ROBOSCREEN DIAGNOSTICS

Instructions for Use

hTDP43 total ELISA

Enzyme immunoassay for quantitative determination of human TDP43 protein in biological samples.

Rev. 0/2021

For research use only

Order Number:

847-0108000107	96 reactions

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Publication No.: IFU_hTDP43 total ELISA_e_rev0 0/2021

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1 Introduction

1.1 Intended use

The hTDP43 total ELISA is designed to detect and quantify the level of human TDP43 (transactive response DNA binding protein 43 kDa) in cell free biological samples. All contents of the hTDP43 total ELISA are produced under the guidelines of quality control accordingly to the DIN EN ISO 13485 requirements.

The hTDP43 total ELISA is for research use only and is not intended as diagnostic test.

1.2 Warranty and technical support

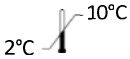
The manufacturer guarantees the correct functioning of the kit for the applications described in the instructions for use (IFU). During the warranty period, hTDP43 total ELISA allows for precise and reproducible data collection in connection with superior sensitivity. Any warranty claims shall only be 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 TDP43.

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
	Expiry date
	Manufactured by
	For single use only

The following abbreviations are used in the IFU:

AD	Alzheimer's disease
CSF	Cerebrospinal fluid
CTRL	Control
ELISA	Enzyme-linked immunosorbent assay
GLP	Good Laboratory Practice
HRP	Horseradish peroxidase
IFU	Instruction for use
mAb	Monoclonal antibody
OD	Optical density
RT	Room temperature (18-25°C)
STD	Standard
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 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 chapter *5 – Kit components, storage and expiry date* 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.

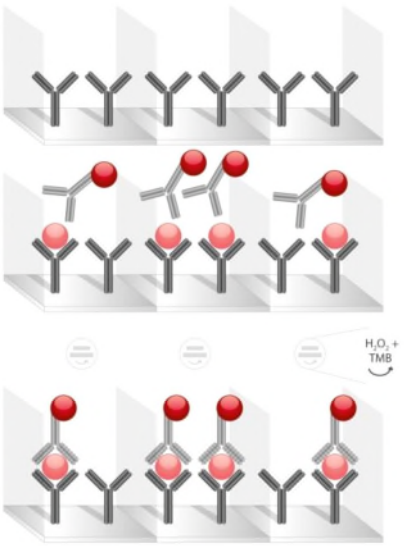
Avoid any contact with stop solution. This may cause irritations to the skin and chemical burns.

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

The hTDP43 total ELISA is based on a sensitive sandwich ELISA using mAb 21B2 for capturing TDP43. The epitope of 21B2 is located in N-terminal region of TDP43 (amino acids 79-91). As detection antibody HRP labelled mAb 2G10 binding in the middle region of TDP43 is used. Amount of bound conjugated antibody is estimated using chromogenic substrate tetramethylbenzidine (TMB). The concentration of TDP43 is proportional to the obtained optical density. Controls are included for the proof of reproducibility and evaluation of the assay within labs.



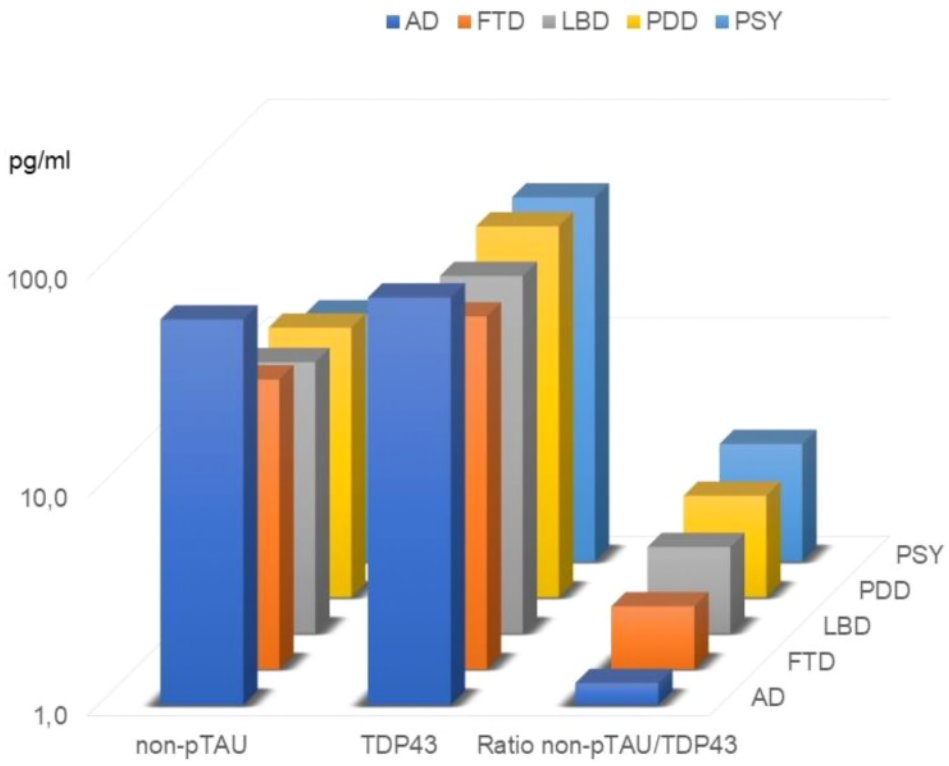
1. Ready-to-use: Monoclonal capture antibody specific to TDP43 coated on well plate

2. Binding of TDP43 simultaneously by capture antibody and HRP-conjugated antibody

3. Direct detection via HRP-conjugated antibody and perox-ide/TMB.

4 Example of use

CSF samples were analyzed from patients classified as Alzheimer dementia (n=37), Frontotemporal dementia (n=30), Parkinsons disease (n=13), Dementia with Lewy Body disease (n=30) and a group w/o signs of neurological disorders (PSY, n=30). hTDP43 total ELISA showed differences between groups but ratio of non-pTAU (cat. No. 847-0108000102) to TDP43 only discriminates all groups significant from each other ($p<0.001$). Tests were kindly performed by J. Escal, member of Dr. Perret-Liaudet group in Hospital Bron (Lyon, France).



5 Kit components, storage and expiry date

5.1 Kit components

Component	Σ 96	Description
D1 Immunostrips	12 x 8	Coated immunostrips containing anti-human TDP43 antibody, blocked and stabilized. Ready to use.
D2 40X Wash buffer	1 x 50 ml	40X Wash buffer containing PBS, detergent and proclin 300.
D3.1 – D3.6 Standards	6 x 3	TDP43 protein standards for preparing a standard curve for quantification of human TDP43 in unknown biological samples. Containing PBS, protein and proclin 300.
D3.1 	3	3500 pg TDP43
D3.2 	3	1300 pg TDP43
D3.3 	3	650 pg TDP43
D3.4 	3	400 pg TDP43
D3.5 	3	150 pg TDP43
D3.6 	3	50 pg TDP43
D5 15X HRP conjugate 	1 ml	HRP conjugated mAb anti-human TDP43, 15X concentrate, containing TRIS buffer, albumin, detergent and stabilizers (Kathon, Bronidox).
D6 Assay buffer	50 ml	Assay buffer containing PBS, protein, detergent and proclin 300. Ready to use.

Component		Σ 96	Description
D7 Control high		3	Human TDP43 high positive control (CTRL), containing PBS, protein and proclin 300.
D8 Control low		3	Human TDP43 low positive control (CTRL), containing PBS, protein and proclin 300.
D9 Staining solution		20 ml	TMB/peroxide solution. Ready to use.
D10 Stop solution		25 ml	1 M sulphuric acid. Ready to use.
Sealing tape		2	
Instruction for use		1	

5.2 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 bag, taking out strips and closing of bag.	At 6 ± 4 °C up to 4 weeks.
D2	Ready to use 1X wash solution.	At 6 ± 4 °C up to 2 weeks.
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.

6 Components not included in the kit:

- Calibrated micropipettes with CV < 3 %
  Volume: 0.5-10 µl; 10-100 µL; 100-1000 µL
- Calibrated 8-channel micro-pipette with reagent reservoirs
- Vortex mixer
- Automated or semi-automated ELISA plate washing system
- Bidistilled or deionized water
- Paper towels, pipette tips and timer
- ELISA plate reader for reading absorbance at 450 and 620 nm
- Polypropylene tubes for sample dilution

7 Preparation of components

Immunostrips **D1**, assay buffer **D6**, staining solution **D9** and stop solution **D10** are **ready to use** components.

7.1 1X Wash solution

Mix 40X wash buffer **D2** by 2-3 x inversing and dilute **D2** with bidistilled or deionized water 1:40 as described below before the first wash step of the immunoassay.

Number of immunostrips	Volume of 1X Wash solution	Volume of 40X Wash buffer D2	Volume of bidistilled or de-ionized water
1-4	400 ml	10 ml	390 ml
5-8	600 ml	15 ml	585 ml
9-12	800 ml	20 ml	780 ml

7.2 Standards D3.1 – D3.6

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

7.3 Controls D7 and D8

Add **0.5 ml** of assay buffer **D6** to each control vial **D7** and **D8** and mix quickly, e.g. within 2 s by vortex.

7.4 1X HRP conjugate

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

Number of immunostrips	Volume of 15X HRP D5	Volume of assay buffer D6
1 – 4	0.3 ml	4.2 ml
5 – 8	0.4 ml	5.6 ml
9 - 12	0.6 ml	8.4 ml

8 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.

1X HRP conjugate D5, staining solution D9 and stop solution D10 should be transferred by 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.

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, R^2 of the standard curve should be ≥ 0.99.

It is recommended to use a pipetting scheme to apply all STD, CTRL and samples.

8.1 Ready to use components

- Allow assay buffer **D6**, staining solution **D9** and stop solution **D10** to reach RT and mix by vortex before use.

8.2 Reconstitution of reagents

- 1X wash solution should be prepared before the first wash step.
- Standards D3.1 – D3.6 and controls D7 and D8 should be reconstituted before starting the test.
- 1X HRP conjugate should be prepared before starting the test.

8.3 Specimen preparation and dilution

- Allow samples to reach RT before use.
- Mix samples before use by vortexing for 6-10 s.
- For dilution of specimen use of known and pre-tested polypropylene tubes only is recommended.
- A sample dilution between **1:100** and **1:1000** for **plasma and between 1:5** and **1:50** for **CSF** is recommended.
- To inhibit reactions from specimen e.g. serum to mouse immunoglobulin Roboscreen offers Blocking solution on request.
- Samples showing an OD higher than OD of highest standard D3.1 should be diluted more using assay buffer D6.
- Samples showing an OD lower than OD of lowest standard D3.6 should be diluted less.

Immunoassay procedure

1. Transfer 50 µl of 1X HRP conjugate in each well.
2. Afterwards pipet 50 µl of each standard, controls and pre-diluted **sample** from polypropylene tube onto Immunostrips.
3. The sequence of pipetting steps can be reversed.

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

4. Mix thoroughly e.g. by pipette at least 5 times.
5. Cover plate with lid or foil.
6. Incubate immune plate for 3 h ± 10 min at RT.
7. Remove cover and wash **5** times with 300 µl/well wash solution manually or by use of a plate washer.

NOTE
Staining should be performed immediately after washing step 7 within 5 min.

8. Pipette 100 µl of **staining solution D9** into each well.
9. Incubate plate at RT in the dark for 30 min.
10. Stop the substrate reaction by adding 150 µl of **stop solution D10** into each well.
11. Measurement of absorbance: Mix plate with shaker of the reader for 3-5 s and let it settle down for 5 s. Measure the OD at 450 nm using 620 nm as reference wave length within 10 minutes after termination of the reaction.

Note
In high concentrated controls or samples staining components may be precipitated some time after termination. In this case additional mixing before reading is recommended.

9 Data analysis

The OD of the measured values is determined as the difference of the measured OD at 450 nm minus the OD at reference wavelength 620 nm ($OD_{450/620\ nm}$).

9.1 Quality criteria of the assay

- $OD_{450/620\ nm}$ values of positive controls **D7** and **D8** should be inside range corresponding to batch specific certificate.
- R^2 of the calibration curve should be ≥ 0.99.

9.2 Calculation of unknown TDP43 concentration

For the determination of the human TDP43 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** (3500 pg/ml) and the lowest standard **D3.6** (50 pg/ml).

Note
Dilution factor has to be included for estimation of real concentration of TDP43 within samples.

Note
Samples with a measured OD smaller than the OD of the lowest standard D3.6 can be reported in terms of TDP43 concentration <50 pg/ml.