Human Tau [pT231] phosphoELISA™ Kit

Catalog Number KHB8051 (96 tests)

Pub. No. MAN0009956 **Rev.** 4.0 (31)

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 [pT231] phosphoELISA™ Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human tau [pT231] in human cerebrospinal fluid (CSF), buffered solution, or cell culture medium. The assay will recognize both natural and recombinant human tau [pT231].

Human Tau [pT231] is a microtubule-associated protein of considerable importance to neuronal axons of vertebrate brain. Human Tau [pT231] exists as six different isoforms that result from alternative splicing of a single transcript derived from a gene located on chromosome 17. The molecular weights of the tau isoforms range from 48–68 kDa. Human Tau [pT231] protein is highly soluble and normally attached to axonal microtubules. Tau stabilizes the microtubules and makes them rigid. Human Tau [pT231] interacts with actin in the cytoskeleton and neuronal outgrowth, anchors enzymes such as protein kinases and phosphatases, and regulates intracellular vesicle transport.

Contents and storage

Upon receipt, store the kit at 2–8°C.

Contents	Cat. No. KHB8051 (96 tests)
Hu Tau [pT231] Standard, lyophilized; contains 0.1% sodium azide. Refer to vial label for reconstitution volume	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide; red dye[1]	25 mL
Antibody Coated Plate; 96-well strip-well plate	1 plate
Hu Tau [pT231] Detection Antibody; contains 0.1% sodium azide; blue dye[1]	11 mL
Anti-Rabbit IgG HRP (100X)	0.125 mL
HRP Diluent; contains 3.3 mM thymol; 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; beakers, flask and cylinders for preparation of reagents
- 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 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 PMSF (0.3 M stock in DMSO) to 1 mM and 50 µL protease inhibitor cocktail (e.g., Sigma Cat. No. P-2714) for each 1 mL of Cell Extraction Buffer.

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 [pT231]. [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. Under these conditions, cell extract dilutions from 1:10 to 1:100 with Standard Diluent Buffer are sufficient for Tau [pT231] detection. 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).

Pre-dilute samples

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

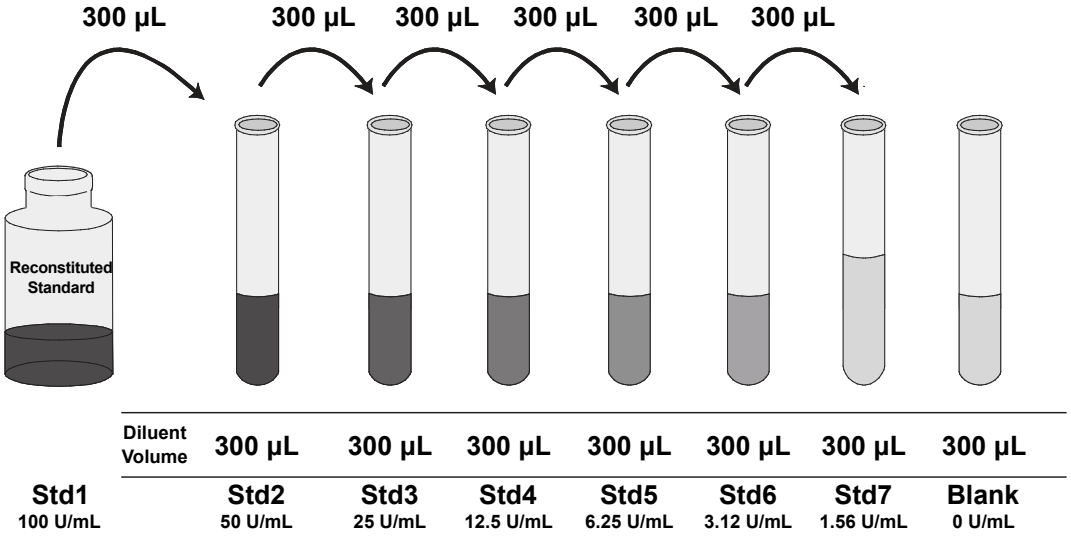
- Perform sample dilutions with Standard Diluent Buffer.
- Dilute samples prepared in Cell Extraction Buffer 1:50 or greater in Standard Diluent Buffer (e.g., 2 µL sample into 98 µL buffer). This dilution is necessary to avoid SDS interference with the assay.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: The Hu Tau [pT231] Phospho was calibrated using recombinant Hu Tau-441 protein expressed in *E. coli* conjugated to phospho-peptide T231.

1. Reconstitute Hu Tau [pT231] Phospho Standard to 100 U/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 100 U/mL human tau [pT231]. **Use the standard within 1 hour of reconstitution.**
2. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 50, 25, 12.5, 6.25, 3.12, 1.56, and 0 U/mL human tau [pT231].
3. Make serial dilutions of the standard as described below 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 Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

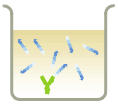
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, and dispense the solution into a tube containing 1 mL of 1X Assay Buffer. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 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



a. Add 100 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. Cover the plate with a plate cover and incubate 2 hours at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add detector antibody

a. Add 100 µL of Hu Tau [pT231] Phospho Detection Antibody solution into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate 1 hour at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 Add IgG HRP

a. Add 100 µL Anti-Rabbit IgG HRP solution (see page 2) 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.

4 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.

5 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 were obtained for the various standards over the range of 0 to 100 U/mL human tau [pT231].

Standard Hu Tau [pT231] Phospho (U/mL)	Optical Density (450 nm)
100	3.46
50	2.31
25	1.33
12.5	0.74
6.25	0.43
3.12	0.25
1.56	0.17
0	0.07

Inter-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	53.92	11.73	3.29
Standard Deviation	4.60	0.61	0.32
% Coefficient of Variation	8.53	5.22	9.60

Intra-assay precision

Samples of known human tau [pT231] concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	50.21	11.51	3.26
Standard Deviation	2.38	0.69	0.17
% Coefficient of Variation	4.74	5.99	5.33

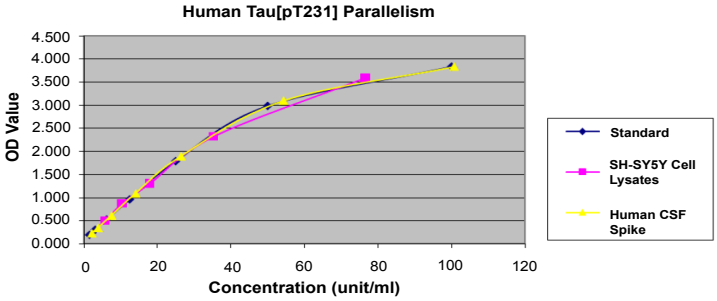
Linearity of dilution

Human CSF samples (spiked with recombinant human tau [pT231] and human tau [pT231] from SH-SY5Y 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 0.999 in CSF spiked with recombinant human tau [pT231] and 0.997 in SH--SY5Y cell lysate.

Dilution	CSF			SH-SY5Y Cell Lysate			
	Measured (U/mL)	Expected		Dilution	Measured (U/mL)	Expected	
		(U/mL)	%			(U/mL)	%
Neat	100.74	100.74	100	1/80	76.55	76.55	100
1/2	54.30	50.37	107.8	1/160	35.3	38.3	92.2
1/4	26.42	25.19	104.9	1/320	17.3	19.1	93.7
1/8	14.07	12.59	111.7	1/640	10.3	9.6	107.7
1/16	7.51	6.30	119.2	1/1280	5.8	4.8	120.6
1/32	3.85	3.15	122.3	—			

Parallelism

Human CSF samples (spiked with recombinant human tau [pT231] and human tau [pT231] from SH-SY5Y cell extracts 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 the full human tau [pT231] content in samples.



Recovery

The recoveries of human tau [pT231] added to human CSF and Cell Extraction Buffer (FNN0011) were measured on the Human Tau [pT231] phosphoELISA™ Kit.

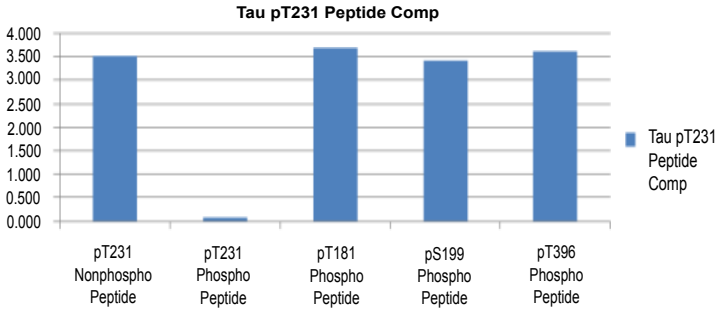
Sample	Average % Recovery
Human CSF	110
Cell Extraction Buffer	104

Sensitivity

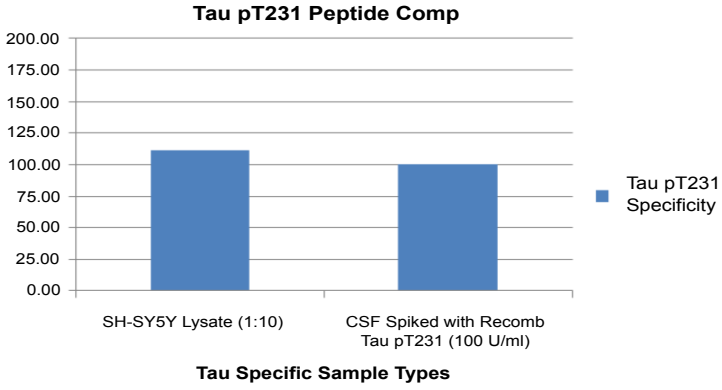
The analytical sensitivity of human tau [pT231] is <1 U/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 48 times over two separate assays.

Specificity

The peptide blocking competition data presented shows that only the phosphopeptide containing phosphorylated threonine 231 can block the ELISA signal. The non-phosphorylated peptide sequence or other phosphopeptides from the Tau sequence did not block the signal.



The Human Tau [pT231] phosphoELISA™ Kit is suitable for the measurement of human tau [pT231] in different sample matrices. 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 [pT231] 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: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.