ROBOSCREEN DIAGNOSTICS

Instructions for Use

BD Tau Neuro IP Kit

Immunoprecipitation kit for pre-analytical enrichment of brain derived Tau protein from peripheral cell free body fluids.

Rev. 1/2025

For research use only

Order Number:

847-0108000110	96 reactions

This documentation describes the state at the time of publishing. It does not necessarily have to match future versions. Subject to change!

Manufacturer:

Roboscreen GmbH
Hohmannstrasse 7
04129 Leipzig
Germany

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

www.roboscreen.com
info@roboscreen.com

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1 Introduction

1.1 Intended use

The BD Tau Neuro IP Kit is designed to efficiently immunoprecipitate and enrich brain derived TAU protein from peripheral cell free body fluids as blood plasma or serum. The enrichment of brain derived TAU makes it reliably measurable without matrix effects in a system of choice e.g. Human BD Tau Luminescence ELISA using Microtiterplate reader with luminescence readout e.g. Infinite (Tecan) or other systems that specifically offer BD Tau measurement.

All contents of the BD TAU Neuro IP Kit are produced under the guidelines of quality control according to the DIN EN ISO 13485 requirements.

The BD TAU Neuro IP Kit is intended for research use only and not for diagnostic purposes.

1.2 Warranty and technical support

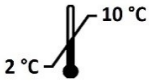
The manufacturer guarantees the correct functioning of the kit for the applications described in the instructions for use (IFU). During the warranty period, Neuro IP Kit allows for precise and reproducible immunoprecipitation of neurological biomarkers from cell free body fluids. Any warranty claims 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 immunoprecipitation of neurological biomarkers from cell free body fluids.

CONSULT INSTRUCTIONS FOR USE
The instruction for use must be read carefully prior to use. Given instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions for use.

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
AUC	Area under the curve
BD Tau	Brain derived TAU
GLP	Good Laboratory Practice
IP	Immunoprecipitation
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 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.

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 Functional principle

Monoclonal antibody 2B8, which is specific for the junction between the amino acids at the end of exon 4 and the amino acids at the beginning of exon 5, is immobilized on the surface area of the magnetic beads. By means of this BD Tau Neuro IP Kit, the brain derived Tau protein can be specifically captured from the sample. This achieves an enrichment of the target protein and minimizes matrix effects. Subsequently, the target protein is measurable in a system of choice e.g. Human BD Tau Luminescence ELISA using Microtiterplate reader with luminescence readout e.g. Infinite (Tecan) or other systems that specifically offer BD Tau measurement.

4 Application examples

4.1 IP of human plasma samples with BD TAU immunobeads and analysis with Human BD Tau Luminescence ELISA

IP of 200 µl EDTA plasma samples was performed according to the protocol using 41 samples from subjects, of whom 16 showed signs of dementia and 25 were classified as Alzheimer's disease subjects. Elution was performed using 50 µl and complete eluate was analyzed using Human BD Tau Luminescence ELISA **(REF# 847-0108000109)**.

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

Figure 1 shows box plots showing differentiation between groups.

The analysis was performed retrospectively without any diagnostic background.

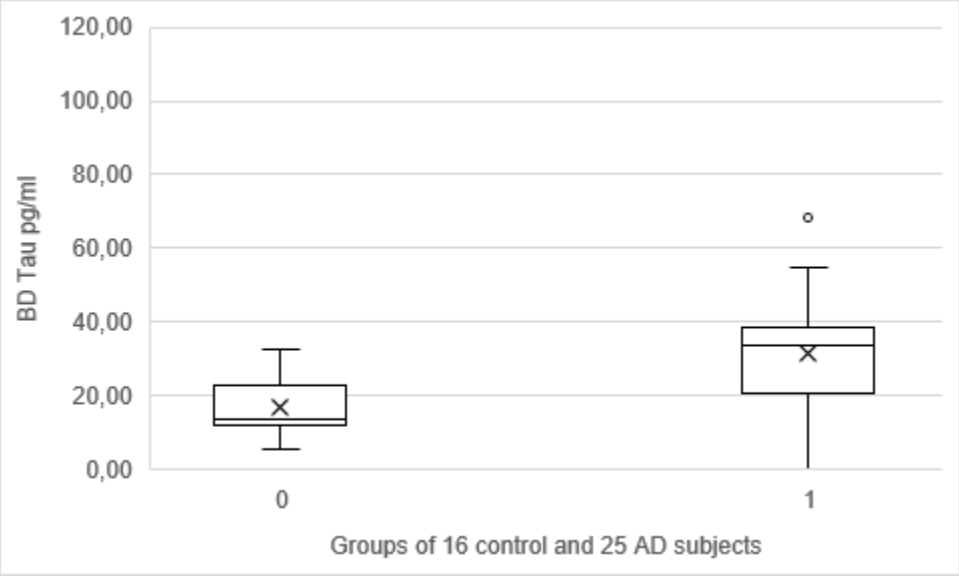


Figure 1: Analysis of 200 µl EDTA plasma after pre-analytical BD-Tau-IP (IP-Eluate) using Human BD Tau Luminescence ELISA.

4.2 Analytical description of the IP of human plasma samples with BD Tau immunobeads and analysis with Human BD Tau Luminescence ELISA

A high concentrated BD TAU sample was spiked 6 x in a very low concentrated BD TAU EDTA plasma sample (3 pg/ml) and diluted within 8 steps between 260 pg/ml and 4 pg/ml. An IP of 200 µl was performed from each dilution according to the protocol. Elution was performed using 50 µl and complete eluate was analyzed using Human BD Tau Luminescence ELISA **(REF# 847-0108000109)**.

The measurements of the 6-dilution series show a high agreement regarding expected concentration combined with an excellent low variation (Table 1).

All series show excellent linearity and a very good correlation between all measurements (Figure 2).

Table 1: Mean, SD and variation coefficient of the 6-dilution series of a high concentrated BD Tau sample in a very low concentrated BD Tau EDTA plasma sample (3 pg/ml) in range from 260 to 4 pg/ml.

Eluate (pg/ml)	Mean (pg/ml)	SD (pg/ml)	CV
260	259,5	14,59	5,6
130	116,1	5,91	5,1
60	56,6	0,81	1,4
30	24,9	0,57	2,3
15	12,6	0,45	3,6
8	7,2	0,36	5,0
6	5,2	0,14	2,7
4	3,7	0,08	2,3

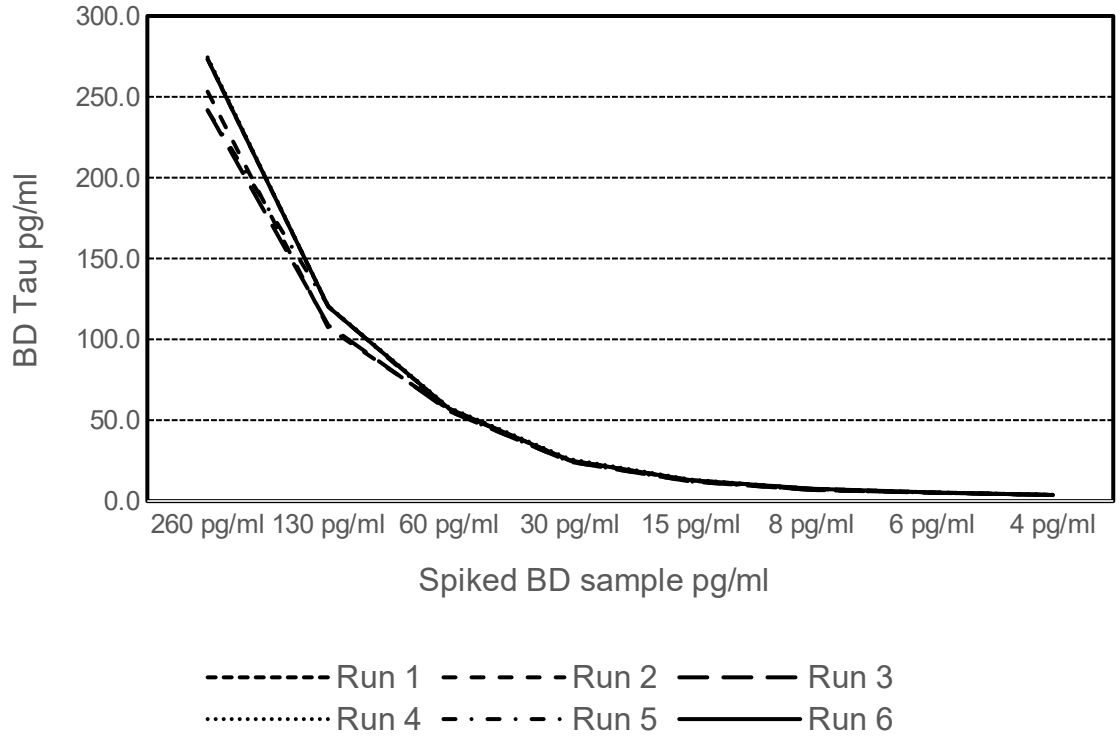


Figure 2: A high concentrated BD Tau sample was spiked 6 x in a very low concentrated BD Tau EDTA plasma sample (3 pg/ml) in range from 260 to 4 pg/ml.

5 Kit components

Table 2: Kit components.

Component	Σ 96	Description
5X Wash buffer **IP WB 5X**	1 x 75 ml	5X Wash buffer containing PBS, protein and Proclin 300.
5X IP buffer **IP B 5X**	1 x 15 ml	IP buffer containing HEPES, NaCl and detergents. Ready to use.
Elution buffer **IP EB 1**	1 x 125 ml	Elution buffer containing PBS and detergent. Ready to use.
Immunobeads BD TAU **IB BD TAU**	4 x 0.7 ml	Immunobeads, brain derived Tau containing magnetic beads coated with murine monoclonal antibody in PBS with 0.1 % BSA, 0.05 % Tween and 0.02 % sodium acide.
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6 Components not included in the kit

- cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail, Roche, Ref# 11836170001
- Low Protein Binding Microcentrifuge Tubes, 1.5 ml, e.g. Eppendorf, Ref# 0030108116
- Magnetic Separation Rack, e.g. Life Technologies, Ref# 12321D
- Rotation Mixer, e.g. Hulamixer™, Invitrogen, Ref# 15920D
- Vortex mixer.
- Centrifuge for 1.5/2.0 ml tubes.
- Thermomixer.
- Calibrated micropipettes with CV < 3%,
- Volume: 10-100 µL; 100-1000 µL.
- Deionized water.
- Paper towels, pipette tips and timer.

7 Recommended Products

Table 3: Recommended Products.

Product	Manufacturer	Order Number
Human BD TAU Luminescence ELISA	Roboscreen GmbH	847-0108000109

8 Preparation of components

8.1 1X Wash buffer solution

Dilute **IP WB 5X** **1:5** using deionized water before starting the IP.

Table 4: Preparation of 1X Wash buffer solution.

Number IPs	Volume 1X Wash buffer solution needed	Volume **IP WB 5X**	Volume deionized water
24	100 ml	20 ml	80 ml
48	200 ml	40 ml	160 ml
72	300 ml	60 ml	240 ml
96	400 ml	80 ml	320 ml

8.2 5X IP buffer + Protease Inhibitor Cocktail

Dissolve ***cOmplete, Mini, EDTA-free Protease inhibitor cocktail tablet*** or alternative inhibitor mixture (not included in the kit) in **IP B 5X** before starting the IP.

Table 5: Preparation of 5X IP buffer + Protease Inhibitor Cocktail.

Number IPs	Volume 5X IP buffer + Protease Inhibitor Cocktail needed	Volume **IP B 5X**	Number of cOmplete, Mini, EDTA-free Protease inhibitor cocktail tablets
20 - 24	4 ml	4 ml	2
44 - 48	8 ml	8 ml	4
68 - 72	12 ml	12 ml	6
92 - 96	16 ml	16 ml	8

9 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:

Table 6: Storage and expiry date of components.

Component	Preparation step	Expiry date
1X Wash buffer solution	**IP WB 5X** diluted 1:5 with deionized water	At 6 ± 4°C up to 2week.
IP B 5X + Protease inhibitor cocktail	*cOmplete, Mini, EDTA-free Protease inhibitor cocktail tablet* dissolved in **IP B 5X**	At 5 ± 3°C up to 2 weeks. At -20 ± 5°C at least 12 weeks.

10 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.

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

11 Specimen preparation

Before using the peripheral cell free samples such as blood plasma or serum in the IP reaction they should be mixed vigorously on a vortex mixer for 5 – 10 s and insoluble material should be pelleted by centrifugation for 5 min at high speed at room temperature in a fixed angle rotor.

12 Immunoprecipitation procedure

1. Per IP reaction transfer 25 µl of magnetic Immunobeads of choice to a 1.5 ml Low Protein Binding Microcentrifuge Tube (table 7).
2. Place the reaction tube into the magnetic separation rack and incubate for 2 min to immobilize beads.
3. Carefully remove the supernatant to avoid loss of beads.
4. Pipet 1000 µl of 1X Wash buffer solution to the beads, mix them by vortexing.
5. Place the reaction tube into the magnetic separation rack and incubate for 2 min to immobilize beads.
6. Carefully remove the supernatant to avoid loss of beads.
7. In the following order add 50 µl 5X IP buffer with protease inhibitor cocktail and 200 µl sample to the beads (table 7), mix them by vortexing.
8. Incubate reaction tube at RT for 60 min in the rotation mixer under continuous vigorous agitation[1]. Ensure that the liquid is in contact with the beads.

Table 7: Example for the composition of an IP reaction[2].

Total IP volume	Immunobeads	**IP B 5X** + Protease inhibitor cocktail	Sample e.g. Plasma
0.25 ml	25 µl[3]	50 µl	200 µl

9. After incubation, shortly spin down the reaction tube by centrifuge to remove bead / liquid residues from the lid.
10. Place the reaction tube into the magnetic separation rack and incubate for 2 min to immobilize beads.

[1] When using the recommended Hulamixer™, use the following settings:
ORBITAL ROTATION: 100 rpm / ORBITAL ROTATION TIME : 02 s | RECIPROCAL MOTION TURNING ANGLE : 45° / RECIPROCAL MOTION TIME : 02 s |VIBRATING MOTION TURNING ANGLE : 5° / VIBRATING MOTION : 2 s

[2] The ratio of 25 µl Immunobeads to 200 µl sample proved to be suitable for enrichment of BD Tau from plasma or serum samples. However, if there is a need to use a larger sample volume to precipitate more target protein, the total IP volume and / or the volume of sample in the IP reaction can be scaled up.

[3] Because 25 µl puffer are removed the complete IP volume is 250 µl not 275 µl.

11. Carefully remove the supernatant to avoid loss of beads.

NOTE
It is possible to use the supernatant for a second subsequent IP reaction. Contact manufacturer for other available Neuro IP Kits and materials.

12. Wash the beads with 1X Wash buffer solution, *following these steps:*
    - Remove reaction tube from magnetic separation rack.
    - Add 1.0 ml 1X Wash buffer solution.
    - Mix well by vortex and shortly spin down by centrifuge.
    - Place reaction tube into magnetic separation rack for 2 min.
    - Carefully remove the supernatant.
13. Repeat the wash step (12) two more times with 1X Wash buffer solution.
14. After that wash the beads one time with Elution buffer as described in step (12).
15. After removing the elution buffer in step (14), leave the tubes in the magnetic rack for another 2 minutes and remove any remaining liquid that may have accumulated.
16. Then carefully and thoroughly resuspend the washed beads in 50 µl of Elution buffer.
17. Heat the resuspended beads to 99 ± 1 °C with open caps while shaking at 900 rpm for 10 min using a thermomixer.
1. Cool reaction tube with closed caps quickly for 2 min on ice or in a cooling rack and shortly spin down by centrifuge.
2. Place reaction tube into magnetic separation rack for 2 min and carefully remove the eluate from beads.

13 References

Morgado B, Klafki HW, Bauer C, **Waniek K**, Esselmann H, Wirths O, Hansen N, **Lachmann I**, **Osterloh D**, Schuchhardt J, Wiltfang J. Assessment of immunoprecipitation with subsequent immunoassays for the blood-based diagnosis of Alzheimer's disease. Eur Arch Psychiatry Clin Neurosci. 2024 Feb 6. doi: 10.1007/s00406-023-01751-2. Epub ahead of print. PMID: 38316685.