

Instructions for Use

Life Science Kits & Assays

pTAU rel ELISA



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1 Introduction

1.1 Intended use

The pTAU rel ELISA is an enzyme immunoassay intended for the quantitative determination of a tau fraction without (w/o) phosphor at T175/T181 in human CSF for supporting diagnosis of Alzheimer's disease. The development of the Alzheimer's disease is characterized by three stages, as defined by the US National Institute on Aging workgroups:

- a preclinical stage of Alzheimer's Disease,
- the mild cognitive impairment (MCI) stage due to AD and
- dementia stage due to AD.

Tau w/o phosphor T175/T181 in CSF shows at least comparable diagnostic specificity and sensitivity to other diagnostic available tests for Alzheimer's disease.

1.2 Warranty and technical support

The manufacturer guarantees the correct functioning of the kit for the applications described in the instructions for use (IFU). During the warranty period, pTAU rel ELISA allows for precise and reproducible data collection in connection with superior sensitivity. Any warranty claims shall only be valid if the general principles of Good Laboratory Practice (GLP) and the manufacturer's recommendations are observed.

To improve the application and design, Analytik Jena AG reserves the right of product replacement or modification. The manufacturer may be contacted at any time for questions and problems or technical support concerning the quantification of tau w/o phosphor T175/T181 in CSF.


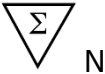
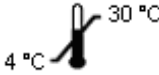




CONSULT INSTRUCTIONS FOR USE



This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.3 Notes on the use of this instructions for use

For easy reference and orientation, the IFU uses the following warning and information symbols as well as the shown methodology:

	REF Catalogue number
	Content Contains sufficient reagents for <N> tests
	Storage conditions
	Consult instructions for use
	Expiration date
	Manufactured by
	For single use only

The following abbreviations are used in the IFU:

AD	Alzheimer's disease
CSF	Cerebrospinal fluid
CV	Coefficient of variation
ELISA	Enzyme-linked immunosorbent assay
GLP	Good Laboratory Practice
HRP	Horseradish peroxidase
OD	Optical density
RT	Room temperature (15 - 25°C)
TMB	Tetramethylbenzidine

2 Safety precautions

We recommend reading this chapter thoroughly before using this kit, to ensure the safety of the user and error-free utilization.

Any safety instructions and additional information of this IFU must be observed at all times.

Read and make sure you understand the operating instructions completely and thoroughly before carrying out the test. Use the currently valid version from the kit.

Notify the respective supplier in writing within one week from receiving the merchandise, should the test pack be substantially damaged. Damaged components must not be used to carry out the assay, however, they should be kept until the transport damages are finally settled.

Comply with Good Laboratory Practice and safety regulations. Wear laboratory coats, disposable Latex gloves and safety goggles whenever the need arises.

Reagents of this kit which contain hazardous substances may cause irritations to the eyes and skin. See indications under COMPONENTS OF THE KIT and on the labels. Safety data sheets of this product are available upon request.

Chemicals and prepared or used reagents shall be disposed of as hazardous waste in compliance with the respective national regulations.

The cleaning staff has to be instructed by the experts with regard to any potential risks and the appropriate handling of such substances.

Avoid any contact with stopping solution. This may cause irritations to the skin and chemical burns.



FOR SINGLE USE ONLY!

This kit is made for single use only!

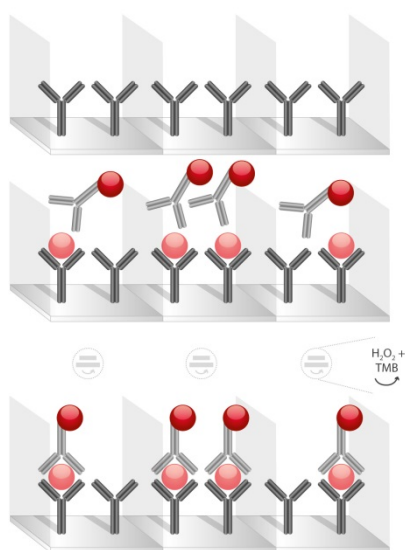
ATTENTION!

Don't eat or drink components of the kit!

The kit shall only be handled by educated personnel in a laboratory environment!

3 Test principle

This kit works by means of monoclonal antibody 1G2 that specifically recognize tau protein w/o phosphorylation at position T175 and T181, immobilized on the surface area of the microtiter plate. Tau protein w/o phosphor at T175/T181 from samples, standards and controls is trapped by this antibody in presence of another peroxidase conjugated monoclonal anti-tau antibody that specifically binds to amino acids 155-165 of human tau protein. Amount of bound conjugated antibody is estimated using chromogenic substrate tetramethylbenzidine (TMB). The concentration of tau protein w/o phosphor at T175/T181 is proportional to the obtained optical density.



1. Ready-to-use: Capture antibody coated on well plate
2. Binding of target antigen by capture antibody and incubation of HRP-conjugated species antibody
3. Direct detection using a HRP-conjugated species antibody

4 Performance assessment

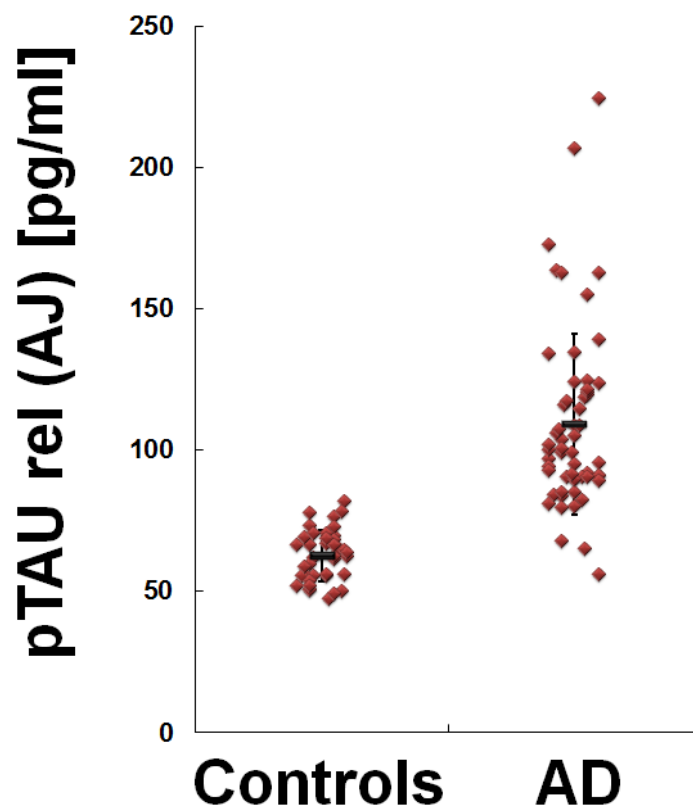
The table below shows typical data for calibration curves. Do not use for calculation!

Standard	Tau (pg/ml)	OD _{mean}	OD/OD _{max} (%)
D3.1	1200	2.682	100
D3.2	600	1.815	68
D3.3	120	0.572	21
D3.4	50	0.409	15
D3.5	25	0.294	11
D3.6	12	0.123	5










The table summarizes sensitivity and specificity related to determined cutoffs.

Analytical Sensitivity (Limit of Detection)	10 pg/mL	Mean signal negative control (blank) + 3SD
Cutoff	78 pg/mL	Each laboratory must establish its own cut off values.
Clinical sensitivity	95 %	AD/MCI patients (n=58)
Clinical specificity	98 %	Controls (n=42)




Verification of significant differences ($p < 0.001$) of concentration tau w/o phosphor at T175/T181 (pTAU rel ELISA) in CSF samples of patients with Alzheimer's disease (n=58) as well as control patients (n=42).



5 Kit components

Component	 96	Description
Immunoplate D1	12 x 8	Coated immuno strips containing 1G2 antibody, blocked and stabilized. Ready to use.
40X Wash buffer D2	1 x 50 ml	40X Wash buffer containing PBS, protein detergent and proclin 300.
Standards	6 x 3	Lyophilized tau standards (LYO) for preparing a standard curve for quantification of TAU in unknown cerebrospinal fluid samples. Containing PBS, protein and proclin 300.
D3.1	 3	1200 pg tau
D3.2	 3	600 pg tau
D3.3	 3	120 pg tau
D3.4	 3	50 pg tau
D3.5	 3	25 pg tau
D3.6	 3	12 pg tau
Negative control D4	 1 ml	Negative control (BLANK), containing PBS, protein, detergent and proclin 300. Ready to use.
15X HRP conjugate D5	 1 ml	Monoclonal anti-Tau antibody conjugated with horseradish peroxidase, 15X concentrate containing PBS, protein, detergent and proclin 300.
Assay buffer D6	50 ml	Dilution buffer containing PBS, protein, detergent and proclin 300. Ready to use.

Kit components

Component		96	Description
Control high D7		3	Tau high positive control. Containing PBS, protein and proclin 300.
Control low D8		3	Tau low positive control. Containing PBS, protein and proclin 300.
Staining solution D9		20 ml	TMB/peroxide solution. Ready to use.
Stop solution D10		25 ml	1 M sulphuric acid. Ready to use.
Sealing tape		1	
Instructions for use		1	

6 Preparation of components

6.1 1X Wash buffer solution

Dilute 40X wash buffer **D2** using de-ionized or bi-distilled water before the first wash step of the immunoassay.

Volume of 1X Wash buffer	Volume of 40X Wash buffer D2	Volume of de-ionized or bi-distillated water
400 ml	10 ml	390 ml
600 ml	15 ml	585 ml
800 ml	20 ml	780 ml
1000 ml	25 ml	975 ml

6.2 Standards D3.1-D3.6

Add 0.5 ml of dilution buffer **D6** to each standard vial **D3.1 – D3.6** and mix quickly, e.g. within 2 s by vortex.

6.3 Controls D7 and D8

Add 1 ml of dilution buffer **D6** to each control vial **D7** and **D8** and mix quickly, e.g. within 2 s by vortex.

Preparation of components

6.4 1X HRP conjugate

Dilute 15X HRP conjugate D5 at 1:15 ratio with dilution buffer D6. Mix by means of shaking the tubes.

Number of immuno strips	Volume of 15 x HRP D5	Volume of dilution buffer D6
1 - 4	0.3 ml	4.2 ml
5 - 8	0.6 ml	8.4 ml
9 - 12	0.9 ml	12.6 ml

7 Storage and expiry date

The kit is delivered at ambient temperature and should be stored at $6 \pm 4^{\circ}\text{C}$. Protect from heat and direct sunlight. Under these conditions, the kit has a life time as indicated on the kit box while retaining its endurance and stability.

Prepared kit components have the following expiry dates:

Component	Preparation step	Expiry date
D1	Coated immuno strips after opening of bag, taking of strips out and closing of bag.	At $6 \pm 4^{\circ}\text{C}$ up to 4 weeks.
D2	1X Ready-to-use washing solution.	At $6 \pm 4^{\circ}\text{C}$ up to 1 week.
D3.1-D3.6	Standards D3.1-D3.6 dissolved in D6.	At $6 \pm 4^{\circ}\text{C}$ up to 4 h.
D7, D8	Controls D7 and D8 dissolved in D6.	At $6 \pm 4^{\circ}\text{C}$ up to 4 h.
D5	Ready-to-use HRP-conjugate 1:15 diluted.	At $6 \pm 4^{\circ}\text{C}$ up to 4 h.

8 Components not included in the kit

- Calibrated micropipettes with $\text{CV} < 3\%$,
Volume: 10-100 μL ; 100-1000 μL .
- 8-channel micropipette with reagent reservoirs.
- Plate shaker 100-1500 rpm e. g. Rotamax 120.
- Vortex mixer.
- Automated or semi-automated ELISA plate washing system.
- Bi-distilled or de-ionized water.
- Paper towels, pipette tips and timer.
- ELISA plate reader for reading absorbance at 450 and 620 nm.
- Polypropylene tubes for sample dilution.

9 Procedure notes

Any improper handling of samples or modification of the test procedure may influence the results. The indicated volumes, incubation times, temperatures and pretreatment steps must be followed strictly regarding this instruction.

Be sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all staining solution **D9** to reach room temperature (21.5 ± 3.5 °C). Mix dilution buffer **D6** and 15X HRP conjugate **D5** by vortex before use.

Avoid contamination of reagents, pipettes and wells/tubes by using of different disposables between different samples and components. Do not interchange caps. Do not re-use any well, tube or reagent.

It is advised to test in duplicate and to use a pipetting scheme.

Solution of 1X HRP conjugate, staining solution **D9** and stop solution **D10** should transferred by 8-channel micropipette to all wells.

Washing should be done by 8-channel micropipette or ELISA plate washer. Avoid drying and stressing of wells and control exact washing of all wells.

10 Specimen collection and storage

The Alzheimer's Biomarker Standardization Initiative provides the following recommendations for the pre-analytical and analytical aspects for AD biomarker testing in CSF (Vanderstichele et al., Standardization of pre-analytical aspects of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: a consensus paper from the Alzheimer's Biomarkers Standardization Initiative. *Alzheimers Dement.* 2012 Jan;8(1):65-73).

10.1 Specimen collection

Lumbar puncture may be performed at the vertebral body L3-L5 with the patient either sitting or lying down. Use a small diameter (0.7 mm and 22 G), preferably not traumatic needle. A small-gauge needle will make a

smaller hole in the Dura mater, aiding healing, and not traumatic needle will reduce the chance of blood contamination in the CSF.

Each laboratory should use one kind of polypropylene tubes only. Glass or polystyrene tubes should in no circumstances be used. Tubes of the smallest volume should be used, and these should be filled to at least 50% of their volume. It is important to have carefully recorded and validated details concerning each stored sample so that any investigator when using these samples has a precise history of the sample.

Centrifugation is only required for visually hemorrhagic samples. Centrifuge soon with recommended 2000 x g at RT for 10 min.

10.2 Specimen storage

It is recommended to freeze samples and store at -80°C for long time storage. It is recommended to limit the number of freeze /thaw cycles to a maximum of 1-2. Samples should be stored no longer than 2 years.

Note

For dilution of CSF use polypropylene tubes or dilute directly onto immuno plate **D1**.

10.3 Specimen dilution

Samples showing an OD higher than OD of highest standard **D3.1** should be diluted before test procedure 1:2 or higher using assay buffer **D6**.

11 Test procedure

1. Pipette 50 μ L of **1X HRP conjugate** in each well.
2. Pipette 50 μ L of each **standard, controls and patient samples** into the respective wells of plate and mix plate using plate mixer (e. g. 10 – 20 s at 700 rpm) or manually using pipette tip 2-3 times.
3. Cover plate with lid or sealing tape.
4. Incubate immuno plate at plate shaker with 150 rpm at RT for 3 h \pm 15 min.
5. Wash plate 5x with 300 μ L/well of **1X wash solution** using an automatic ELISA plate washer.

Note

Alternatively, when performed manually discard incubation solution. Remove excess solution after washing by tapping immuno strip on paper towel.

6. Pipette 100 μ L of **staining solution D9** into each well.
7. Incubate plate at RT in the dark for 30 min.
8. Stop the substrate reaction by adding 150 μ L of **stop solution D10** into each well. Briefly mix contents by gently shaking the plate.
9. Measure optical density with a photometer at 450 nm against 620 nm as reference wave length within 15 min after stopping.

Note

In samples with a high concentration of specific antibodies, the dye that forms may be precipitated, due to intensive staining. Therefore, 10 min time lapse until the measurement takes place are recommended.

12 Data analysis

12.1 Quality criteria of the assay

- OD_{450/620 nm} of the **negative control D4 (BLANK)** should be < 0.1.
- Concentration of **positive controls D7 and D8** should be inside range corresponding to lot specific certificate.
- OD_{450/620 nm} of the **standard D3.1** should be > 2.0.
- For each case calculate R² of the calibration curve that should be > 0.99.

12.2 Calculation of unknown concentration of tau w/o phosphor at T175 or T181

Use logarithmic values (LN) of standard ODs and standard concentrations for plotting them onto x-axis (OD) and y-axis (concentration) of a linear diagram or for using them in linear regression analysis to estimate concentration of each sample. Logarithmic values of measured sample OD's have to be used for this regression analysis followed by exponentiation to calculate concentration in pg/ml or ng/l.

Note

Dilution factor of 2 has to be included for estimation of real concentration of tau w/o phosphor at T175 or T181 within samples and for **CTRL D7 and D8** after exponentiation.

An automated method performed by common reader software could be also used for quantification; 4 or 5 parameter logistics or logit-log methods are recommended. The calibration curve typically shows a linear part between a plateau for highest standard **D3.1** (1200 pg/ml) and plateau for lowest standard **D3.6** (12 pg/ml).

Note

Measured samples with OD below OD of lowest standard D3.6 without option to measure it more concentrated could be calculated for unknown tau w/o phosphor at T175 or T181 concentration as $\frac{1}{2}$ of this lowest standard concentration = 6 pg/ml.

13 Expected values

Note

Expected values are calculated from data of first clinical validation stage. All data should to be handled as preliminary.

For a cohort of 58 AD patients a level of 109 ± 32 pg/ml could be found with quartile range of 90 – 120 pg/ml, whereas the level of control cohort (n=42) was 62 ± 9 pg/ml with a quartile range of 55 – 69 pg/ml.

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