3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Tau ELISA, Mouse

Product Number CS0430
Storage Temperature 2-8 °C

Technical Bulletin

Product Description
Tau ELISA, Mouse is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) for the *in vitro* quantitative determination of mouse Tau in mouse brain homogenates, cell extracts, buffered solutions, or cell culture media. The assay will recognize both natural and recombinant mTau.

A monoclonal antibody specific for Tau has been coated onto the wells of the multiwell strips provided. Samples, including standards of known mTau content, control specimens, and unknowns, are pipetted into these wells. During the first incubation, the mTau antigen binds to the immobilized (capture) antibody on one site. After washing, a rabbit polyclonal antibody specific for Tau is added. During the second incubation, this antibody binds to the immobilized mTau 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 mTau 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-3ß, 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. Circulating tau is detected in cerebrospinal fluid (CSF). Levels of tau and phosphorylated tau are reportedly increased in Alzheimer's disease and other neurodegenerative diseases. The Tau ELISA, Mouse kit is designed for research use only and provides a sensitive method to measure mouse tau levels (both phosphorylated and nonphosphorylated) in samples of tissue culture supernatants, brain homogenates, or cell extracts.

Reagents

- **Mouse Tau Standard, 2 vials, Product No. T 3575-** full-length recombinant mouseTau, expressed in *E. coli.* . 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-Tau Detection Antibody, 11 mL, Product No. T 3200-** A detection antibody, produced in rabbit. Contains sodium azide. Ready to use.
- **Anti-Rabbit IgG- HRP concentrate (100X), 1 vial Product No. I 9408**- 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 5663-**. Contains 0.1% Kathon® 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 µL and 1,000 µL. (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 mouse Tau ELISA 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 duplicates.

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 $Na_4P_2O_7$
  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)
  PMSF is very unstable and must be added prior to use, even if added previously.
  Protease inhibitor cocktail (Product. No. P 2714) Add 250 µL 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 °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 °C.
6. Aliquot the clear lysate to clean microcentrifuge tubes. Lysates can be stored at – 70 °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 µL 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 °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 cell 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 to make the final concentration 5 M with 8.2 M guanidine HCl/82 mM Tris-HCl, pH 8.0.

Reagent Preparation

Mouse Tau Standard
1. The standard is a full length recombinant mouse Tau, calibrated against the mass of a highly purified recombinant Tau protein expressed in *E. coli.*
2. Reconstitute one vial of Standard to 10,000 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 10,000 pg/mL
5. Prepare serial standard dilutions as follows:

Tube #	Standard Diluent Buffer	Standard from tube #:	Final mouseTau concentration pg/mL
1	Reconstitute according to label instructions		10,000 pg/mL
2	0.480 mL	0.120 mL (1)	2000
3	0.300 mL	0.300 mL (2)	1000
4	0.300 mL	0.300 mL (3)	500
5	0.300 mL	0.300 mL (4)	250
6	0.300 mL	0.300 mL (5)	125
7	0.300 mL	0.300 mL (6)	62.5
8	0.300 mL	0.300 mL (7)	31.2
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 µL 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 µL	2 mL
4	40 µL	4 mL
6	60 µL	6 mL
8	80 µL	8 mL
10	100 µL	10 mL
12	120 µL	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

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 4th 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

Mouse Tau ELISA Assay Summary

1) 100 µL of Standards or Samples (samples diluted 1:10 in Standard Diluent Buffer)
Incubate 2 hours at RT, aspirate and wash 4x

2) Add 100 µL Detection Antibody
Incubate 1 hour at RT, aspirate and wash 4x

3) Add 100 µL Anti-Rabbit IgG-HRP
Incubate 30 min at RT, aspirate and wash 4x

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

5) Add 100 µL 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

1st incubation

a Add 100 µL Standard Diluent to zero wells.
b Add 100 µL standards, samples or controls to the appropriate wells.
c 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.*
d Cell culture supernatants or buffered solutions; dilute 1:2 in *Standard Diluent Buffer* (50 µL buffer + 50 µL sample).
e Tap gently on the plate to mix, cover with Plate Cover and incubate 2 hours at room temperature.
f Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells for a total of 4 times following washing instructions

2nd incubation

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

3rd incubation

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

Substrate incubation

a Add 100 µL of Stabilized Chromogen into all wells. *The liquid in the wells will begin to turn blue.*
b Do not cover the plate
c 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

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

Absorbance reading

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

Results

1. The results may be calculated using any immunoassay software package.
2. The four-parameter algorithm provides the best curve fit.
3. 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 2000 pg/mL standard should be further diluted and assayed again.

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.

Tau Standard pg/mL	Optical Density 450 nm
0	0.064
31.2	0.080
62.5	0.092
125	0.136
250	0.246
500	0.530
1000	1.250
2000	2.650

Limitations:

- Do not extrapolate the standard curve beyond the 2000 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.
- 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 Tau is <15 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 Tau concentration were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	124	437	1592
Standard Deviation (SD)	4.6	9.2	47.1
% Coefficient of Variation	3.7	2.1	3.0

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)	116	420	1505
Standard Deviation (SD)	11.0	20.2	97.8
Coefficient of Variation %	9.5	4.8	6.5

Linearity of Dilution

Cell Extraction Buffer and tissue culture medium containing 10% fetal calf serum were spiked with recombinant mTau 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 Extraction Buffer**			**Cell Culture**		
	Measured pg/mL	Expected pg/mL	% Expected	Measured pg/mL	Expected pg/mL	% Expected
Neat	1932	-	-	1986	-	-
1:2	1031	970	106	1069	993	108
1:4	506	485	104	523	497	105
1:8	242	243	100	263	248	106
1:16	125	121	103	129	124	104

Recovery

a The recovery of mTau added to Cell Extract Buffer averaged 107%.

b The recovery of mouseTau added to homogenate buffer (1:10 dilution) averaged 90%.

c The recovery of mouse Tau added to tissue culture medium containing 1% fetal calf serum averaged 92%, while the recovery of mouse Tau added to tissue culture medium containing 10% fetal calf serum averaged 110%.

Parallelism

Natural mouseTau, from mouse brain homogenate, 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 1 below and indicated that the standard accurately reflects natural mouse Tau content in samples.

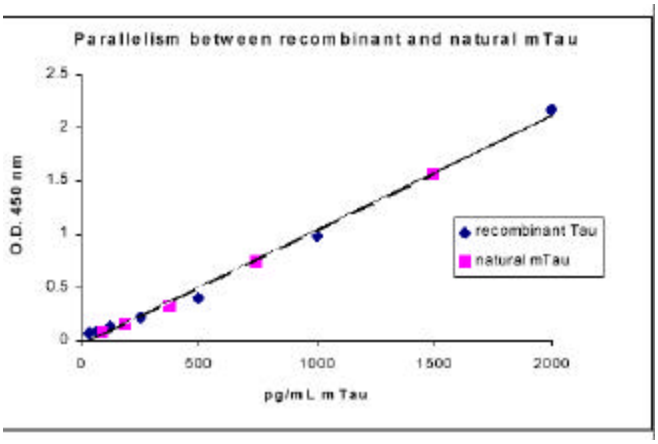

Figure 1

Specificity

Buffered solutions of a panel of substances at 20,000 pg/mL were assayed with the Mouse Tau ELISA.

- The following substances were tested and found to have no cross-reactivity:
  Human ß Amyloid 1-40 and ß Amyloid 1-42, α-Synuclein, ß-Synuclein.
- Human Tau showed variable reactivity in the Mouse Total Tau ELISA kit ranging from 48-72% in human brain homogenates and human SHSY-5Y neuroblastoma cell extracts.
- In human cerebrospinal fluid (CSF) samples the variable reactivity in the Mouse Total Tau kit ranged from 0-87%.

Expected values

Samples	Total Protein mg/mL	Total Tau (ng Tau/mg total protein)
Mouse brain Homogenates 1:500	4.2	60
Mouse neuro 2a cell line 1:200	6.1	15

References

1. Liu, F., et al., Involvement of aberrant glycosylation in phosphorylation of tau by CDK 5 and GSK-3β., FEBS Lett., **530**, 209-214. (2002).
2. Sawamura, N., et al. Site-specific phosphorylation of tau accompanied by activation of mitogen-activated protein kinase (MAPK) in brains of Niemann-Pick type C mice. J. Biol. Chem. **276**, 10314-10319 (2001).
3. Mandelkow, E., Alzheimer's disease. The tangled tale of tau. Nature, **402**, 588-589 (1999).
4. Sontag, E., et al. Molecular interactions among protein phosphatase 2A, tau, and microtubules. Implications for the regulation of tau phosphorylation and the development of tauopathies. J. Biol. Chem., **274**, 25490-25498 (1999).
5. Wang, J.Z., et al., Tau is phosphorylated by GSK-3 at several sites found in Alzheimer disease and its biological activity markedly inhibited only after it is prephosphorylated by Akinase. FEBS Lett., **436**, 28-34 (1998).
6. Papasozomenos, S.C., et al., Testosterone prevents the heat shock-induced overactivation of glycogen synthase kinase-3β but not of cyclin-dependent kinase 5 and c-Jun NH2-terminal kinase and concomitantly abolishes hyperphosphorylation of tau: implications for Alzheimer's disease. Proc. Nat'l. Acad. Sci. USA, **99**, 1140-1145 (2002).
7. Ishiguro, K., et al., Phosphorylated tau in human cerebrospinal fluid is a diagnostic marker for Alzheimer's disease. Neurosci. Lett., **270,** 91-94 (1999).
8. Gotz, J., et al. Tau filament formation in transgenic mice expressing P301L tau., J. Biol. Chem., **276,** 529-534 (2001).

AH/PHC 08/04