Human TAU(pT217) ELISA Kit
Catalogue Number: OM641673
(96Tests)
Store all reagents at 2-8°C
Collect sample: serum or blood plasma
Assay range ： 0.19-12.5 ng/mL

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!
PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

INTENDED USE

This assay employs the Sandwich enzyme immunoassay technique. Determine the level of Human pTAU in the sample. Add standard and sample to the microplate that have been pre-coated with Anti-Human pTAU antibody. After incubation, add Biotin-conjugated anti-Human pTAU antibody. It is then combined with HRP-conjugated streptavidin to form an immune complex, then incubated and washed to remove unbound enzyme, and then added to the chromogenic substrate TMB to produce a blue color, and converted to the final yellow under the action of acid. Finally, the absorbance (OD) value was measured at 450 nm. The concentration of Human pTAU in the sample was proportional to the OD value. The concentration of Human pTAU in the sample can be calculated by drawing a standard curve.

PRINCIPLE OF THE ASSAY

For the determination of Human pTAU concentrations in serum, plasma and other related biological fluids.

REAGENTS PROVIDED

All reagents provided are stored at 2-8° C. Refer to the expiration date on the label.

1. Pre-coated micro well plates	96 wells	1 plate
2. Biotinylated Antibody	0.03mL	1 tube
3. Standard (25 ng/mL)	1 mL	1 tube
4.Streptavidin labeled HRP	0.03mL	1 tube
5. 10x Assay Buffer	30 mL	1 tube
6. Sample Diluent	30.0 mL	1 vial
7. Antibody diluent	30.0 mL	1 vial
8.TMB Substrate	18.0 mL	1 vial
9. Stop Solution	8 mL	1 vial
10. Instruction	1 copy	

SAMPLE COLLECTION AND STORAGE

Serum – Use serum separation tubes (SST). Allow the sample to clot for 30 minutes, then centrifuge at approximately 1000 × g for 15 minutes. Assay the separated serum immediately or aliquot and store at -20°C or -80°C.

Plasma – Collect plasma using EDTA or heparin as anticoagulant. Centrifuge at 1000 × g for 15 minutes at 2-8°C within 30 minutes of collection. Store the sample at -20°C or -80°C and avoid repeated freeze-thaw cycles.

Cell Culture Supernatant & Other Biological Fluids – Centrifuge to remove particulates and assay immediately, or aliquot and store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Note: Serum, plasma, and cell culture supernatant samples intended for use within 7 days may be stored at 2-8°C; otherwise, they must be stored at -20°C (for ≤2 months) or -80°C (for ≤6 months) to prevent loss of bioactivity and contamination. Avoid freeze-thaw cycles. For assay, allow the samples to equilibrate to room temperature slowly.

DO NOT USE HEAT-TREATED SPECIMENS.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. A Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes for transferring volumes ranging from 2 µL to 1 mL.
3. An adjustable 10 µL -100 µL pipettes for reagent preparation.
4. 100 mL and 1 L graduated cylinders.
5. Calibrated, adjustable precision pipettes, recommended to be used with disposable plastic tips. (Multi-channel pipettes are advisable for large-scale

assays.)
6. Absorbent blotting paper.
7. A 37°C constant temperature incubator.
8. Distilled or deionized water.
9. Data analysis and graphing software.
10. Test tubes for preparing standard solutions or sample dilutions.

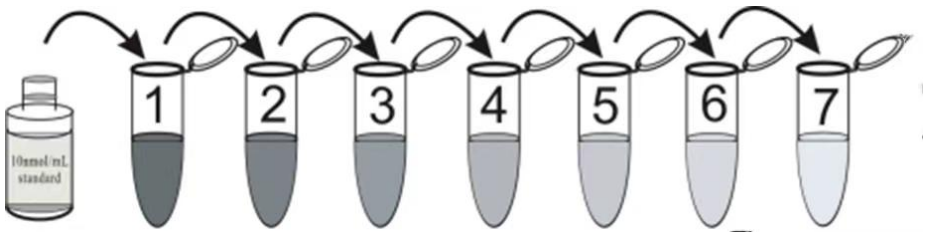
PRECAUTIONS

1. Do not interchange or mix reagents from different kit lots. The standards, labels, and microplate are matched to ensure optimal performance; use only the reagents provided by the manufacturer.
2. Bring all reagents and materials to room temperature (20-25°C) before use. Do not thaw samples or reagents using a water bath.
3. Do not use any kit components beyond their expiration date.
4. Use only deionized or distilled water for diluting reagents.
5. Remove the microplate strips from the protective pouch only immediately before use. Any unused strips should be resealed in the original pouch with the desiccant and stored at 2-8°C.
6. Use a fresh, disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid with sodium hypochlorite solutions.
8. Serum and plasma should be regarded as potentially hazardous materials capable of transmitting infectious agents. Disposable gloves must be worn during the assay procedure, as no method can fully guarantee that canine blood-derived products are free of transmissible pathogens. Therefore, all blood-derived products should be handled as potential sources of infection in accordance with good laboratory practices.
9. All samples should be processed using methods capable of inactivating viruses.
10. Solid Waste: Autoclave at 121°C for 60 minutes.
11. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste liquid must stand for at least 30 minutes to inactivate viruses before disposal.
12. Substrate Solution is susceptible to contamination. Do not use if it appears blue before use.
13. Chromogen Solution B contains 20% acetone. Keep away from heat sources or open flames.
14. Remove all kit reagents from the refrigerator and allow them to equilibrate to room temperature (20-25°C).

REAGENT PREPARATION

1. Bring all kit components and samples to room temperature (20-25℃) before use.

2.Standards - Centrifuge the standard at 10,000 × g for 1 min. This reconstitution produces a working solution of 25 ng/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 12.5, 6.25, 3.125, 1.56 , 0.78, 0.39, 0.19 ng/mL. Dilution method: Take 7 EP tubes, add 500 µl of Standard/Sample diluent to each tube. Pipette 500 µl of the 25 ng/mL working solution to the first tube and mix up to produce a 12.5 pg/mL working solution. Pipette 500 µl of the solution from the former tube into the latter one according to these steps.



3.Wash Buffer - Dilute 30 mL of Wash Solution concentrate (10×) with 270 mL of deionized or distilled water to prepare 300 mL of Wash Solution (1×). If crystals have formed in the solution, warm to room temperature and mix gently until the crystals have been completely dissolved. The 1×wash solution is stable for 2 weeks at 2-8℃.

ASSAY PROCEDURE

1. Remove the required strips from the aluminum foil bag equilibrated at room temperature for 20 minutes. Seal the remaining strips in a resealable bag and store at 4℃.
2. Set up standard wells and sample wells. Add 100 µ L of standard solutions at different concentrations to the standard wells, and 100 µ L of the test samples to the sample wells. Leave the blank wells empty. Incubate in a constant temperature incubator at 37℃ for 60 minutes.
3. Discard the liquid, blot dry on absorbent paper, add 250 µ L of wash buffer to each well, let stand for 1 minute, discard the wash buffer, and blot dry on absorbent paper. Repeat the wash process three times (an automated plate washer may also be used).
4. Add 100 µ L of detection antibody to each well and incubate in a constant temperature incubator at 37℃ for 60 minutes.
5. Discard the liquid, blot dry on absorbent paper, add 250 µ L of wash buffer to each well, let stand for 1 minute, discard the wash buffer, and blot dry on absorbent paper. Repeat the wash process three times (an automated plate washer may also be used).
6. Add 100 µ L of 1:2000 diluted secondary antibody to each well and incubate in a constant temperature incubator at 37℃ for 60 minutes.
7. Discard the liquid, blot dry on absorbent paper, add 250 µ L of wash buffer to

each well, let stand for 1 minute, discard the wash buffer, and blot dry on absorbent paper. Repeat the wash process four times (an automated plate washer may also be used).

8. Add 100 µ L of substrate chromogen solution (TMB) to each well and incubate at 37℃ in the dark for 15 minutes.

9. Add 50 µ L of stop solution to each well. Within 15 minutes, measure the optical density (OD) of each well at a wavelength of 450 nm.

TYPICAL DATA

CALCULATION OF RESULTS

Calculate the average optical density (OD) for each standard, control, and sample replicate. Subtract the average OD value of the zero standard from these averages. To generate the standard curve, plot the mean OD value for each standard on the y-axis against its concentration on the x-axis. Draw the best-fit curve through the plotted points. This method yields adequate, though less precise, fitting results. If samples were diluted, the concentration value obtained from the standard curve must be multiplied by the corresponding dilution factor.

