ab325894 – Human Tau (phospho S396) SimpleStep ELISA® Kit – Extracellular, Chemiluminescent

For the quantitative measurement of Tau (phospho S396) in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, and CSF.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab325894

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Limitations: All data, except typical standard curve and sensitivity, serum from individual healthy female donors, serum/plasma (EDTA)/CSF from individual Alzheimer's Disease donors, and CSF from pooled normal donors, were collected using the colorimetric version of this kit (ab324328).

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Human Tau (phospho S396) Capture Antibody 10X	600 µL	+4°C
Human Tau (phospho S396) Detector Antibody 10X	600 µL	+4°C
Human Tau (phospho S396) Lyophilized Chemically Ligated Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI2	6 mL	+4°C
Sample Diluent NS	12 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
ChemiHRP Reagent A	3 mL	+4°C
ChemiHRP Reagent B	3 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

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

Luminometer with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

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.

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.

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.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

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).

1. Reconstitute the Tau (phospho S396) standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 112,000 pg/mL **Stock Standard** Solution.
2. Label nine tubes, Standards 1– 9.
3. Add 309 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-9.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	27	309	112,000	9,000
2	Standard#1	75	150	9,000	3,000
3	Standard#2	75	150	3,000	1,000
4	Standard#3	75	150	1,000	333.33
5	Standard#4	75	150	333.33	111.11
6	Standard#5	75	150	111.11	37.04
7	Standard#6	75	150	37.04	12.35
8	Standard#7	75	150	12.35	4.12
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	≤ 50%
Plasma – Citrate	≤ 25%
Plasma – EDTA	≤ 25%
Plasma – Heparin	≤ 25%
SH-SY5Y Cell Culture Supernatant*	≤ 100%
CSF	≤ 50%

*Media is DMEM containing 10% fetal bovine serum.

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. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.
Plasma Collect plasma using citrate, EDTA, or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:4 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.
Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Assay, or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.
Cerebrospinal Fluid (CSF) Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

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.

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.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
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.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 30 minutes at room temperature on a plate shaker set to 750 rpm.
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 30 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.
7. Add 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

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For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human Tau (phospho S396). This pair does not recognize human Tau that is non-phosphorylated at S396. The amino acids numbering is based on Tau-F (2N4R, Uniprot P10636-8).

The standard protein in this kit is full length human Tau-A (Uniprot P10636-3) ligated with a peptide identical to the region surrounding S396, containing phosphorylated S396.

Native signal was detected in individual post-mortem CSF sample types.

Spiked protein experiments were used to validate serum, plasma (citrate), plasma (EDTA), plasma (heparin), and cell culture supernatant sample types.

50% pooled serum, 25% pooled plasma (citrate, EDTA, Heparin) and 50% pooled CSF samples from healthy donors were measured in duplicate. All values were below the detectable range of the assay.

50% serum samples from ten individual healthy human female donors was measured in duplicate. One donor interpolated dilution factor corrected value was 125.5 pg/mL. All remaining donors values were below the detectable range of the assay.

50% serum from five individual Alzheimer's Disease donors, 25% plasma (EDTA) from four individual Alzheimer's Disease donors and 50% CSF from six individual Alzheimer's Disease donors were measured in duplicate. All values were below the detectable range of the assay.

CROSS REACTIVITY

25,600 pg/mL of the following recombinant full length human Tau-A proteins each ligated with a peptide identical to the region surrounding the respective modified amino acid and containing the phosphorylated amino acid were tested for cross reactivity:

Recombinant Protein	% Cross Reactivity
Human Tau-A non-phosphorylated S396	none
Human Tau-A phospho T181	none
Human Tau-A phospho S199	6.2%
Human Tau-A phospho T217	none
Human Tau-A phospho T231	none

INTERFERENCE

12,800 pg/mL of the following recombinant full length human Tau-A proteins each ligated with a peptide identical to the region surrounding the respective modified amino acid and containing the phosphorylated amino acid were tested for interference with 1,600 pg/mL Human Tau (phospho S396):

Recombinant Protein	% Interference
Human Tau-A non-phosphorylated S396	-3.4%
Human Tau-A phospho T181	+4.1%
Human Tau-A phospho S199	+18.4%
Human Tau-A phospho T217	-8.8%
Human Tau-A phospho T231	-3.5%

SPECIES REACTIVITY

Other species reactivity was determined by measuring brain tissue extract samples of various species using Human Tau (phospho S396) ELISA kit ab324329 which uses the same antibody pair.

Reactivity was observed for the following other species: Mouse and Rat
Other species reactivity not determined.

50% mouse CSF sample was measured in duplicate. The interpolated dilution factor corrected value (to neat sample) was 494 pg/mL.

50% rat and bovine CSF samples were measured in duplicate. All values were below the detectable range of the assay.

CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence 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.
- Note: Most chemiluminescence 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.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted chemiluminescence 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.
- Samples generating chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence values less than that of the lowest standard should be retested in a less dilute form.

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 (pg/mL)	RLU		Mean RLU
	1	2	
0	3,345	3,456	3,401
4.12	4,681	5,010	4,846
12.35	8,133	7,919	8,026
37.04	17,867	18,133	18,000
111.11	44,199	43,784	43,992
333.33	132,730	136,340	134,535
1,000	428,740	431,130	429,935
3,000	1,252,400	1,302,700	1,277,550
9,000	3,728,700	3,775,800	3,752,250

Table 1. Example of human Tau (phospho S396) standard curve in Sample Diluent NS. The Tau (phospho S396) standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 2.27 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=16) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of Tau (phospho S396) 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 (%)
50% Serum	81	78 - 84
25% Plasma – Citrate	93	90 - 97
25% Plasma – EDTA	85	83 - 88
25% Plasma – Heparin	87	82 - 89
100% SH-SY5Y Cell Culture Supernatant*	100	98 - 103
50% CSF	90	87 - 95

*Media is DMEM 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 Tau (phospho S396) 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	50% Post-Mortem CSF
Undiluted	pg/mL	951.5
	% Expected value	100%
2	pg/mL	425.1
	% Expected value	89%
4	pg/mL	210.6
	% Expected value	89%
8	pg/mL	105.1
	% Expected value	88%

Recombinant Tau (phospho S396) was spiked into the following biological samples and then diluted in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Serum	25% Plasma (Citrate)	25% Plasma (EDTA)	25% Plasma (Heparin)	100% SH-SY5Y Supernatant
Undiluted	pg/mL	1,133.7	1,670.1	1,583.4	1,506.2	1,686.4
	% Expected value	100%	100%	100%	100%	100%
2	pg/mL	585.2	811.8	772.2	792.2	857.6
	% Expected value	103%	97%	98%	105%	102%
4	pg/mL	328.6	438.5	398.5	410.1	420.0
	% Expected value	116%	105%	101%	109%	100%
8	pg/mL	170.1	220.6	217.0	211.0	209.3
	% Expected value	120%	106%	110%	112%	99%
16	pg/mL	83.1	117.1	115.3	113.0	108.7
	% Expected value	117%	112%	117%	120%	103%

Precision

Mean coefficient of variations of interpolated values of Tau (phospho S396) from two concentrations of recombinant protein spiked into serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	4.6	1.1

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

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