Version 3a Last updated 8 May 2024

ab269557 Cleaved Tau SimpleStep ELISA® Kit (Human Asp738/Mouse Asp713)

For the quantitative measurement of Cleaved Tau in human and mouse serum, plasma, cell culture supernatant, cerebrospinal fluid, cell and tissue extract.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Cleaved Tau *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Cleaved Tau protein in human and mouse serum, plasma, cell culture supernatant, cerebrospinal fluid, cell and tissue extract.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Tau promotes microtubule assembly and stability; it might be involved in the establishment and maintenance of neuronal polarity. The C-terminus of Tau binds axonal microtubules while the N-terminus binds neural plasma membrane components, suggesting that Tau functions as a linker protein between both. Axonal polarity is predetermined by Tau localization (in the neuronal cell) in the domain of the cell body defined by the centrosome. Tau is expressed predominantly in neurons. Human Tau is expressed at least as 9 isoforms. The long PNS-tau isoform is expressed in the peripheral nervous system while the others are expressed in the central nervous system. The short isoforms allow plasticity of the cytoskeleton whereas the longer isoforms may preferentially play a role in its stabilization. Tau is phosphorylated at various serine and threonine residues. Tau phosphorylation impairs the Tau ability to bind microtubules and leads to microtubule depolymerization. Hyperphosphorylated Tau is the major component of paired helical

filaments, the building block of neurofibrillary lesions in Alzheimer's disease (AD) brain. Tau truncation can affect Tau pathologic characteristics, including its ability to acquire AD-related conformations and to assemble into filaments. Amyloid-beta protein can trigger caspase activation and cellular apoptosis. Several activated caspases, including caspase-3, caspase-7, and caspase-8, can cleave Tau at a highly conserved sequence present in all isoforms generating Cleaved Tau with …SSTGSIDMVD sequence at the carboxy terminus. This carboxy terminal Asp corresponds to, for example, Asp738 of human canonical PNS isoform, Asp421 of human Tau-F isoform, or Asp713 of mouse canonical PNS isoform. The cleavage of Tau at this site is an important inducer of Tau polymerization in AD. This kit is designed to detect all Tau isoforms only when cleaved at the above described site.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells

Add 50 µL Antibody Cocktail to all wells

Incubate at room temperature for 1 hour

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL TMB Development Solution to each well and incubate for 10 minutes.

Add 100 µL Stop Solution and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Cleaved Tau Capture Antibody 10X	600 µL	+4°C
Cleaved Tau Detector Antibody 10X	600 µL	+4°C
Cleaved Tau Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI2*	6 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

*Note: Antibody Diluent CPI2- This buffer has been reformulated to enhance stability after freeze-thaw cycles while producing data equivalent to the original formulation of antibody diluent CPI previously used in this kit.
While we run stock down, you may receive kits containing antibody diluent CPI. This does not affect the way you should use the kit.
If you have any questions please contact Abcam Scientific Support.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

- The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.
- To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Cell Extraction Buffer PTR (For cell and tissue extracts only):

Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL Cell Extraction Buffer PTR 5X and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. If required protease inhibitors can be added.

Alternative – Enhancer may be added to 1X Cell Extraction Buffer PTR after extraction of cells or tissue. Refer to note in the Troubleshooting section.

9.2 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

9.3 Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CPI2. Mix thoroughly and gently.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

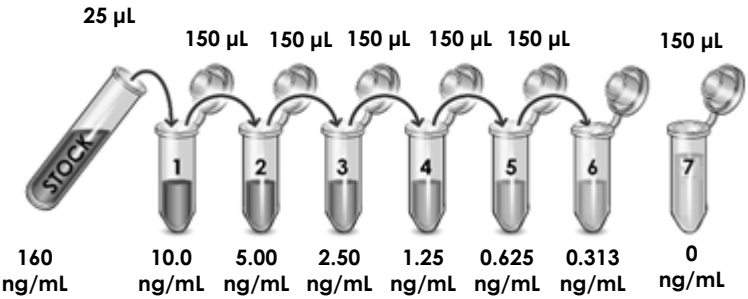
10.1 **IMPORTANT**: If the protein standard vial has a volume identified on the label, reconstitute the Cleaved Tau protein by adding that volume of dilutant indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the Cleaved Tau protein standard by adding 500 µL dilutant. Hold at room temperature for 10 minutes and mix gently. This is the 160ng/mL **Stock Standard** Solution.

10.2 For **serum, plasma, cell culture supernatant, cerebrospinal fluid samples measurements**, reconstitute the Cleaved Tau protein standard in Sample Diluent NS.
For **cell and tissue extract samples measurements**, reconstitute the Cleaved Tau protein standard in 1X Cell Extraction Buffer PTR.

10.2.1 Label seven tubes, Standards 1– 7.

10.2.2 Add 375 µL of appropriate diluent (see step 10.1) into tube number 1 and 150 µL of appropriate diluent into numbers 2-7.

10.2.3 Use the Stock Standard to prepare the following dilution series. Standard #7 contains no protein and is the Blank control:



11. Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum*	<100%
Plasma – Citrate*	<100%
Plasma – EDTA*	<100%
Plasma – Heparin*	<100%
Mouse Brain Supernatant	6.25-25%
Cell Culture Media*	<100%
Cerebrospinal Fluid*	<20%
Mouse Brain Extract	31 - 250 µg/mL
Staurosporine Treated Neuro-2a Extract	16 - 250 µg/mL
Mock Treated Neuro-2a Extract	500 µg/mL
2-hr Staurosporine Treated SH-SY5Y Extract	63 - 1,000 µg/mL
4-hr Staurosporine Treated SH-SY5Y Extract	16 - 250 µg/mL
Mock Treated SH-SY5Y Extract	125 - 1,000 µg/mL

*Based on spiked sample

11.1 Plasma:

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Assay neat or dilute samples into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Assay neat or dilute samples into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants:

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.4 Cerebrospinal Fluid (CSF):

Dilute cerebrospinal fluid at least 1:5 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.5 Preparation of extracts from cell pellets:

11.5.1 Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.

11.5.2 Rinse cells twice with PBS.

11.5.3 Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR.

11.5.4 Incubate on ice for 20 minutes.

11.5.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.5.6 Transfer the supernatants into clean tubes and discard the pellets.

11.5.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.5.8 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.6 Preparation of extracts from adherent cells by direct lysis (alternative protocol):

11.6.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.6.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate).

11.6.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.

11.6.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.6.5 Transfer the supernatants into clean tubes and discard the pellets.

11.6.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.6.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.7 Preparation of extracts from tissue homogenates:

11.7.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

11.7.2 Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.

11.7.3 Incubate on ice for 20 minutes.

11.7.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.7.5 Transfer the supernatants into clean tubes and discard the pellets.

11.7.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.7.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes. Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

13.9 Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed

time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Δ **Note**: that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

13.10 Analyze the data as described below.

14. Calculations

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

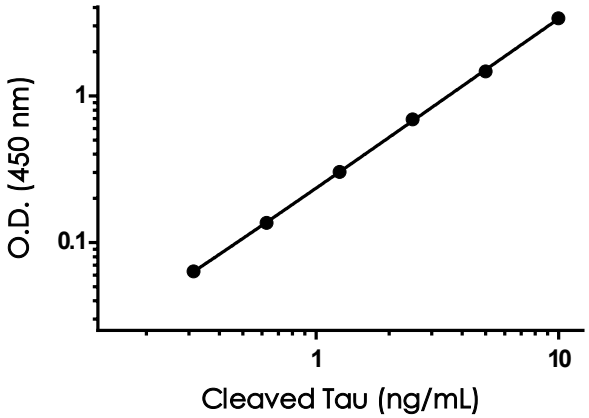
Δ **Note:** Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

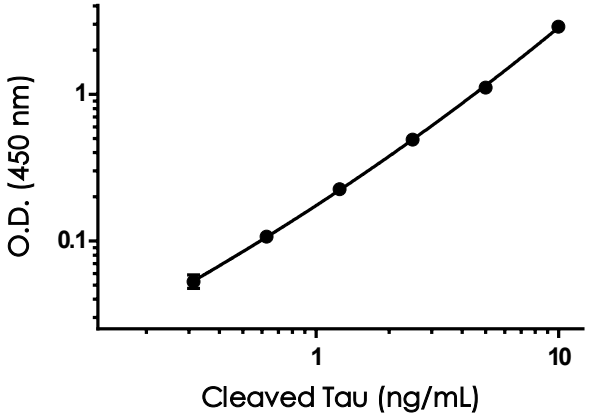
15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Concentration (ng/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.050	0.049	0.050
0.313	0.114	0.112	0.113
0.625	0.187	0.185	0.186
1.25	0.360	0.345	0.352
2.50	0.761	0.719	0.740
5.00	1.447	1.598	1.522
10.0	3.351	3.497	3.424

Figure 1. Example of human and mouse Cleaved Tau standard curve in Sample Diluent NS. The Cleaved Tau standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Standard Curve Measurements			
Concentration (ng/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.053	0.052	0.053
0.313	0.101	0.11	0.105
0.625	0.157	0.163	0.160
1.25	0.280	0.275	0.278
2.50	0.515	0.575	0.545
5.00	1.145	1.180	1.163
10.0	2.976	2.919	2.947

Figure 2. Example of human and mouse Cleaved Tau standard curve in 1X Cell Extraction Buffer PTR. The Cleaved Tau standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The MDD was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	n=	Minimal Detectable Dose
Sample Diluent NS	16	0.075 ng/mL
1X Cell Extraction Buffer PTR	16	0.084 ng/mL

RECOVERY –

Three concentrations of Cleaved Tau recombinant protein were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
100% Human Serum	105	103 - 109
100% Human Plasma - Citrate	95	91 - 100
100% Human Plasma - EDTA	93	90 - 97
100% Human Plasma - Heparin	107	103 - 113
100% Mouse Serum	95	90 - 105
100% Mouse Plasma - Citrate	81	80 – 82
100% Mouse Plasma - EDTA	103	98 – 110
100% Mouse Plasma - Heparin	99	90 – 105
25% Mouse Brain Supernatant	88	83 - 92
100% Cell Culture Media*	118	113 - 125
20% Cerebrospinal Fluid	95	93 - 97
100 µg/ mL Mouse Brain Extract	92	87 - 100
150 µg/mL Staurosporine Treated Neuro-2a Extract	85	82 - 90
500 µg/mL Mock Treated Neuro-2a Extract	82	77 - 86
500 µg/mL 2-hr Staurosporine Treated SH-SY5Y Extract	86	69 - 117
50 µg/mL 4-hr Staurosporine Treated SH-SY5Y Extract	87	80 - 95
500 µg/mL Mock Treated SH-SY5Y Extract	94	86 - 101

*Media is RPMI 1640 containing 10% fetal bovine serum.

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Cleaved Tau was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	25% Mouse Brain Supernatant
Undiluted	ng/mL	1.148
	% Expected value	100
2	ng/mL	0.583
	% Expected value	102
4	ng/mL	0.298
	% Expected value	104

Recombinant Cleaved Tau was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	100% Human Serum	100% Human Plasma-Citrate	100% Human Plasma-EDTA	100% Human Plasma-Heparin	20% Human CSF	100% Cell Culture Media
Undiluted	ng/mL	6.93	5.79	7.08	6.72	6.99	7.35
	% Expected value	100	100	100	100	100	100
2	ng/mL	3.43	2.99	3.33	3.44	3.25	3.56
	% Expected value	99	103	94	103	93	97
4	ng/mL	1.70	1.52	1.67	1.74	1.64	1.73
	% Expected value	98	105	94	104	94	94
8	ng/mL	0.821	0.763	0.793	0.828	0.831	0.916
	% Expected value	95	105	90	99	95	100
16	ng/mL	0.398	0.402	0.416	0.449	0.415	0.461
	% Expected value	92	111	94	107	95	100

100% pooled human and mouse serum and plasma (EDTA, Heparin, Citrate) samples was measured in duplicate. All values were below the detectable range of the assay.

Native Cleaved Tau was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	250 µg/mL Mouse Brain Extract	250 µg/mL STS Treated Neuro-2a Extract	1,000 µg/mL 2-hr STS Treated SH-SY5Y Extract	250 µg/mL 4-hr STS Treated SH-SY5Y Extract	1,000 µg/mL Mock Treated SH-SY5Y Extract
Undiluted	ng/mL	1.94	5.53	7.65	5.24	2.29
	% Expected value	100	100	100	100	100
2	ng/mL	1.06	3.18	3.49	2.80	1.17
	% Expected value	109	115	91	107	102
4	ng/mL	0.581	1.57	1.96	1.46	0.618
	% Expected value	120	114	103	111	108
8	ng/mL	0.294	0.79	1.07	0.764	0.343
	% Expected value	122	114	112	117	120
16	ng/mL	ND	0.421	0.537	0.389	ND
	% Expected value	ND	122	112	119	ND

ND – Not Determined, interpolated values were below the 6th standard.

PRECISION –

Mean coefficient of variations of interpolated values of Cleaved Tau from two concentrations of spiked human serum within the working range of the assay.

	Intra-Assay	Inter-Assay
n =	8	3
CV (%)	4.5	5.3

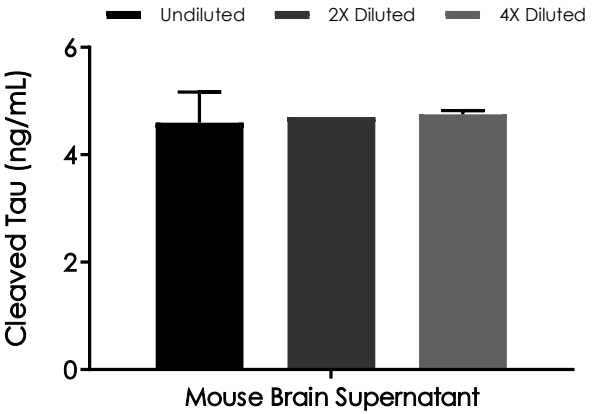


Figure 3. Interpolated concentrations of native Cleaved Tau in mouse brain supernatant sample. The concentrations of Cleaved Tau were measured in duplicates, interpolated from the Cleaved Tau standard curves and corrected for sample dilution. Undiluted sample is as follows: mouse brain supernatant 25%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Cleaved Tau concentration was determined to be 4.68 ng/mL in neat brain supernatant.

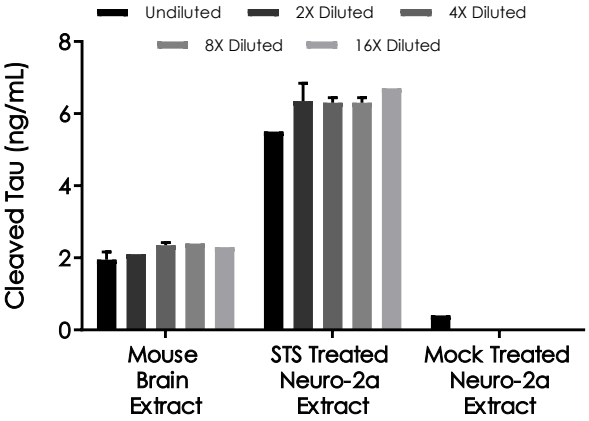


Figure 4. Interpolated concentrations of native Cleaved Tau in mouse brain tissue extract, 4 hours 1 µM staurosporine treated and mock treated Neuro-2a cell extracts samples based on 250 µg/mL, 250 µg/mL and 500 µg/mL extract loads, respectively. The concentrations of Cleaved Tau were measured in duplicate and interpolated from the Cleaved Tau standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Cleaved Tau concentration was determined to be 2.19 ng/mL in mouse brain extract, 6.27 ng/mL in staurosporine treated Neuro-2a extract and 0.426 ng/mL in mock treated Neuro-2a extract.

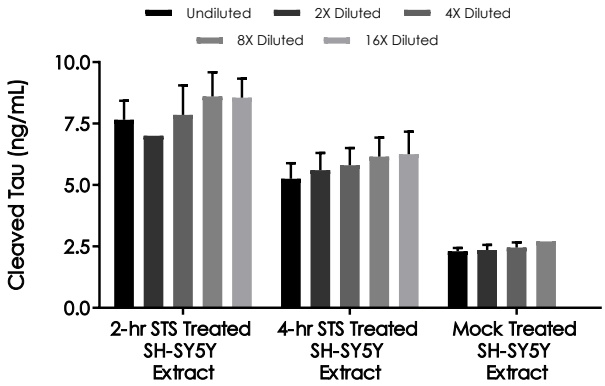


Figure 5. Interpolated concentrations of native Cleaved Tau in 2 hours 1 µM staurosporine treated SH-SY5Y, 4 hours 1 µM staurosporine treated SH-SY5Y and mock treated cell extract samples based on 1,000 µg/mL, 250 µg/mL and 1,000 µg/mL extract loads, respectively. The concentrations of Cleaved Tau were measured in duplicate and interpolated from the Cleaved Tau standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Cleaved Tau concentration was determined to be 7.93 ng/ mL in 2 hours staurosporine treated SH-SY5Y extract, 5.80 in 4 hours staurosporine treated SH-SY5Y extract and 2.42 in mock treated SH-SY5Y extract.

17. Assay Specificity

This kit recognizes both native and recombinant human and mouse Cleaved Tau protein in serum, plasma, cell culture supernatant, cerebrospinal fluid, and cell and tissue extract samples only. This kit is designed to detect all Tau isoforms only when cleaved. For cleavage definition see the Overview section.

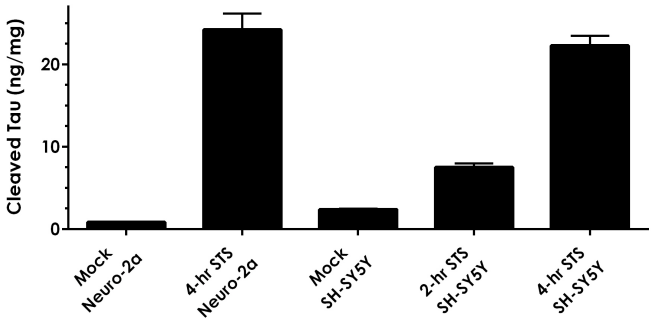


Figure 6. Comparison of staurosporine treated and mock treated Neuro-2a and SH-SY5Y cell extracts. SH-SY5Y cells were cultured in the presence of 1 µM staurosporine for 2 hours and 4 hours, or in the presence of staurosporine solvent (mock) for 4 hours. Neuro-2a cells were cultured in the presence of 1 µM staurosporine for 4 hours, or in the presence of staurosporine solvent (mock) for 4 hours. The concentrations of Cleaved Tau were measured in three different dilutions of the cell extract samples in duplicates and interpolated from the Cleaved Tau standard curve. The interpolated dilution factor corrected values are plotted in ng of Cleaved Tau per mg of extract (mean +/- SD, n=3). The mean Cleaved Tau concentration was determined to be 0.85 ng/mg in Neuro-2a (mock), 24.6 ng/mg in Neuro-2a (4-hours STS), 2.37 ng/mg in SH-SY5Y (mock), 7.49 ng/mg in SH-SY5Y (2-hours STS), 22.2 ng/mg in SH-SY5Y (4-hours STS) cell extracts.

Urine, milk and saliva samples have not been tested with this kit.

CROSS REACTIVITY

Recombinant mouse MAPT fragment (aa598-713) with carboxy-terminal tag, human MAPT 316 (TAU-A isoform) and mouse MAPT 732 (PNS-Tau isoform) were prepared at 10 ng/mL and 5 ng/mL and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE

Recombinant mouse MAPT fragment (aa598-713) with carboxy-terminal tag, human MAPT 316 (TAU-A isoform) and mouse MAPT 732 (PNS-Tau isoform) were prepared at 50 ng/mL and 5 ng/mL and tested for interference. 12% interference with mouse MAPT fragment (aa598-713) with carboxy-terminal tag was observed. No significant interference with human MAPT 316 and mouse MAPT 732 was observed.

18. Species Reactivity

This kit recognizes human and mouse Cleaved Tau protein.

Other species reactivity not determined.

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB Development Solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)