ROBOSCREEN DIAGNOSTICS

Instructions for Use

Human p217 Tau Luminescence ELISA

Immunoassay for quantification of p217 Tau protein

from peripheral cell-free body fluids.

Rev. 1/2026

Order Number:

847-0108000111	96 reactions

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1 Introduction

1.1 Intended use

The Human p217 Tau Luminescence ELISA is an enzyme immunoassay intended for the quantitative determination of p217 Tau protein from peripheral cell-free body fluids, such as blood plasma or serum, after enrichment of BD Tau using the BD Tau Neuro IP Kit (**REF 847-0108000110**). For research use only. Not for use in diagnostic procedures.

1.2 Warranty and technical support

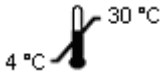
The manufacturer guarantees the correct functioning of the kit for the applications described in the instructions for use (IFU). During the warranty period, the Human p217 Tau Luminescence ELISA enables precise and reproducible data collection combined with superior sensitivity. Any warranty claims shall 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, problems, or technical support concerning the quantification of p217 Tau in samples.

CONSULT INSTRUCTIONS FOR USE
This package insert must be read carefully before 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 these 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
CV	Coefficient of variation
ELISA	Enzyme-linked immunosorbent assay
GLP	Good Laboratory Practice
HRP	Horseradish peroxidase
RT	Room temperature (18-25°C)

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 in 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 of 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 experts with regard to any potential risks and the appropriate handling of such substances.

Avoid any contact with the 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 using monoclonal antibodies immobilized on the surface area of the microtiter plate that specifically recognize human Tau protein between amino acids 155-181 from eluates obtained by enrichment using the BD Tau Neuro IP Kit (**REF 847-0108000110**). Tau protein from samples, standards, and controls is trapped by this antibody, followed by detection by horseradish peroxidase conjugated monoclonal anti-p217 Tau antibody 9G8 that specifically binds to amino acids phosphorylated T217 of human brain-derived Tau protein (BD Tau, 2N4R). Amount of bound conjugated antibody is estimated using chemiluminescence substrate and peroxide. The concentration of p217 Tau is proportional to the obtained relative light units (RLU).

4 Application example

IP of BD Tau from plasma and analysis with the Human p217 Tau Luminescence ELISA

IP of 200 µl EDTA plasma samples was performed according to the protocol of **BD Tau Neuro IP Kit** (**REF 847-0108000110**) using 62 samples from subjects, of whom 42 were Aß-positive (AD, n=42) and 20 were Aß-negative (CTRL, n=20). Elution was performed using 50 µl, and the complete eluate was analyzed in 2 repeats using the Human p217 Tau Luminescence ELISA.

All samples were measured, and the concentration of p217 Tau was quantified. A significant difference was found between the two groups (Students T Test, $p < 0.001$).

Figure 1 shows box plots showing differentiation between the two groups.

The analysis was performed retrospectively without any diagnostic background.

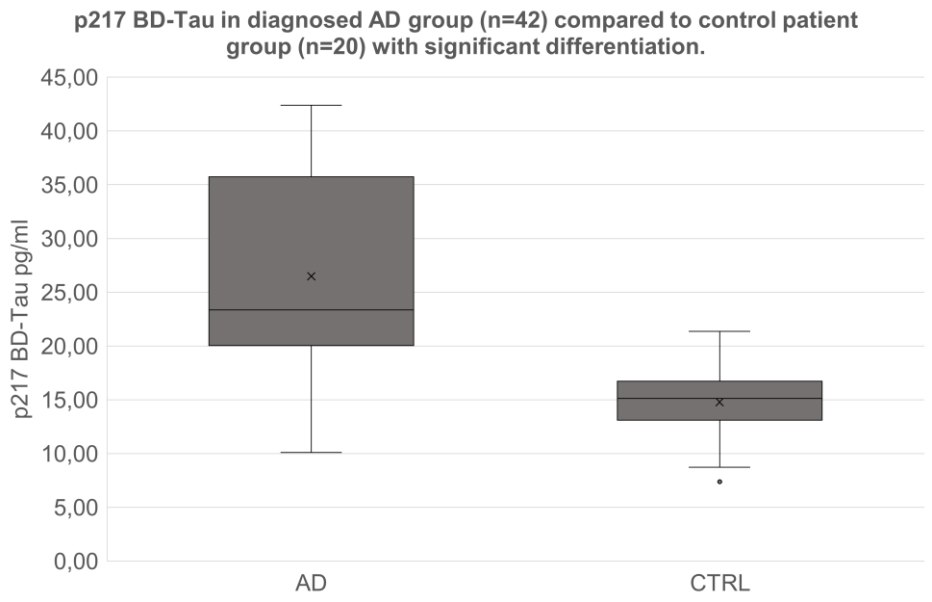


Figure 1: Analysis of 200 µl EDTA plasma after pre-analytical BD-Tau-IP (IP-Eluate) using Human p217 Tau Luminescence ELISA.

5 Kit components

Component		Σ 96	Description
Microtiterplate **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 p217 Tau in unknown samples. Containing PBS, protein and proclin 300.
D3.1		3	100 pg p217 Tau
D3.2		3	40 pg p217 Tau
D3.3		3	20 pg p217 Tau
D3.4		3	10 pg p217 Tau
D3.5		3	5 pg p217 Tau
D3.6		3	1 pg p217 Tau
20X HRP conjugate **D5**		1.0 ml	Monoclonal anti-p217 Tau antibody conjugated with horseradish peroxidase, 20X concentrate containing PBS, protein, detergent and proclin 300.
Assay buffer **D6**		50 ml	Dilution buffer containing PBS, detergent and proclin 300. Ready to use.

Component	Σ 96	Description
Control high **D7**	3	Lyophilized p217 Tau high positive control (CTRL), containing PBS, protein and proclin 300.
Control low **D8**	3	Lyophilized p217 Tau low positive control (CTRL), containing PBS, protein and proclin 300.
Enhancer CL **D9**	8 ml	Luminol solution. Ready to use.
Peroxide CL **D10**	8 ml	Peroxide solution. Ready to use.
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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 vortexing.

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 vortexing.

6.4 HRP conjugate

Dilute 20X HRP conjugate D5 at a 1:20 ratio with dilution buffer D6. Mix by shaking the tubes.

Number of immunostrips	Volume of 20 x HRP D5	Volume of dilution buffer D6
1 – 4	0.25 ml	4.75 ml
5 – 8	0.5 ml	9.5 ml
9 - 12	0.75 ml	14.25 ml

6.5 CL working solution

Number of immunostrips	Volume of Enhancer D9	Volume of Peroxide D10
1 – 4	2.5 ml	2.5 ml
5 – 8	4 ml	4 ml
9 - 12	6 ml	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 the bag, taking out strips, and closing the bag.	At 6 ± 4°C for up to 4 weeks.
D2	1X Ready-to-use washing solution.	At 6 ± 4°C for up to 1 week.
D3.1-D3.6	Standards D3.1-D3.6 dissolved in D6.	At 6 ± 4°C for up to 4 h.
D7, D8	Controls D7 and D8 dissolved in D6.	At 6 ± 4°C for up to 4 h.
D5	Ready-to-use HRP-conjugate diluted 1:20.	At 6 ± 4°C for up to 4 h.
CL Working solution	Mixed working solution within a brown tube.	At room temperature for up to 8 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 a timer.
- Microtiterplate reader with luminescence readout e.g. Infinite (Tecan).
- 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 15).

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 the required reagents, materials, and devices are prepared and ready at the appropriate time. Allow all staining solutions to reach room temperature (21.5 ± 3.5 °C). Mix assay buffer D6 and 20X HRP conjugate D5 by vortexing before use.

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

All measurements can be made in a single determination; however, determining in duplicate increases the safety of the results and allows for additional evaluations of the precision of the measurements. For double determination, 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, mixed staining solutions should be transferred by an 8-channel micropipette or a pipette with reservoir (multistep pipette) to all wells of the immunostrips.

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

10 Sample preparation, storage and dilution of eluates

10.1 Sample preparation

Immunoprecipitation of BD Tau from cell-free peripheral samples e.g. blood plasma or serum, is recommended using **BD Tau Neuro IP Kit** (**REF 847-0108000110**). For the appropriate work of Human BD Tau Luminescence ELISA, IP of 200 µl sample is recommended.

After elution with 50 µl of elution buffer, a volume of 25 -35 µl is expected in tubes containing eluates.

10.2 Eluate storage

It is recommended to freeze eluates and store them at -80°C for storage for more than 6 hours.

It is recommended to limit the number of freezing and thawing cycles to 2.

10.3 Specimen dilution

For appropriate measurement of p217 Tau protein concentration in eluates, add 25 µl of dilution buffer D6 to each complete eluate (see 10.1) and transfer the diluted eluate completely to the assay as described.

Samples, e.g. blood plasma showing a signal higher than 2500 RLU, should be pre-diluted before starting the repeat of sample preparation. For pre-dilution use of bi-distilled or de-ionized water is recommended.

11 Test procedure

1. Pipette 50 µl of **1X HRP-conjugate** followed by 50 µl standards, controls and complete pre-diluted eluates in each well.
2. Mix thoroughly by pipetting is recommended.
3. Cover the plate with a lid or foil.
4. Incubate plate using a plate shaker with **150 rpm** ± 15 rpm at **RT** for **3 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.

5. Wash plate 5 x with 300 µl/well of **1X wash solution** using an automatic ELISA plate washer.
6. Pipette 100 µl of **CL working solution** into each well.
7. Measure maximal luminescence according to the settings in the Magellan software for the Infinite Luminescence mode with a waiting time of 1 min, automatic attenuation, integration time of 100 ms, and rest time of 0 ms.

12 Data analysis

12.1 Quality criteria of the assay

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

12.2 Calculation of unknown p217 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 highest standard **D3.1** (100 pg/ml) and the lowest standard **D3.6** (1 pg/ml).

13 References

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