Human Tau [pT181] phosphoELISA™ ELISA Kit

Catalog Number KHO0631 (96 tests)

Pub. No. MAN0004022 **Rev.** 2.0

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Human Tau [pT181] phosphoELISA™ ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human tau [pT181] in human cerebrospinal fluid (CSF), buffered solution, or cell culture medium. The assay recognizes both natural and recombinant human tau [pT181].

Human tau exists as six different isoforms that result from alternative splicing of a single transcript. The molecular weights of the tau isoforms range from 48 kDa to 68 kDa. Tau protein is highly soluble and normally attached to axonal microtubules, but circulating tau can be detected in cerebrospinal fluid (CSF) under certain conditions. Tau stabilizes the microtubules and makes them rigid.

Tau is regulated though phosphorylation at sites including threonine 181 by numerous serine/threonine kinases. The hyperphosphorylated form of tau is the major component of paired helical filaments (PHFs).

Contents and storage

Upon receipt, store the kit at 2 to 8°C.

Contents	Cat. No. KHO0631 (96 tests)
Hu Tau [pT181] Standard, lyophilized; contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide and red dye[1]	25 mL
Antibody Coated Wells, 96-well strip-well plate	1 plate
Hu Tau [pT181] Detection Antibody; contains 0.1% sodium azide and blue dye[1]	6 mL
Anti-Rabbit IgG HRP (100X); contains 3.3 mM thymol	0.125 mL
HRP Diluent; contains 3.3 mM thymol and yellow dye[1]	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

[1] To help monitor the addition of reagents to the reaction wells and avoid any pipetting errors, we provide colored Standard Diluent Buffer, Detection Antibody, and HRP Diluent. The colored dye does not interfere with the test results.

Required materials not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions
- Cell Extraction Buffer (Cat. No. FNN0011, or see "Prepare Cell Extraction Buffer")

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Prepare Cell Extraction Buffer

Note: See the *ELISA Technical Guide* for detailed information on preparing Cell Extraction Buffer.

1. Prepare 5 mL of Cell Extraction Buffer.

   Cell Extraction Buffer consists of 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, and 0.5% deoxycholate.
2. Immediately before use, add 1 mM PMSF (0.3 M stock in DMSO) and 250 µL protease inhibitor cocktail (e.g., Sigma Cat. No. P-2714).

Prepare cell lysate

1. Collect cells by centrifugation (non-adherent cells) or scraping from culture flasks (adherent cells), then wash cells twice with cold PBS.
2. Remove and discard the supernatant and collect the cell pellet. The pellet can be stored at −80°C and lysed at a later date if desired.
3. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes, on ice. Vortex at 10-minute intervals.

   Note: The volume of Cell Extraction Buffer used depends on the number of cells in the cell pellet, and expression levels of human tau [pT181]. [FOR EXAMPLE, 10^7 SH-SY5Y cells can be extracted in 0.5 mL of Cell Extraction Buffer to recover 1 mg/mL of total protein. Researchers must optimize the extraction procedures for their own applications].
4. Transfer the lysate into microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C.
5. Transfer the supernatant into clean microcentrifuge tubes. Samples can be stored at −80°C (avoid multiple freeze-thaw cycles).

Prepare brain homogenate

Note: See the *ELISA Technical Guide* for detailed information on preparing brain homogenates.

1. Weigh out ~100 mg (wet mass) of mouse brain sample in a microcentrifuge tube.
2. Add 8 × the brain mass of cold 5 M guanidine-HCl/50 mM Tris, pH 8.0 by 50–100 µL aliquots. Homogenize thoroughly after each addition.
3. Mix the homogenate at room temperature for 3–4 hours.
4. Dilute the sample ten-fold with cold PBS with 1X protease inhibitor cocktail (e.g., Sigma Cat. No. P-2714).
5. Centrifuge at 16,000 × *g* for 20 minutes at 4°C.
6. Transfer the supernatant into clean microcentrifuge tubes and keep on ice, or store at −80°C.

Pre-dilute samples

Because conditions may vary, we recommend that each investigator determine the optimal dilution for each application.

- Perform sample dilutions with Standard Diluent Buffer.
- Dilute samples prepared in 5 M guanidine-HCl/50 mM Tris, pH 8.0 1:10 to 1:100 with Standard Diluent Buffer.
- Dilute samples prepared in Cell Extraction Buffer 1:5 or greater in Standard Diluent Buffer (e.g., 10 µL sample into 40 µL buffer).

  This dilution is necessary to reduce the matrix effect of the Cell Extraction Buffer. SDS concentration should be less than 0.01% before adding to the plate. While a 1:5 sample dilution has been found to be satisfactory, higher dilutions such as 1:10 or 1:20 may be optimal.

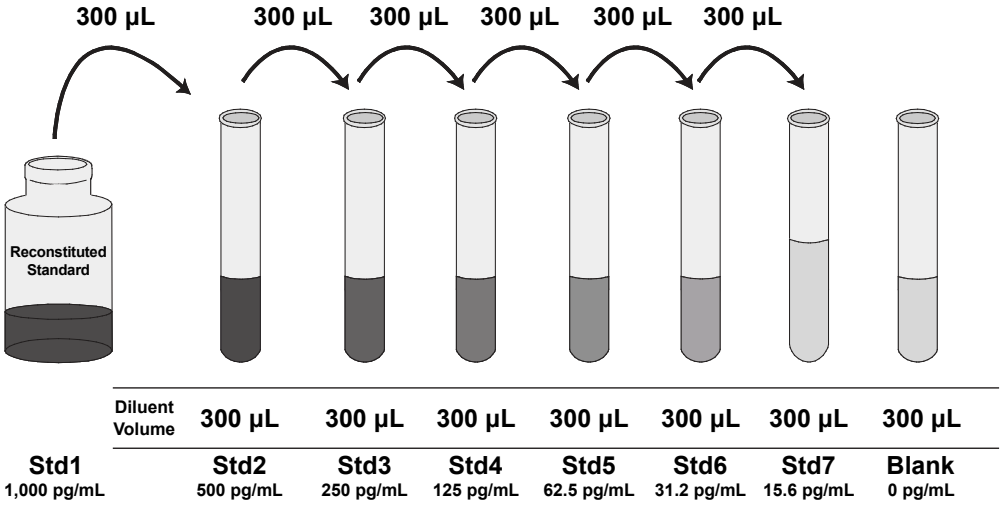
  For 10^7 SH-SY5Y cells, use 0.5–10 µL of the clarified lysate diluted to 50 µL in Standard Diluent Buffer for each well.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: The Hu Tau [pT181] Standard was calibrated using GSK-3β-phosphorylated, recombinant Hu Tau-441 protein expressed in *E. coli*, and SMCC conjugated to phosphopeptide T181.

1. Reconstitute Hu Tau [pT181] Standard to 1,000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 1,000 pg/mL human tau [pT181]. **Use the standard within 1 hour of reconstitution.**
2. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 500, 250, 125, 62.5, 31.2, 15.6, and 0 pg/mL of human tau [pT181].
3. Make serial dilutions of the standard as shown in the dilution diagram. Mix thoroughly between steps.
4. Remaining reconstituted standard should be discarded or frozen in aliquots at -80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



Prepare 1X Anti-Rabbit IgG HRP solution

Note: Prepare 1X Anti-Rabbit IgG HRP solution within 15 minutes of usage.

The Anti-Rabbit IgG HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

1. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
2. Return the unused Anti-Rabbit IgG HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 15 to 19 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 **Bind antigen and add detector**

a. Add 50 µL of standards, controls, or samples (see "Pre-dilute samples"on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Add 50 µL of Hu Tau [pT181] Detection Antibody solution into each well except the chromogen blanks.
c. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate 14 to 18 hours at 4°C.
d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 **Add IgG HRP**

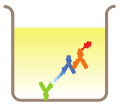
a. Add 100 µL 1X Anti-Rabbit IgG HRP solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 **Add Stabilized Chromogen**

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

4 **Add Stop Solution**



Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data was obtained for the various standards over the range of 0 to 1,000 pg/mL Hu Tau [pT181].

Standard Human Tau [pT181] (pg/mL)	Optical Density (450 nm)
1,000	3.34
500	1.26
250	0.65
125	0.34
62.5	0.22
31.2	0.17
15.6	0.13
0	0.10

Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	33.1	124.3	480.9
Standard Deviation	3.21	4.58	15.29
% Coefficient of Variation	10	4	3

Intra-assay precision

Samples of known Hu Tau [pT181] concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	35.6	126.5	492.2
Standard Deviation	2.11	4.60	15.98
% Coefficient of Variation	6	4	3

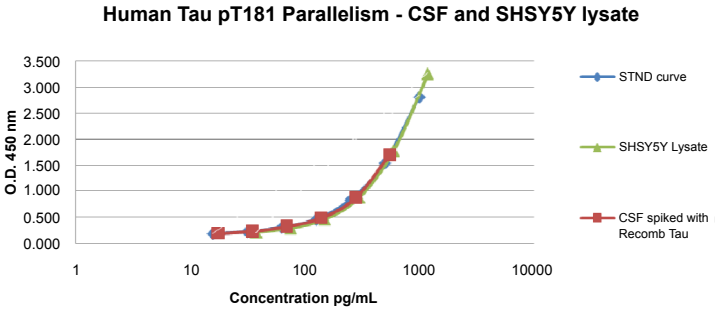
Linearity of dilution

Human CSF samples spiked with human tau [pT181] and natural human tau [pT181] from SH-SY5Y neuroblastoma cell extracts were 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.

Dilution	CSF			SH-SY5Y Cell Lysate			
	Measured (pg/mL)	Expected (pg/mL)	%	Dilution	Measured (pg/mL)	Expected (pg/mL)	%
Neat	556.99	556.99	100	1/64	1,196.90	1,196.90	100
1/2	261.60	278.50	93.93	1/128	588.14	598.45	98
1/4	128.66	139.25	92.39	1/256	266.44	299.22	89
1/8	70.82	69.62	101.72	1/512	120.43	149.61	80
1/16	37.10	34.81	106.57	1/1024	59.89	74.81	80
1/32	20.27	17.41	116.44	1/2048	30.55	37.40	82

Parallelism

Human CSF was spiked with Hu Tau [pT181], and both CSF samples and natural Hu Tau [pT181] from SH-SY5Y neuroblastoma cell extract were serially diluted in Standard Diluent Buffer over the range of the assay. The optical density of each dilution was plotted against the standard curve. The standard accurately reflects natural Hu Tau [pT181] content in samples.



Sensitivity

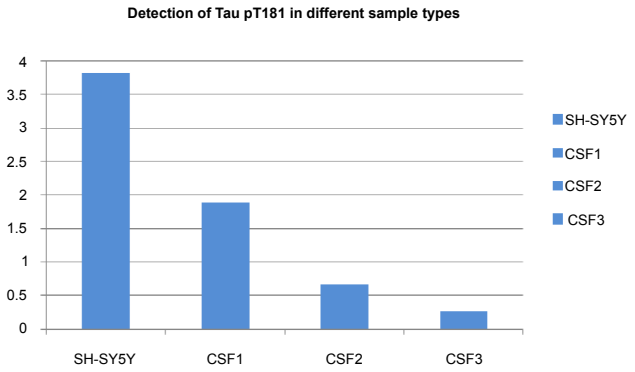
The analytical sensitivity of the assay is <10 pg/mL human tau [pT181]. This was determined by adding two standard deviations to the mean O.D. obtained from 64 assays of the zero standard.

Specificity

The peptide blocking competition data presented shows that only the phosphopeptide containing the phosphorylated threonine 181 could block the ELISA signal. The non-phosphorylated peptide sequence or other phosphopeptides from the human tau [pT181] sequence did not block the signal.

	Non-Phospho-peptide	pT181 Phospho-peptide	Non-specific phospho-peptides			
			pS396	pT231	pS199	pS214
Standard 0	0.05	0.05	0.06	0.06	0.06	0.06
Standard 1	1.98	2.00	2.04	1.95	1.90	2.02
Peptide Blocking	1.91	0.33	2.04	1.89	1.93	2.06
	1.97	0.32	2.09	2.01	1.96	2.15

The Human Tau [pT181] phosphoELISA™ ELISA Kit is suitable for the measurement of human tau [pT181] in different sample matrixes. Human CSF and cell extract from neuroblastoma, were analyzed. Human CSF samples were spiked at various concentrations prior to performing assay. The data presented show that the kit detects various concentrations of human tau [pT181] in different sample types.



Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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