# Phospho-Tau (Thr181) ELISA Kit (Human) (OKGD00202)

## Instructions for use

For the quantitative measurement of Tau p181 in human serum and plasma and cell lysates.

Lot to lot variations can occur. Refer to the manual provided with the kit.

This product is intended for research use only.

# Table of Contents

# 1. Background

## Principle

Aviva Systems Biology's Phospho-Tau (Thr181) ELISA Kit (Human) (OKGD00202) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. A monoclonal antibody specific for Tau p181 has been pre-coated onto a 96-well plate. Standards or test samples are added to the wells, incubated and washed. A Biotin conjugated detector antibody specific for Tau p181 is added, incubated, and followed by wash. Next it is incubated with horseradish peroxidase (HRP)-Streptavidin, followed by wash. An enzymatic reaction is produced through the addition of TMB substrate which is catalyzed by HRP generating a blue color product that changes yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm is quantitatively proportional to the amount of sample Tau p181 captured in well.
Prot ID of human Tau p181, 2N4R isoform

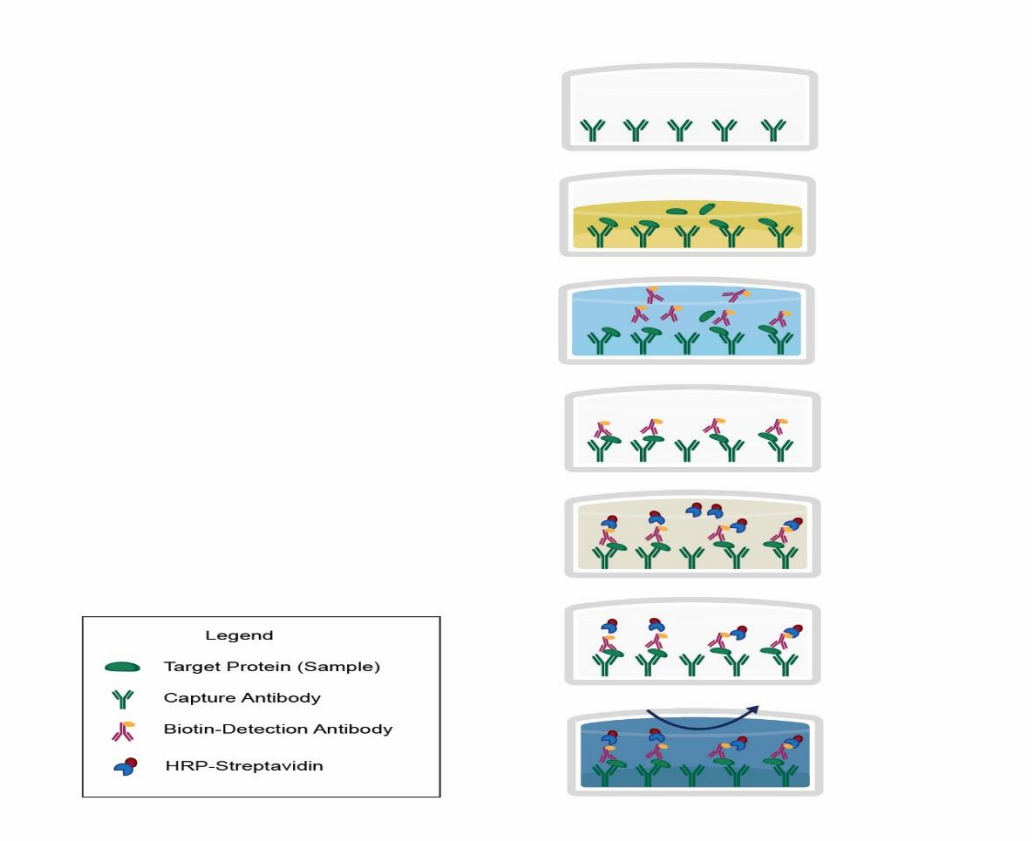
## Target Background

Microtubule-associated protein Tau p181, encoded by the MAPT gene, plays a critical role in microtubule assembly and cytoskeletal stability. Multiple isoforms are produced by alternative splicing, and these different isoforms carry out a variety of functions in many tissues. This protein is highly expressed in neurons of the central nervous system and is involved in establishing and maintaining neuronal polarity and cytoskeletal stabilization. In pathological conditions, such as Alzheimer's disease, Tau p181 undergoes hyperphosphorylation, leading to a reduction in its microtubule-stabilizing ability, aggregation into paired helical filaments, and formation of neurofibrillary tangles, contributing to neuronal degeneration and cognitive decline.

## General Specifications

| General Specifications | |
|---|---|
| Range | 15.63 - 1000 pg/ml |
| Limit of Detection (LOD) | ≤2 pg/mL (Derived by linear regression of $OD_{450}$ of the Mean Blank + 2xSD) |
| Target Information | Human Tau p181<br>UniProt ID: P10636-8<br>Gene ID:4137<br>Target Alias: MAPT, DDPAC, FTDP-17, MAPTL, MSTD, MTBT1, MTBT2, PPND, PPP1R103, microtubule associated protein tau p181 |
| Avg. Recovery | 102.23% |

## 2. Assay Summary




## 3. Storage and Stability

Upon receipt, **store Standard at -70°C**, Assay Diluent at -20°C, and the rest of kit components at 4°C until expiration date.

## 4. Kit Components

The following reagents are the provided contents of the kit:

| Assay Components | Quantity | Storage Condition |
|---|---|---|
| Microplate Coated with anti-pTau 181 | 1 x 96 Wells Plate | 4°C |
| Human pTau Standard | 10 ul | -70°C |
| Assay Diluent | 1 x 40 mL | -20°C |
| 500X Detector | 1 x 45 uL | 4°C |
| 100X Conjugate | 1 x 120 uL | 4°C |
| 20X Wash Buffer | 1 x 30 mL | 4°C |
| TMB Substrate | 1 x 12 mL | 4°C |
| Stop Solution | 1 x 12 mL | 4°C |

## 5. Precautions

- Read instructions fully prior to beginning use of the assay kit.
- Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For information on hazardous substances included in the kit refer to the Material Safety Data Sheet (MSDS).
- Kit cannot be used beyond the expiration date on the label.

## 6. Required Materials Not Supplied

- Microplate reader capable of reading absorbance at 450 nm.
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Distilled or deionized ultrapure water.
- 37°C Incubator
- Shaker

## 7. Technical Application Tips

- Do not mix or substitute components from other kits.
- To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
- Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
- Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.
- Replicate wells are recommended for standards and samples.
- Cover microplate while incubating to prevent evaporation.
- Do not allow the microplate wells dry at any point during the assay procedure.
- Do not reuse tips or tube to prevent cross contamination.
- Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
- Completely remove of all liquids when washing to prevent cross contamination.
- Prepare reagents immediately prior to use and do not store, apart from the top standard.
- For optimal results for inter- and intra-assay consistency, equilibrate all materials (except for standards) to room temperature prior to performing assay and perform all incubations at 37°C.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.
- Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
- Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
- Samples that are hemolytic, lipemic, or contain precipitates, fibrin strands or bilirubin may cause inaccurate results due to interfering factors.
- TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

# 8. Reagent Preparation

- Equilibrate all reagents and materials to ambient room temperature, except Standard, detector and conjugate. Keep Standard, detector and conjugate on ice.
- Prepare the Tau p181 standard immediately before plating in the 96 well plate. Standards should be held on ice until use in the experiment.

## 8.1 Human Tau Standard

**Preparation**

**8.1.1** Prepare the Tau p181 Standard (2N4R isoform) by performing pre-dilution step.

Pre-dilute using 2uL of stock and 398uL of Assay diluent.

Afterwards, using cold Assay Diluent. Add 2 µL of pre-diluted p181 standard to 2 mL of Assay Diluent **Standard #1**. Close, and **vortex gently**. Avoid exposure of standards to RT, keep on ice.

**8.1.2** Prepare a set of serially diluted standards as follows:

8.1.2.1 Label tubes 1—8 with each respective standard number.

8.1.2.2 Add 500 µL of **Assay Diluent** to the remaining tubes.

8.1.2.4 Prepare **Standard #2** by adding 500 µL of **Standard #1** from Tube #1 to Tube #2. **Vortex gently** to mix.

8.1.2.5 Prepare **Standard #3** by adding 500 µL of **Standard #2** from Tube #2 to Tube #3. **Vortex gently** to mix.

8.1.2.6 Prepare further serial dilutions in the remaining tubes. Reference the table below as a guide for serial dilution scheme.

8.1.2.7 The last tube is a blank standard (only **Assay Diluent**), which should be included with every experiment.

| Standard Number (Tube) | Standard To Dilute (pg/mL) | Volume Standard to Dilute (µL) | Volume Assay Diluent (µL) | Total Volume (µL) | Final Concentration (pg/mL) |
|---|---|---|---|---|---|
| 1 | Pre-diluted Tau p181 Standard | 2 uL of pre-dilution | 2000 | 2000 | 1000.000 |
| 2 | 1000.000 | 500 | 500 | 1000 | 500.000 |
| 3 | 500.000 | 500 | 500 | 1000 | 250.000 |
| 4 | 250.000 | 500 | 500 | 1000 | 125.000 |
| 5 | 125.000 | 500 | 500 | 1000 | 62.500 |
| 6 | 62.500 | 500 | 500 | 1000 | 31.250 |
| 7 | 31.250 | 500 | 500 | 1000 | 15.630 |
| 8 | NA | 0 | 500 | 1000 | 0.000 |

## 8.2 Detector Working Solution

**8.2.1** For each 96 wells to be used in the experiment prepare 11 mL of **1XTau p181 Detector Working Solution** by adding 44 uL of **100X Detector** to 10.956 mL of **Assay Diluent**. Detector solution must be at RT.

**8.2.2** Mix thoroughly and gently. Hold no longer than 5 minutes prior to use in procedure. Do not store 1X concentration for future use.

## 8.3 Conjugate Working Solution

**8.3.1** For each 96 wells to be used in the experiment prepare 11 mL of **1X Conjugate Working Dilution** add 110 µL of **100X Conjugate** to 10.89 mL of **Assay Diluent**. Reporter solution must be at RT.

## 8.4 1X Wash Buffer

**8.4.1** If crystals have formed in the **20X Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.
**8.4.2** Add the entire 30ml contents of the **20X Wash Buffer** bottle to 570ml of ultra-pure water in a clean bottle.
**8.4.3** Mix gently and thoroughly by magnetic stirrer. **Avoid foaming or bubbles.**
**8.4.4** Hold the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 2 weeks. Do not freeze.

## 8.5 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Any unused well strips should be sealed in the original packaging and stored at 4°C with desiccant.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

# 9. Sample Preparation

## 9.1 Sample Preparation and Storage

- Store samples to be assayed at 2-8°C for 24 hours prior to being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.
- This assay is intended for serum, plasma and other biological fluids. The sample preparation protocols below have been provided for your reference:

    - **Serum** - Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

    - **Plasma** - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

    - **Cell culture supernatants** – Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Note: Samples that are hemolytic, lipemic, or contain precipitates, fibrin strands or bilirubin may cause inaccurate results due to interfering factors

## 9.2 Sample Dilution

- Target protein concentration must be estimated, and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.
    - Dilute cell culture supernatant or recombinant protein samples using **Assay Diluent**. Special assay diluent (not included) may be required for serum, plasma and tissue homogenates samples.
    - Mix diluted samples gently and thoroughly.
    - Pipetting less than 2 µL is not recommended for optimal assay accuracy.

## 10. Assay Procedure

Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure, except Standard, detector and conjugate. Keep detector and conjugate on ice. Standard is to be used immediately after preparation from -70°C storage.

**10.1** Add 100 µL of the serially diluted Tau p181 **Standards**, diluted samples or blank into wells of the **Anti-Tau p181 Microplate**. At least two replicates of each standard, sample or blank, is recommended.

**10.2** Cover the plate with a plate seal and incubate for 120 minutes at 37°C.

**10.3** Wash plate 3 times with 300 µL **1X Wash Buffer** using plate washer.

**10.4** Add 100 µL of prepared **1X Detector Working Solution** to each well.

**10.5** Cover the plate with plate seal and incubate for 60 minutes at room temperature with gentle shaking.

**10.6** Wash plate **5 times** with 300 µL **1X Wash Buffer**

**10.7** Add 100 µL of prepared **1X Conjugate Working Solution** to each well.

**10.8** Cover the plate with plate seal and incubate for 30 minutes at room temperature, gently shaking.

**10.9** Wash plate **5 times** with 1X Wash Buffer using plate washer. Tap the plate to remove any liquid inside wells.

Add 100 µL of TMB Substrate to each well, seal with cover and incubate for 5-30 minutes at RT in the dark. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time. (NOTE: TMB Substrate must be pre-warmed to room temperature before adding to the plate. Incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells. The blank (final standard point) should be faint blue to clear.)

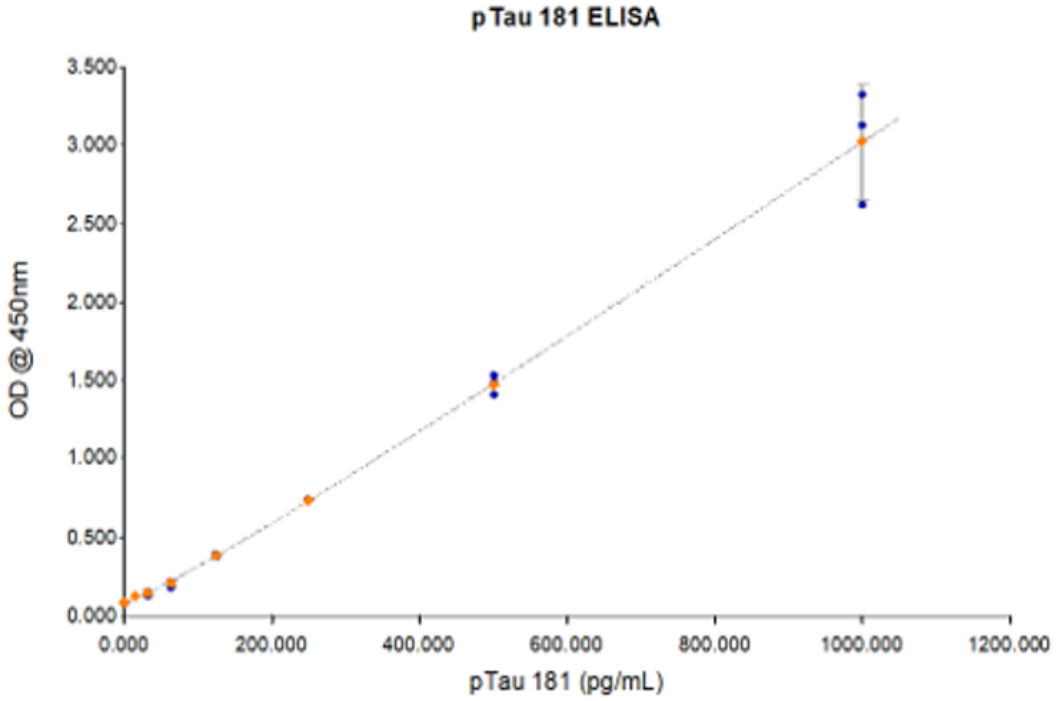
**10.10** Add 100 µL of **Stop Solution** to each well. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for the **TMB Substrate.**

**10.11** Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction.

## 11. Calculation of Results

For analysis of the assay results, average the duplicate readings for each standard and samples, then subtract average zero standard optical density.
Plot a four or five parameter logistic curve with Standard concentration on the x-axis and OD values on the Y-axis.
If the OD of the sample surpasses the upper limit of the standard curve, the sample should be diluted further and re-tested.

### 11.1 Typical data




| Curve Name | Curve Formula | A | B | C | D | R2 |
|---|---|---|---|---|---|---|
| StdCurve | Y = (A-D)/(1+(X/C)^B) + D | 0.0808 | 1.12 | 1.07E+04 | 45.5 | 1 |

## 12. Technical Resources

**Technical Support:**

For optimal service please be prepared to supply the lot number of the kit used.

USA

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