Assay Summary