ab325447 – Human Tau (phospho T217) SimpleStep ELISA® Kit, Chemiluminescent

For the quantitative measurement of Tau (phospho T217) 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.

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, Sensitivity, individual post-mortem CSF samples, and individual serum and CSF Alzheimer's disease samples, were collected using the colorimetric version of this kit (ab318936).

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Human Tau (phospho T217) Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Human Tau (phospho T217) Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Human Tau (phospho T217) Lyophilized Chemically Ligated Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent CPI2	6 mL	10 x 6 mL	+4°C
Sample Diluent NS	12 mL	2 x 50 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
ChemiHRP Reagent A	3 mL	50 mL	+4°C
ChemiHRP Reagent B	3 mL	50 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	10 x 96 Wells	+4°C
Plate Seal	1	10	+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.
Plate shaker for all incubation steps.
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 T217) 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 240 ng/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 304 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 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**	80	304	240,000	50,000
2	Standard#1	75	150	50,000	16,666.7
3	Standard#2	75	150	16,666.7	5,555.6
4	Standard#3	75	150	5,555.6	1,851.9
5	Standard#4	75	150	1,851.9	617.3
6	Standard#5	75	150	617.3	205.8
7	Standard#6	75	150	205.8	68.6
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	≤ 25%
Plasma – Citrate	≤ 25%
Plasma – EDTA	≤ 25%
Plasma – Heparin	≤ 25%
SH-SY5Y Cell Culture Supernatant*	≤ 12.5%
Cell Culture Media*	≤ 100%
Cerebrosprinal Fluid	≤ 100%

*Media is RPMI 1640 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 1:4 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. Dilute samples at least 1:8 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.
Cerebrospinal Fluid (CSF) Assay or dilute 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:
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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human Tau (phospho T217). This pair does not recognize tau that is non-phosphorylated at T217. 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 T217, containing phosphorylated T217.

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

25% pooled serum, 25% pooled plasma (citrate, EDTA, Heparin) and 100% pooled CSF samples from healthy donors were measured in duplicate. All values were below the detectable range of the assay.

25% serum from ten individual healthy human female donors were measured in duplicate. All values were below the detectable range of the assay.

100% post-mortem CSF from six donors were measured in duplicate. All values were below the detectable range of the assay.

25% serum from five individual Alzheimer's disease donors and 100% CSF from four individual Alzheimer's disease donors were measured in duplicate. All values were below the detectable range of the assay.

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

For the measurement of Tau (phospho T217) in cell extract and tissue extract sample types, use Human Tau (phospho T217) ELISA kit ab318935.

CROSS REACTIVITY

100 ng/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:

Human Tau-A non-phosphorylated T217
Human Tau-A phospho T181
Human Tau-A phospho S199
Human Tau-A phospho T231
Human Tau-A phospho S396

No cross reactivity was observed.

INTERFERENCE

100 ng/mL of recombinant full length human Tau-A proteins each ligated with a peptide containing a specific phosphorylated amino acid were tested for interference with 10 ng/mL of full length human Tau-A ligated with a peptide containing phosphorylated T217 (human Tau phospho-T217):

Tested Interferent	Decreased Interpolated Value of Human Tau-A phospho-T217
Human Tau-A non-phosphorylated T217	1.34-fold (25%)
Human Tau-A phospho T181	1.31-fold (23%)
Human Tau-A phospho S199	1.19-fold (16%)
Human Tau-A phospho T231	1.26-fold (20%)
Human Tau-A phospho S396	1.14-fold (13%)

SPECIES REACTIVITY

Other species reactivity was determined by measuring brain tissue extract samples of various species using Human Tau (phospho T217) ELISA kit ab318935 which uses the same antibody pair.

Reactivity was observed for the following species: Human, Mouse, and Rat

Other species reactivity not determined.

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	1,178	1,404	1,291
68.6	5,741	5,812	5,777
205.8	17,339	18,378	17,859
617.3	48,577	48,754	48,666
1,851.9	159,410	169,160	164,285
5,555.6	536,150	565,110	550,630
16,666.7	1,605,400	1,586,500	1,595,950
50,000	4,459,700	4,450,200	4,454,950

Table 1. Example of human Tau (phospho T217) standard curve in Sample Diluent NS. The Tau (phospho T217) 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 17.1 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=8) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of Tau (phospho T217) 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 (%)
25% Serum	84	80 - 87
25% Plasma – Citrate	82	76 - 89
25% Plasma – EDTA	82	77 - 85
25% Plasma – Heparin	82	77 - 87
12.5% SH-SY5Y Supernatant	86	81 - 92
100% Cell Culture Media*	89	87 - 94
50% Cerebrospinal Fluid	108	104 - 113

*Media is RPMI 1640 containing 10% fetal calf 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.

Recombinant Tau (phospho T217) was spiked into the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	25% Serum	25% Plasma (Citrate)	25% Plasma (Heparin)	100% CSF
Undiluted	ng/mL	11.44	10.87	11.70	25.96
	% Expected value	100%	100%	100%	100%
2	ng/mL	6.36	5.93	5.92	13.17
	% Expected value	111%	109%	101%	101%
4	ng/mL	2.91	2.91	3.11	7.15
	% Expected value	102%	107%	106%	110%
8	ng/mL	1.52	1.51	1.72	3.81
	% Expected value	106%	111%	118%	117%
16	ng/mL	0.80	0.82	0.86	1.89
	% Expected value	112%	120%	117%	116%

Dilution Factor	Interpolated value	25% Plasma (EDTA)	12.5% SH-SY5Y Supernatant	100% Cell Culture *Media
Undiluted	ng/mL	11.07	3.27	21.11
	% Expected value	100%	100%	100%
2	ng/mL	6.30	1.64	11.22
	% Expected value	114%	100%	106%
4	ng/mL	3.19	0.91	5.87
	% Expected value	115%	112%	111%
8	ng/mL	1.64	0.43	3.00
	% Expected value	118%	106%	114%

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

Precision

Mean coefficient of variations of interpolated values of Tau (phospho T217) from two concentrations of recombinant protein spiked into human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	4.5	2.9

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Figure 1 Standard Curve.
Standard curve comparison between the Chemiluminescent Human Tau (phospho T217) SimpleStep ELISA and colorimetric Human Tau (phospho T217) SimpleStep ELISA (ab318936) in Sample Diluent NS. Background-subtracted data values (mean +/- SD) are graphed.

Figure 2 Range Comparison.
Dynamic range comparison between the Chemiluminescent Human Tau (phospho T217) SimpleStep ELISA and colorimetric Human Tau (phospho T217) SimpleStep ELISA (ab318936).

Figure 3 Linearity of Dilution.
Recombinant Tau (phospho T217) was spiked into the following human samples and diluted in a 2-fold dilution series in Sample Diluent NS. Undiluted samples are 25% serum, plasma (citrate), plasma (EDTA), and plasma (heparin). The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

Figure 4 Linearity of Dilution.
(mean +/- SD, n=2).
Recombinant Tau (phospho T217) was spiked into the following samples and diluted in a 2-fold dilution series in Sample Diluent NS. Undiluted samples are as follows: SH-SY5Y cell culture supernatant 12.5%, cell culture media (RPMI1640 containing 10% fetal bovine serum)100%, and CSF 100%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

Figure 5 Specificity and Interference.
100 ng/mL of recombinant human Tau-A proteins each ligated with a peptide containing a specific phosphorylated amino acid (non-phosphorylated Tau, Tau pT181, Tau pS199, Tau pT231 and Tau pS396) were tested for specificity, and for interference with 10 ng/mL of recombinant Tau-A ligated with a peptide containing phosphorylated T217 (Tau pT217). The interpolated dilution-corrected values, expressed in % of input (for specificity) and in % of Tau pT217 control (for interference), are plotted (mean +/- SD, n=2).

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