ELISA Kit
Catalog #KMB7011

Mouse
Tau (Total)

www.invitrogen.com
Invitrogen Corporation
542 Flynn Road, Camarillo, CA 93012
Tel: 800-955-6288
E-mail: techsupport@invitrogen.com

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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 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-3β, 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 Mouse Tau (Total) ELISA 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.

PURPOSE

The Invitrogen Mouse (Ms) Tau (Total) ELISA is to be used for the quantitative determination of Ms Tau in mouse brain homogenates, cell extracts, buffered solutions, or cell culture media. The assay will recognize both natural and recombinant Ms Tau.

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Read entire protocol before use.

PRINCIPLE OF THE METHOD

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

During the first incubation, the Ms Tau 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 Ms Tau 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 Ms Tau present in the original specimen.

REAGENTS PROVIDED

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

Reagent	***96 Test Kit***	***192 Test Kit***
Ms Tau (Total) Standard, full length recombinant Tau expressed in *E. coli*. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials
Standard Diluent Buffer. Contains 0.1% sodium azide; 25 mL per bottle.	1 bottle	2 bottles
Antibody Coated Wells, 12x8 Well Strips.	1 plate	2 plates
Ms Tau (Total) Detection Antibody. Rabbit anti-Tau. Contains 0.1% sodium azide; 11 mL per bottle.	1 bottle	2 bottles
Anti-Rabbit IgG HRP (100X). Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial	2 vials
HRP Diluent. Contains 0.1% Kathon® CG/ICP; 25 mL per bottle.	1 bottle	1 bottle
Wash Buffer Concentrate (25X); 100 mL per bottle.	1 bottle	1 bottle
Stabilized Chromogen, Tetramethylbenzidine (TMB); 25 mL per bottle.	1 bottle	1 bottle
Stop Solution; 25 mL per bottle.	1 bottle	1 bottle
Plate Covers, adhesive strips.	3	6

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 large amounts of particulate matter are 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 >2000 pg/mL should be diluted with *Standard Diluent Buffer*. Cell extract and brain homogenate samples containing Tau 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 stability of 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 into 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 level. For example, 10^{7} mouse Neuro-2a cells can be extracted in 0.5 mL of Extract 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^{o}C.
6. Aliquot the clear lysate into clean microfuge tubes. 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 in ELISA.

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 (e.g., 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 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/82 mM Tris-HCl (pH 8.0).

REAGENT PREPARATION AND STORAGE

This *Ms Tau (Total) Standard* was calibrated against the mass of a highly purified recombinant Tau protein expressed in *E. coli*.

A. Reconstitution and Dilution of Mouse Tau (Total) Standard

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

1. Reconstitute standard to 10,000 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 2000 pg/mL Ms Tau. Mix.
3. Add 0.300 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 1000, 500, 250, 125, 62.5, and 31.2 pg/mL Ms Tau.
4. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of Mouse Tau (Total) Standard

Standard:	Add:	Into:
2000 pg/mL	Prepare as described in Step 2.	
1000 pg/mL	0.300 mL of the 2000 pg/mL std.	0.300 mL of the Diluent Buffer
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
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 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 plus 90 µL buffer). For samples such as cell culture medium or buffered 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 *(Total) 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 HRP IgG 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 concentrations for unknown samples and controls from the standard curve plotted in step 16. **Multiply value(s) obtained for cell culture media or buffered solutions by 2 to correct for the 1:2 dilution in step 3.** If cell extract or brain homogenates were diluted further (1:10 or greater), multiply the concentration by the appropriate dilution factor. Samples producing signals greater than that of the highest standard (2000 pg/mL) should be further 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 2000 pg/mL Ms Tau.

Standard Ms Tau (pg/mL)	Optical Density (450 nm)
0	0.066
	0.063
31.2	0.078
	0.082
62.5	0.093
	0.092
125	0.138
	0.135
250	0.250
	0.242
500	0.540
	0.521
1000	1.261
	1.243
2000	2.657
	2.645

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 2000 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >2000 pg/mL with *Standard Diluent Buffer*; reanalyze these and multiply results by the appropriate dilution factor.

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.

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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The minimum detectable dose of Ms 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 Ms 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
SD	4.6	9.2	47.1
%CV	3.7	2.1	3.0

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)	116	420	1505
SD	11.0	20.2	97.8
%CV	9.5	4.8	6.5

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 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.

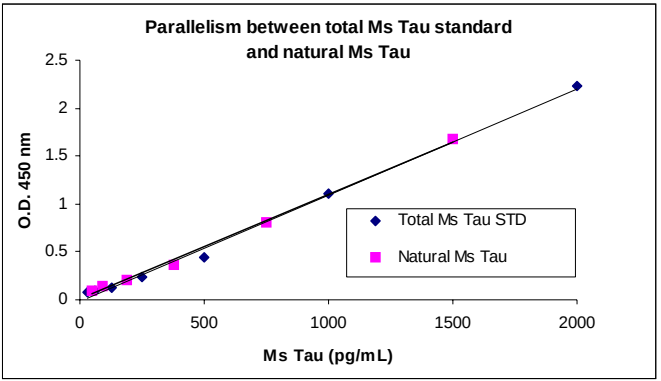
Dilution	Cell Extract Buffer			Cell Culture		
	Measured (pg/mL)	Expected (pg/mL)	% Expected	Measured (pg/mL)	Expected (pg/mL)	% Expected
neat	1941	-	-	1986	-	-
1/2	1031	970	106	1069	993	108
1/4	506	485	104	523	497	105
1/8	242	243	100	263	248	106
1/16	125	121	103	129	124	104

RECOVERY

The recovery of Ms Tau added to Cell Extract Buffer averaged 107%. The recovery of Ms Tau added to homogenate buffer (1:10 dilution) averaged 90%. The recovery of Ms Tau added to tissue culture medium containing 1% fetal calf serum averaged 92%, while the recovery of Ms Tau added to tissue culture medium containing 10% fetal calf serum averaged 110%.

PARALLELISM

Natural Ms Tau, 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 below and indicated that the standard accurately reflects natural Ms Tau content in samples.



SPECIFICITY

Buffered solutions of a panel of substances at 20,000 pg/mL were assayed with the Invitrogen Mouse Tau (Total) 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. Human Tau showed variable reactivity in the Mouse Tau (Total) ELISA kit, ranging from 48-72% in human brain homogenates and human SHSY-5Y neuroblastoma cell extracts. Human cerebrospinal fluid (CSF) samples showed variable reactivity in the Mouse Tau (Total) kit ranging 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

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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 (Total) Assay Summary