BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit with Pre-coated Plates

Human/Mouse/Rat Phospho-Tau (Thr217)

Cat. No. 433107

ELISA Kit for Accurate Quantitation of Phospho-Tau (Thr217) from Human, Mouse, and Rat Brain Lysates

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biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

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Introduction:

Tau is a 441-amino acid protein that binds along microtubules in order to stabilize them. Phosphorylation of Tau reduces its affinity for microtubules, leading to microtubule destabilization. Hyperphosphorylated Tau can assemble into higher order structures, including oligomers and neurofibrillary tangles (NFT). NFTs are a hallmark of several neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and frontotemporal dementia. The level of phosphorylated Tau, therefore, is an important biomarker for the progression of these diseases. Phosphorylation of Tau at threonine 217 has become an important marker in Alzheimer's disease, for example, because it can be detected years ahead of typical disease symptoms and correlates well with disease severity.

The BioLegend LEGEND MAX™ Human/Mouse/Rat Phospho-Tau (Thr217) ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a mouse monoclonal Tau antibody. The standard is composed of full-length human Tau conjugated to a 15-aa phospho-Tau (Thr217) peptide via a sulfo-SMCC linker. The detection antibody is a biotinylated rabbit polyclonal phospho-Tau (Thr217) antibody. After washing away any unbound biotinylated detection antibody, a streptavidin-polymer HRP is used for detection. This kit is specifically designed for the accurate quantitation of phospho-Tau (Thr217) from human, mouse, and rat brain lysates. This kit is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
Phospho-Tau (Thr217) Pre-coated 96-well Strip Microplate	1 plate		750004043
Phospho-Tau (Thr217) Detection Antibody	1 bottle	12 mL	750004045
Phospho-Tau (Thr217) Lyophilized Standard	1 vial	lyophilized	750004047
Streptavidin-Polymer HRP-M	1 bottle	12 mL	750004042
Assay Buffer B	1 bottle	25 mL	79128
Wash Buffer (20X)	1 bottle	50 mL	78233
Substrate Solution F	1 bottle	12 mL	79132
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	1 pack		78101

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Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate shaker
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

<table>
<tr><th colspan="2">Opened or Reconstituted Components</th></tr>
<tr><td>Microplate wells</td><td>If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.</td></tr>
<tr><td>Standard</td><td>The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Detection Antibody</td><td rowspan="6">Store opened reagents between 2°C and 8°C and use within one month.</td></tr>
<tr><td>Streptavidin-Polymer HRP-M</td></tr>
<tr><td>Assay Buffer B</td></tr>
<tr><td>Wash Buffer (20X)</td></tr>
<tr><td>Substrate Solution F</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye, and

clothing contact.

3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma, and other biological fluids in accordance with NCCLS regulations.
4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling:

The conditions listed below for sample collection and storage are meant to be general guidelines. Sample dilutions may need to be optimized to ensure that they are within the linear range of the ELISA.

Tissue Lysates: Rinse tissue with PBS, cut it into small pieces (1-2 mm), and homogenize with a tissue homogenizer. Lyse the homogenized tissue with an equal amount of lysis buffer (ex. 0.5% NP40 lysis buffer) for 30 minutes on ice. Agitate the lysate periodically during lysis. Then, centrifuge the lysate to remove any debris. Use the sample immediately; samples can be aliquoted and stored at ≤ -70°C for later use. Repeated freeze-thaw of samples should be avoided.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized Phospho-Tau (Thr217) Standard by adding the volume of Assay Buffer B to make the standard stock solution (refer to LEGEND MAX™ Kit Lot-Specific Certificate of Analysis). Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.
3. The brain lysate dilution factor should be determined by the end-user. If dilutions are required, use Assay Buffer B as the diluent.

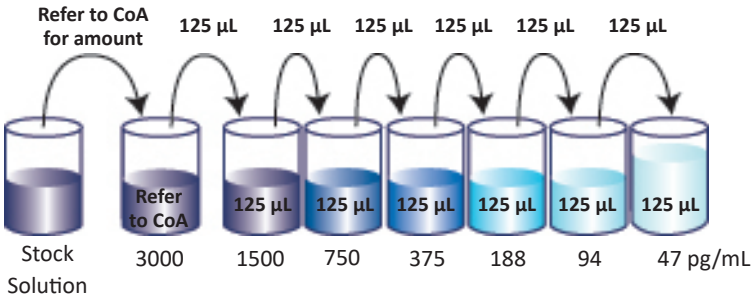
Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.

3. Prepare 250 µL of the 3000 pg/mL top standard by diluting the appropriate amount of the standard stock solution in Assay Buffer B (refer to the LEGEND MAX™ Kit Lot-Specific Certificate of Analysis). Perform six two-fold serial dilutions of the 3000 pg/mL top standard in separate tubes using Assay Buffer B as the diluent. Thus, the phospho-Tau (Thr217) standard concentrations in the tubes are 3000 pg/mL, 1500 pg/mL, 750 pg/mL, 375 pg/mL, 188 pg/mL, 94 pg/mL and 47 pg/mL, respectively. Assay Buffer B serves as the zero standard (0 pg/mL).



4. Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.

5. Add 50 µL of Assay Buffer B to each well that will contain either standard dilutions or samples.

6. Add 50 µL of standard dilutions or samples to the appropriate wells

7. Seal the plate with a plate sealer included in the kit and incubate the plate at room temperature for 2 hours with shaking.

8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

9. Add 100 µL of Phospho-Tau (Thr217) Detection Antibody to each well, seal

the plate and incubate at room temperature for 1 hour with shaking.

10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
11. Add 100 µL of Streptavidin-Polymer HRP-M to each well, seal the plate, and incubate at room temperature for 30 minutes with shaking.
12. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
13. Add 100 µL of Substrate Solution F to each well and incubate for 10 minutes* **in the dark**. Wells containing phospho-Tau (Thr217) should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
14. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.
15. Read absorbance at 450 nm within 15 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

*** Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers.**

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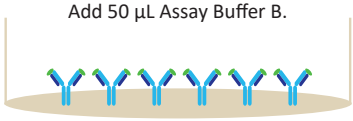
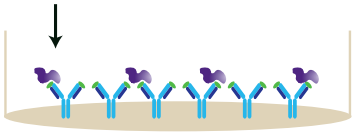
Assay Procedure Summary

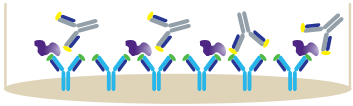
1. Wash 4 times.
Add 50 µL Assay Buffer B.



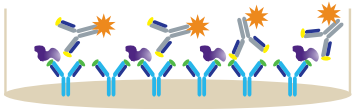
2. Add 50 µL diluted standards or samples.
Incubate 2 hr, RT on plate shaker.



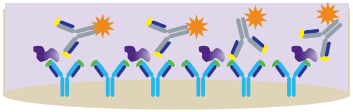
3. Wash 4 times.
Add 100 µL Detection Antibody.
Incubate 1 hr, RT on plate shaker.



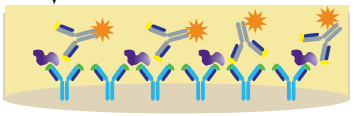
4. Wash 4 times.
Add 100 µL Streptavidin-Polymer HRP-M.
Incubate 30 min, RT on plate shaker.



5. Wash 5 times.
Add 100 µL Substrate Solution F.
Incubate 10 min*, RT on benchtop, **in the dark.**



6. Add 100 µL Stop Solution.



7. Read absorbance at 450 nm and 570 nm.

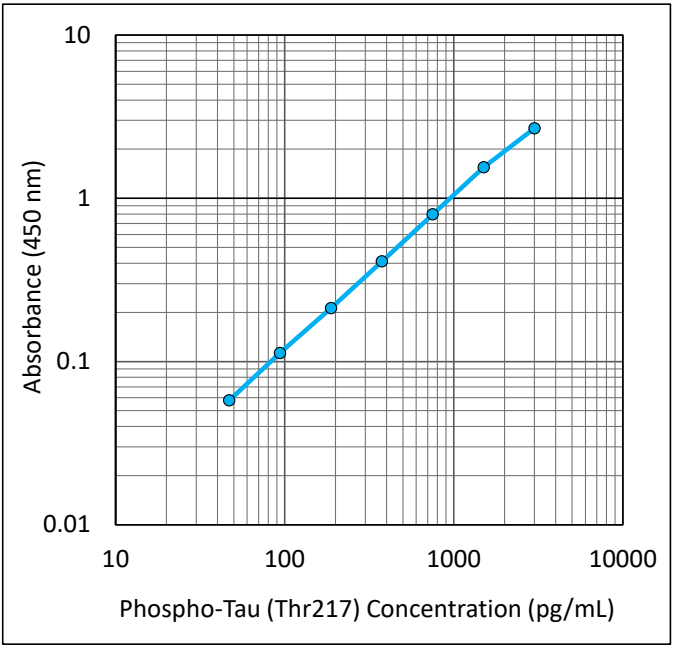
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If the appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with analyte concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown analyte concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the analyte concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution, as appropriate.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

Specificity: No cross reactivity was observed when this kit was used to analyze unconjugated full-length human Tau, a human phospho-Tau (Thr181) conjugate, and a human phospho-Tau (Ser262) conjugate at 50 ng/mL.

Sensitivity: The minimum detectable concentration of phospho-Tau (Thr217) is 8.63 ± 1.71 pg/mL.

Recovery: Human phospho-Tau (Thr217) conjugate was spiked into human, mouse, and rat brain lysates at three different concentrations. Then, sample recovery was analyzed with the LEGEND MAX™ Human/Mouse/Rat Phospho-Tau (Thr217) ELISA kit.

Sample Type	N	Recovery (%)
Human Brain Lysate	2	102.2%
Mouse Brain Lysate	3	103.8%
Rat Brain Lysate	1	100.6%

Linearity: Human, mouse, and rat brain lysates were serially diluted two-fold to produce samples with concentrations within the dynamic range. Then, linearity was analyzed with the LEGEND MAX™ Human/Mouse/Rat Phospho-Tau (Thr217) ELISA kit.

Sample Type	N	Linearity (%)
Human Brain Lysate	2	102.2%
Mouse Brain Lysate	3	97.4%
Rat Brain Lysate	1	114.1%

Intra-Assay Precision: Sixteen replicates of each of two human brain lysate samples containing different phospho-Tau (Thr217) concentrations were tested in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	1799.94	232.10
Standard Deviation	94.15	8.44
%CV	5.23	3.64

Inter-Assay Precision: Two human brain lysate samples containing different concentrations of phospho-Tau (Thr217) were tested in ten independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	10	10
Mean Concentration (pg/mL)	1843.30	226.99
Standard Deviation	133.40	19.51
%CV	7.24	8.60

Biological Samples: Human brain lysate from healthy controls and from Parkinson's disease patients was tested using the LEGEND MAX™ Human/Mouse/Rat Phospho-Tau (Thr217) ELISA kit to determine the concentrations of phospho-Tau (Thr217). Total protein concentrations were determined using a bicinchoninic acid assay (BCA), and the concentration of phospho-Tau (Thr217) was normalized to the total protein concentration. The normalized phospho-Tau (Thr217) concentration in the healthy control brain lysate is 7.13 pg/µg total protein, and the normalized phospho-Tau (Thr217) concentration in the Parkinson's disease brain lysate is 9.32 pg/µg total protein.

Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Notes