

Instructions for Use

Life Science Kits & Assays



hTAU total ELISA



Order No.:

847-0108000101 96 reactions

Publication No.: Manual_hTAU total ELISA_e_rev1(

This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!

Print-out and further use permitted with indication of source.

© Copyright 2017, Analytik Jena AG, AJ Roboscreen GmbH

Manufacturer:

AJ Roboscreen GmbH
Hohmannstraße 7
04129 Leipzig
Made in Germany!

Phone +49 341 989734 0
Fax +49 341 989734 199

Distribution/Publisher:

Analytik Jena AG
Konrad-Zuse-Straße 1
07745 Jena · Germany

www.analytik-jena.com
info@analytik-jena.com

Contents

1	Introduction.....	2
1.1	Intended use.....	2
1.2	Warranty and technical support	2
1.3	Notes on the use of this instructions for use.....	3
2	Safety precautions.....	4
3	Test principle.....	6
4	Performance assessment.....	7
5	Kit components	9
6	Preparation of components.....	11
6.1	1X Wash buffer solution	11
6.2	Standards D3.1-D3.6	11
6.3	Controls D7 and D8	11
6.4	HRP conjugate.....	12
7	Storage and expiry date.....	13
8	Components not included in the kit	13
9	Procedure notes	14
10	Specimen collection and storage	14
10.1	Specimen collection.....	14
10.2	Specimen storage.....	15
10.3	Specimen dilution.....	15
11	Test procedure	16
12	Data analysis	17
12.1	Quality criteria of the assay	17
12.2	Calculation of unknown tau concentration	17
13	Expected values.....	18

1 Introduction

1.1 Intended use

The hTAU total ELISA is an enzyme immunoassay intended for the quantitative determination of total tau protein in human CSF for supporting diagnosis of Alzheimer's disease (AD). 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 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, hTAU total 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 total tau protein 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
 N	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 (18-25°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!

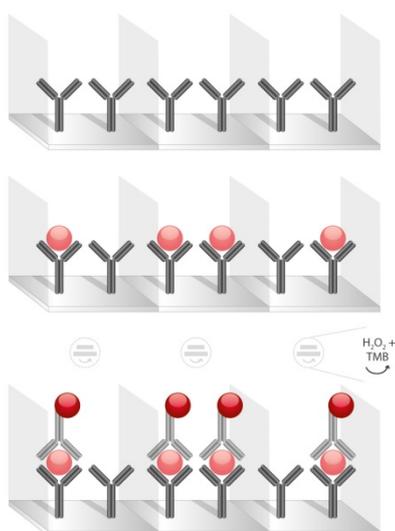
ATTENTION!

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 a monoclonal antibody that specifically recognizes amino acid 155-165 of human tau protein, immobilized on the surface area of the microtiter plate. Tau protein from samples, standards and controls is trapped by this antibody followed by detection by another horseradish peroxidase conjugated monoclonal anti-tau antibody that specifically binds to amino acids 110-120 of human tau protein. Amount of bound conjugated antibody is estimated using chromogenic substrate tetramethyl-benzidine (TMB). The concentration of tau protein is proportional to the obtained optical density.



1. **Ready-to-use: Capture antibody coated on well plate**
2. **Binding of human tau protein by capture antibody.**
3. **Detection of bound tau by HRP-conjugated antibody specific for human tau protein.**

4 Performance assessment

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

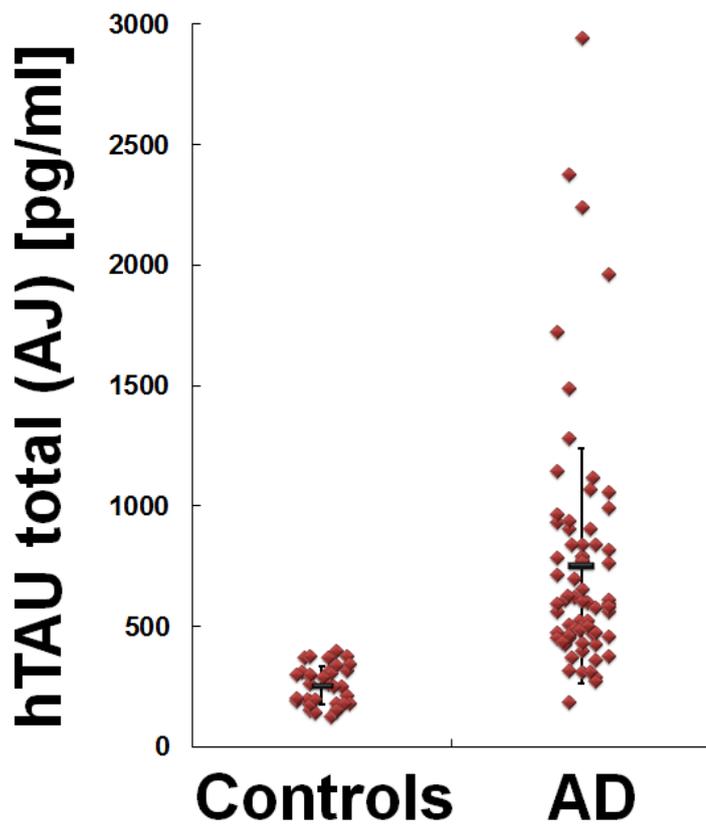
Standard	Tau (pg/ml)	OD _{mean}	OD/OD _{max} (%)
D3.1	1000	3.073	100
D3.2	500	1.664	54.1
D3.3	250	0.747	24.3
D3.4	100	0.333	10.8
D3.5	50	0.245	7.8
D3.6	25	0.149	4.8

The table summarizes sensitivity and specificity related to determined cutoffs.

Analytical Sensitivity (Limit of Detection)	25 pg/mL	Mean signal negative control (blank) + 3SD
Cutoff	399 pg/mL	Each laboratory must establish its own cut off values.
Clinical sensitivity	86 %	AD/MCI patients (n=58)
Clinical specificity	100 %	Controls (n=42)

Performance assessment

Verification of significant differences ($p < 0.001$) of concentration tau protein (hTAU total 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 anti-tau antibody, blocked and stabilized. Ready to use.
40X Wash buffer D2	1 x 50 ml	40X Wash buffer containing PBS, detergent and proclin 300.
Standards	6 x 3	Lyophilized tau standards (STD) for preparing a standard curve for quantification of TAU in unknown cerebrospinal fluid samples. Containing PBS, protein and proclin 300.
D3.1	 3	1000 pg tau
D3.2	 3	500 pg tau
D3.3	 3	250 pg tau
D3.4	 3	100 pg tau
D3.5	 3	50 pg tau
D3.6	 3	25 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 (CTRL), containing PBS, protein and proclin 300.
Control low D8		3	Tau low positive control (CTRL), 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		2	
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 1.0 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 1.0 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 HRP conjugate

Dilute 15X HRP conjugate **D5** at 1:15 ratio with dilution buffer **D6**. Mix by shaking of 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 assay 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 pipette 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 over 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

For appropriate measurement of tau protein concentration in CSF dilute specimen using assay buffer **D6** by means of ratio 1:4 (1 part of specimen e.g. 25 µl and 3 parts of **D6** e.g. 75 µl) before starting test. Use of known and pre-tested polypropylene tubes only is recommended or dilute specimen directly onto ELISA plate by transfer of 75 µl **D6** followed by transfer of 25 µl of each specimen per well.

Samples showing an OD higher than OD of highest standard **D3.1** should be diluted more than 1:4 using assay buffer **D6**.

11 Test procedure

1. Pipette 100 µl of standard, control, blank and pre-diluted patient samples in each well. Alternatively, transfer 75 µl of assay buffer **D6** followed by 25 µl patient sample and mix it by pipetting for 2-3 times.
2. Cover plate with lid or foil and incubate plate using a plate shaker with 150 rpm at RT for 2 h ± 10 min.
3. Wash plate 5 x with 300 µl/well of **1X wash solution** using an automatic ELISA plate washer.
4. Pipette 100 µl of 1X HRP conjugate in each well.
5. Cover plate with lid or foil and incubate immuno plate at RT for 90 min ± 10 min.
6. Wash plate 5 x with 300 µl/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.

7. Pipette 100 µl of **staining solution D9** into each well.
8. Incubate plate at RT in the dark for 30 min.
9. Stop the substrate reaction by adding 150 µl of **stop solution D10** into each well. Briefly mix contents by gently shaking the plate inside reader.
10. 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 tau protein 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 tau concentration

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 4 has to be included for estimation of real concentration of tau within samples 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** (1000 pg/ml) and plateau for lowest standard **D3.6** (25 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 concentration as ½ of this lowest standard concentration = 12.5 pg/ml.

13 Expected values

Note

The German Society for CSF diagnostics and Clinical Neurochemistry e. V. in the German Society of Neurology recommends the following reference ranges:

<50 Years	51-70 Years	>70 Years
<300 pg/mL	<450 pg/mL	<500 pg/mL

Headquarters

Analytik Jena AG
Konrad-Zuse-Str. 1
07745 Jena · Germany

Phone +49 3641 77 70
Fax +49 3641 77 9279
info@analytik-jena.com
www.analytik-jena.com

Pictures: Analytik Jena AG
Subject to changes in design and scope of delivery as well as further technical development!