ROBOSCREEN DIAGNOSTICS

Instructions for Use

hTAU total ELISA

Rev. 16/2020

CE

IVD

Order Number:

847-0108000101	96 reactions

CE

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Contents

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

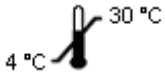
The manufacturer guarantees the correct functioning of the kit for the applications described in the instructions for use (IFU). During the warranty period, hTAU total ELISA allows for precise and reproducible data collection in connection with superior sensitivity. Any warranty claims shall only be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

To improve the application and design, Roboscreen GmbH 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
	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 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 experts with regard to any potential risks and the appropriate handling of such substances.

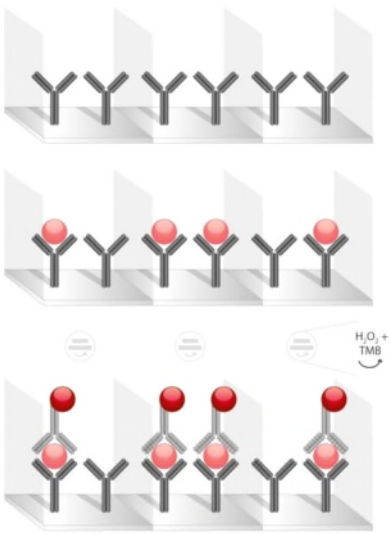
Avoid any contact with stopping solution. This may cause irritations to the skin and chemical burns.

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 acids 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 117-124 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

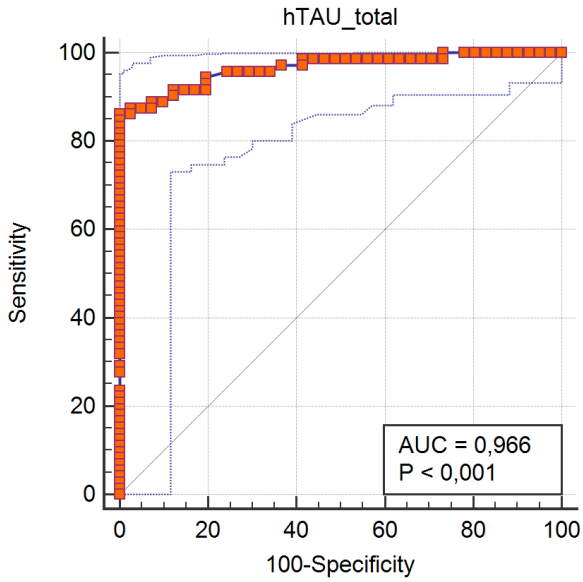
The table below shows typical data for calibration curves. Do not use for calculation!

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

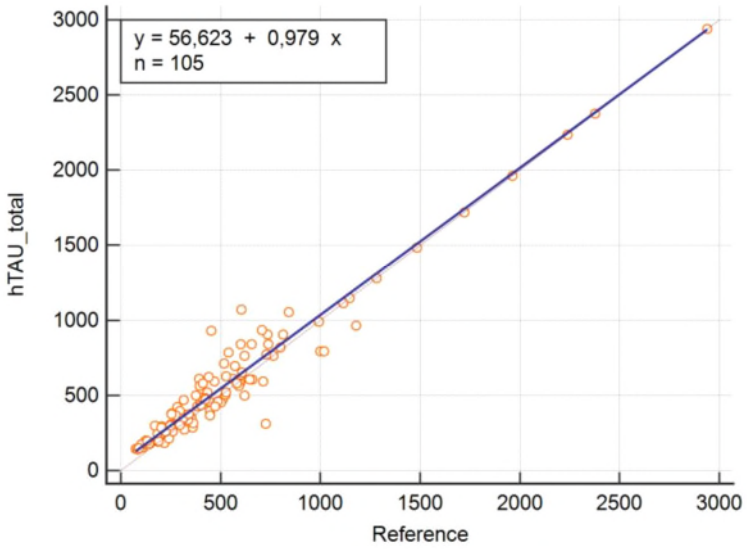
The table summarizes sensitivity and specificity related to determined cutoff.

Analytical Sensitivity (Limit of Detection)	25 pg/mL	Mean signal negative control (blank) + 3xSD
Cutoff	399 pg/mL	Each laboratory must establish its own cut off values.
Clinical sensitivity	86 %	AD/MCI patients (n=72)
Clinical specificity	98 %	Controls (n=41)

The analysis of tau CSF concentrations using hTAU total ELISA in patients with Alzheimer's disease (n = 72) and control patients (n = 41) showed a significant difference between the patient groups (p <0.001) with a sensitivity of 86.1 % and specificity of 98%. The associated ROC analysis showed 96.6% with p <0.001 and a Youden index of 0.86.



A comparative analysis (Deming regression) of the measurements of the hTAU total ELISA to an available commercial reference method shows a good correlation with a correlation coefficient (Pearson) of 0.97 with p <0.0001.



5 Kit components

Component	Σ 96	Description
Immunostrips **D1**	12 x 8	Coated immunostrips 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
15X HRP conjugate **D5** 	1.6 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	Σ 96	Description
Control high **D7** 	3	Lyophilized Tau high positive control (CTRL), containing PBS, protein and proclin 300.
Control low **D8** 	3	Lyophilized 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.

6.4 HRP conjugate

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

Number of immunostrips	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 ± 4°C. Protect from heat and direct sunlight. Under these conditions, the kit has a shelf life 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 immunostrips after opening of the bag, taking out strips and closing the bag.	At 6 ± 4°C up to 4 weeks.
D2	1X Ready-to-use washing solution.	At 6 ± 4°C up to 1 week.
D3.1-D3.6	Standards D3.1-D3.6 dissolved in D6.	At 6 ± 4°C up to 4 h.
D7, D8	Controls D7 and D8 dissolved in D6.	At 6 ± 4°C up to 4 h.
D5	Ready-to-use HRP-conjugate 1:15 diluted.	At 6 ± 4°C up to 4 h.

8 Components not included in the kit

- Calibrated micropipettes with 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.

*Shakers have different forces due to their specifications (deflection in mm) at a frequency of 150 rpm (see note on page 19).

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 immunostrips/tubes by use of different disposables between different samples and components. Do not interchange caps. Do not re-use any well, tube or reagent.

All measurements can be done in **single determinations**; however a dual determination increases the safety of the results and allows additional evaluations for the precision of the measurements.

For duplicate determinations, R2 of the standard curve should be ≥ 0.99. It is recommended to use a pipetting scheme to apply all STD, CTRL and samples.

Solution of 1X HRP conjugate **D5**, staining solution D9 and stop solution D10 should transferred by a 8-channel micropipette or a pipette with reservoir (multistep pipette) to all wells of the immunostrips.

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., 2012).

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. Usage of a non-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 immunostrips 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. Alternatively, specimen can be diluted directly onto immunostrips 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 standards, controls and pre-diluted patient samples in each well. Alternatively, transfer 75 µl of assay buffer **D6** followed by 25 µl patient sample.
2. Mix thoroughly by pipetting for at least 5 times.
3. The order of the pipetting steps can be reversed.
4. Cover plate with lid or foil.
5. Incubate plate using a plate shaker with 150 rpm ± 15 rpm at RT for 2 h ± 10 min.

NOTE

Shakers have different forces due to their deflection at a frequency of 150 rpm. The calculation of the acceleration (a) according to the formula $a = 4 \pi^2 rn^2$ should give a value of 2.5 m / s^2.

Radius (mm)	Number of revo-lutions (rpm)
10	150
5	212
3	274
1,5	387
0.5	671

π^2 = 9.87, r in m (10 mm = 0.01 m) and n in r/s (150 rpm/60 = 2.5 r/s)

6. Wash plate 5 x with 300 µl/well of **1X wash solution** using an automatic ELISA plate washer.
7. Pipette 100 µl of 1X HRP conjugate in each well.
8. Cover plate with lid or foil.
9. Incubate immune plate at RT for 90 min ± 10 min.

10. 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 immunostrips on paper towel.

11. Pipette 100 µl of **staining solution D9** into each well.
12. Incubate plate at RT in the dark for 30 min.
13. Stop the substrate reaction by adding 150 µl of **stop solution D10** into each well. Briefly mix contents by gently shaking the plate inside the plate reader.
14. Measure optical density with a photometer at 450 nm minus the reference wave length at 620 nm 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, a maximum of 10 min time lapse until the measurement takes place are recommended.

12 Data analysis

12.1 Quality criteria of the assay

- Concentration of positive controls D7 and D8 should be inside range corresponding to lot specific certificate.

12.2 Calculation of unknown tau concentration

For the determination of the tau concentration in controls and samples the automatic data analysis by means of reader software, usually the logistic regression with 4 or 5 parameters or logit-log method is recommended. The standard curve typically shows a linear progression between the plateau of the highest standard **D3.1** (1000 pg/ml) and the lowest standard **D3.6** (25 pg/ml).

Note
Dilution factor of 4 has to be included for estimation of real concentration of tau within samples after exponentiation.

Note
Samples with a measured OD smaller than the OD of the lowest standard D3.6 can be reported in terms of TAU protein concentration <25 pg / ml.

13 Expected values

Note
The expected values were calculated from the first clinical validation of the test. The data collected must be considered provisional.

Variable	hTAU total
Classification variable	AD_CO
Sample size	113
AD group	72 (63,72%)
Control group	41 (36,28%)
Area under the ROC curve (AUC)	0,966
Standard Error [a]	0,0147
95% Confidence interval [b]	0,914 to 0,991
z statistic	31,663
Significance level P (Area=0.5)	<0,0001
Youden index J	0,8611
95% Confidence interval [a]	0,7485 to 0,9167
Associated criterion	>399
95% Confidence interval [a]	>378 to >399
Sensitivity (%)	86,11
Specificity (%)	98,00

14 References

Vanderstichele H, et al. 2012. Standardization of preanalytical aspects of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: a consensus paper from the Alzheimer's Biomarkers Standardization Initiative. *Alzheimers Dement.* Jan; 8(1):65-73. doi: 10.1016/j.jalz.2011.07.004. Epub 2011 Nov 2., 2012.