Human Tau ELISA Kit

Enzyme Immunoassay for the quantification of human Tau in cell culture supernatant, serum, cell culture extracts, plasma, cerebral spinal fluid.

Catalog number: ARG82994

Package: 96 wells

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INTRODUCTION

This gene encodes the microtubule-associated protein tau (MAPT) whose transcript undergoes complex, regulated alternative splicing, giving rise to several mRNA species. MAPT transcripts are differentially expressed in the nervous system, depending on stage of neuronal maturation and neuron type. MAPT gene mutations have been associated with several neurodegenerative disorders such as Alzheimer's disease, Pick's disease, frontotemporal dementia, cortico-basal degeneration and progressive supranuclear palsy. [provided by RefSeq, Jul 2008]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique for the quantification of human Tau protein in Cell culture supernatant, serum, plasma, cell / tissue extracts. Standards or samples are pipetted into the wells and then add the tagged anti-Tau antibody and HRP-conjugated anti-Tau antibody mixture. Human tau protein will bind to the tagged anti-Tau antibody and HRP-conjugated anti-Tau antibody. The antibodies and Tau protein complex will then bind to the anti-tag antibody coated on the well. After washing away any unbound materials, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of human Tau protein bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of human Tau protein in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	96 well	4°C
Protein Standards	2 vials (23.77ng)	4°C, Lyophilized
Sample Diluent	50 ml	4°C
10X anti-Tau antibody concentrate	600 µl	4°C
10X HRP-conjugated anti-Tau antibody concentrate	600 µl	4°C
Antibody Diluent	6 ml	4°C
5X Cell Extraction Buffer	10 ml	4°C
10X Wash buffer	20 ml	4°C
TMB Substrate	12 ml	4°C (Protect from light)
STOP solution	12 ml	4°C
Plate sealer	1	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Plate shaker for the incubation steps (~400 rpm)
- Automated microplate washer (optional)
- Phenylmethylsulfonyl Fluoride (PMSF) or other protease inhibitors (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- The plate should be sealed or covered in the incubation period.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Always add samples or standards to the well before the addition of the antibodies mixture to avoid high background occurred.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants- Remove particulates by centrifugation for 10 min at 2000 x g at 4°C and aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles. Dilute samples at least 1:2 with Sample Diluent before assay.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30

minutes before centrifugation for 10 minutes at 2000 x g. Collect serum, and assay immediately. (Samples can be diluted into Sample Diluent and the dilution rate depending on the Tau concentration in the samples, it is recommended diluted serum samples at least 1:2 with Sample Diluent before assay). Or aliquot and store the undiluted samples at ≤-20 °C. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 10 minutes at 2000 x g within 30 minutes of collection. Assay immediately (Samples can be diluted into Sample Diluent and the dilution rate depending on the Tau concentration in the samples, it is recommended diluted plasma samples at least 1:4 with Sample Diluent before assay) or aliquot and store the undiluted samples at ≤-20 °C. Avoid repeated freeze-thaw cycles.

Cell pellets extraction- Collect cells and centrifuge for 5 minutes at 500 x g at 4 °C. Wash cells twice with PBS by centrifugation (5 minutes, 500 x g at 4 °C). Resuspend the pellet in ice-cold 1X Cell Extraction solution (per ml for $2x10^7$ cells). Incubate on ice for 20 minutes followed by centrifuging for 20 minutes at 18,000 x g at 4 °C. Transfer the supernatants into clean tubes. The protein concentrations in the extract could be quantified using a standard protein assay. Samples could be diluted by **1X Cell Extraction solution** to appropriate concentration. Assay the extract immediately or aliquot and store at -80°C. Avoid repeated freeze-thaw cycles.

Tissue homogenates extraction- Tissues should be minced in to small pieces by coarse mincing of the tissue with scissors, scalpels, or a mincer, and then thoroughly washed with PBS to remove blood. Then a homogenizer is used to disrupt these tissues (dounce homogenizer is recommended). Extract 100- 200

µg of homogenized tissue by adding 0.5-1 ml of ice-cold 1X Cell Extraction solution and mix the tissues and buffer by pipetting up and down gently for a few times. Incubate on ice for 20 minutes followed by centrifuging for 20 minutes at 18,000 x g at 4 °C. Transfer the supernatants into clean tubes. The protein concentrations in the extract could be quantified using a standard protein assay. Samples could be diluted by **1X Cell Extraction solution** to appropriate concentration. Assay the extract immediately or aliquot and store at-80°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- Prepare only as much reagent as is needed on the day of the experiment.
- **1X Wash Buffer**: Dilute **10X** Wash buffer into **distilled water** to yield 1X Wash buffer. (E.g., add 10 mL of 10X Wash Buffer into 90 mL of distilled
- water to a final volume of 100 mL). The 1X Wash Buffer can be stored at
- room temperature.
- **1X Cell Extraction solution**: Dilute **5X** Cell Extraction Buffer into **distilled water.** (e.g. For 20 ml Cell Extraction solution: 4 ml Cell Extraction Buffer + 16ml distilled water) Gently mix the solution before use. Protease inhibitors can be added if needed.
- **1X Antibody Mixture**: Dilute 10X Anti-Tau antibody concentrate and 10X HRP-conjugated anti-Tau antibody concentrate into Antibody Diluent. (e.g. For 6 ml Antibody Mixture: 0.6 ml of 10X anti-Tau antibody concentrate + 0.6 ml of 10X HRP-conjugated anti-Tau antibody concentrate + 4.8 of Antibody Diluent). Prepare only as much antibody as is needed on the day of the experiment.

- **Samples**: If the initial assay found samples contain Tau protein higher than the highest standard, the samples can be diluted with Sample Diluent or 1X Cell Extraction solution and then re-assay the samples.

  For the calculation of the concentrations this dilution factor has to be taken into account.

  Example:

  a) Dilution 1:10: 10 µl Serum + 90 µl Sample Diluent (mix thoroughly).

  b) Dilution 1:100: 10 µl 1:10 diluted **a)** + 90 µl Sample Diluent (mix thoroughly).

- **Standards**: Reconstitute the lyophilized standard protein by adding 0.5 ml Sample Diluent (for serum, plasma and cell culture supernatants sample measurement) or 0.5 ml 1X Cell Extraction solution (for cell and tissue extract samples measurement). Stand at room temperature for 10 minutes while mixing gently to yield a stock concentration of 4754 pg/ml. Make sure the standard is dissolved completely before making serial dilutions. The appropriate diluent buffer serves as zero standard (0 pg/ml). Add 21 µl of stock standard and 363 µl appropriate diluent buffer (serum and plasma: Sample Diluent; cell and tissue extract: 1X Cell Extraction solution) into the first standard tube to yield a standard concentration of 2600 pg/ml rest of the standard serial dilution can be diluted by appropriate diluent buffer as according to the suggested concentration below: 2600 pg/ml, 1300 pg/ml, 650 pg/ml, 325 pg/ml, 162.5 pg/ml, 81.25 pg/ml and 40.63 pg/ml.

Dilute human Tau standard as according to the table below:

Standard	Tau Conc. (pg/ml)	µl of diluent	µl of standard
S7	2600	363	21 (4754 pg/ml Stock)
S6	1300	150	150 (S7)
S5	650	150	150 (S6)
S4	325	150	150 (S5)
S3	162.5	150	150 (S4)
S2	81.25	150	150 (S3)
S1	40.63	150	150 (S2)
S0	0	150	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards and samples should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl** of **standards and samples** in duplicate into wells. (Standards and samples should add before antibody mixture)
3. Add **50 µl** of the **Antibody mixture** into wells.
4. Cover with plate sealer and incubate the plate for **1 hour at room temperature** on a plate shaker set to 400 rpm.
5. Aspirate each well and wash, repeating the process 2 times for a total **3 washes**. Wash by filling each well with **350 µl of 1X Wash Buffer** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting

against clean paper towels. Take care that none of the wells dry out before the next reagent is dispensed.

6. Add 100 µl of TMB substrate subsequently to each well. Incubate for 10 minutes at room temperature in dark on a plate shaker set to 400 rpm.

   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.

   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0

7. Add 100 µl of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix.
8. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the initial assay found samples contain Tau protein higher than the highest standard, the samples can be diluted with Sample Diluent or 1X Cell Extraction solution and then re-assay the samples.
7. If the initial assay found samples contain Tau protein lower than the lowest standard, the samples can be less diluted with Sample Diluent and then re-assay the samples.

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Tau ranged from 40.63-2600 pg/ml. The mean MDD was 20 pg/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 5.5% and inter-assay precision was 2.7%.

Recovery

70% - 111%