



Instructions for Use

## Neuro IP Kit

**Immunoprecipitation kit for pre-analytical enrichment of neurological biomarkers  
from cell free body fluids.**



Rev. 0/2023

For research use only

**Order Number:**

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847-0108000108

24 reactions

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## 1 Introduction

### 1.1 Intended use

The Neuro IP Kit is designed to efficiently immunoprecipitate and enrich neurological biomarkers from cell free body fluids as blood plasma or serum and cerebrospinal fluid (CSF). The enrichment of neurological biomarkers makes them reliably measurable and without matrix effects in a system of choice, e.g. SIMOA, LUMIPULSE or ELISA.

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

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

To use this Neuro IP Kit Roboscreen Immuno Beads are recommended (see chapter 7).

### 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 AD 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 AD biomarkers from cell free body fluids.

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#### 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.

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### 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
A $\beta$	Beta-Amyloid
p50-BD-Tau	threonine-50 phosphorylated, brain derived Tau
CSF	Cerebrospinal fluid
GLP	Good Laboratory Practice
IP	Immunoprecipitation
RT	Room temperature (18-25°C)

## 2 Safety precautions

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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.

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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!

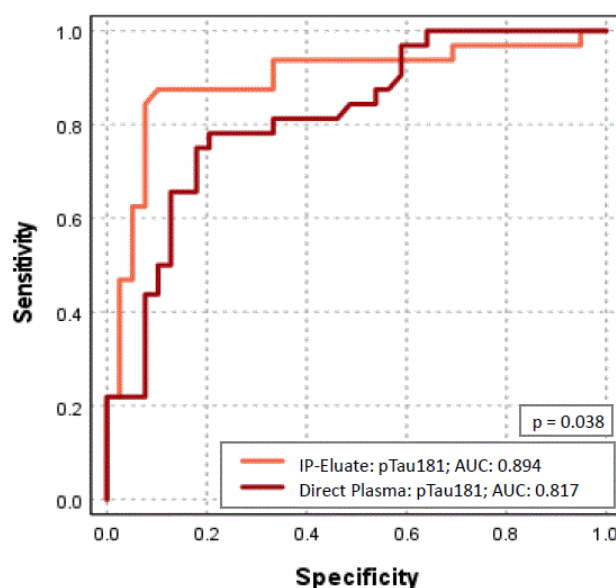
### 3 Functional principle

The Neuro IP Kit works in combination with the recommended Immuno Beads (see chapter 7). Monoclonal antibodies are immobilized on the surface area of the magnetic Immuno Beads. These monoclonal antibodies are directed against various forms of human tau protein or beta-amyloid, other targets are available on request. By using the Neuro IP Kit in combination with Immuno Beads, the target 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. SIMOA, LUMIPULSE, or ELISA.

### 4 Examples of use

#### 4.1 IP with Immuno Beads total Tau and analysis with LUMIPULSE® G600II

IP with Immuno Beads total Tau (Roboscreen GmbH, Order number: 847-060100010[x]) was performed from 200 µl plasma in a total IP volume of 500 µl. The samples used were EDTA plasmas of 71 subjects (32 Aβ-positive subjects and 39 Aβ-negative subjects according to the CSF Aβ42/40 ratio). Elution after IP was performed with 58 µl elution buffer. 50 µl of the eluates as well as the plasmas directly were analyzed with the Lumipulse® G pTau 181 using the fully automated LUMIPULSE® G600II System. Compared to direct plasma measurement pre-analytical Tau IP significantly improves the diagnostic contrast between Aβ-positive and Aβ-negative subjects (p=0.038; Figure 1; Morgado B, 2023)

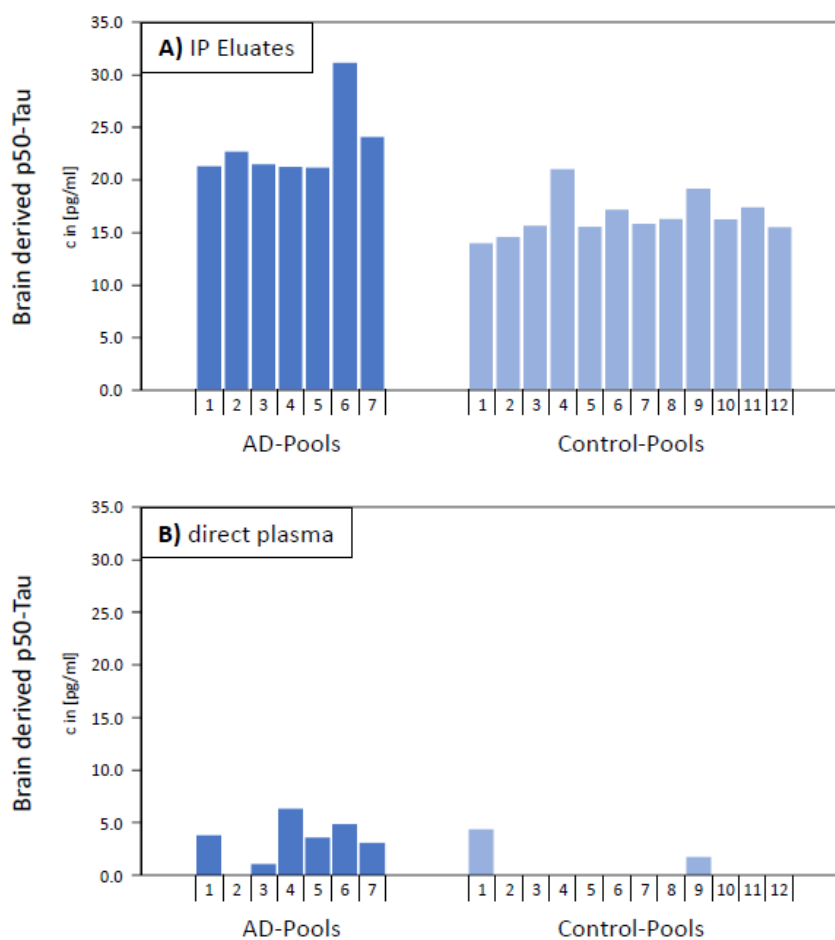


**Figure 1:** Pre-analytical Tau-IP (IP-Eluate) compared to direct plasma measurement increases the diagnostic ability of the Lumipulse® G pTau 181 assay significantly from AUC (area under the curve) 0.817 to AUC 0.894 ( $p = 0.038$ ).

#### 4.2 IP with Immuno Beads total Tau and analysis with *Simoa*™ Quanterix SR-X™

IP with Immuno Beads total Tau (Roboscreen GmbH, Order number: 847-060100010[x]) was performed from 200 µl plasma in a total IP volume of 500 µl. 7 AD citrate plasma pools and 12 control citrate plasma pools each consisting of 3 to 9 individual samples were used as samples. Elution after IP was performed with 35 µl elution buffer. The complete eluates as well as the same volume of the plasma pools directly were analyzed with an in-house *Simoa*™ Assay for threonine-50 phosphorylated, brain derived Tau (p50-BD-Tau) on the *Simoa*™ Quanterix SR-X™. The p50-BD-Tau measurement in the IP eluates shows complete separation between AD and control samples with a mean concentration of 23.4 pg/ml in AD samples compared to a mean concentration of 16.6 pg/ml in control samples (Figure 2, A). With direct plasma measurement, p50-BD-Tau can only be measured in 8 of the 19 plasma pools. In addition, an overlap between AD and control pools is shown for the direct plasma measurement (Figure 2, B).







**Figure 2: A.** Pre-analytical Tau-IP (IP-Eluates) allows differentiation between AD and Control pools using an in-house SIMOA assay for threonine-50 phosphorylated, brain derived Tau (p50-BD-Tau). **B.** For direct measurement, only 8 of the 19 plasma samples can be measured at all, with an overlap of AD and Control pools.

## 5 Kit components

**Table 1:** Kit components.

Component	 96	Description
5X Wash buffer <b>IP WB 5X</b>	1 x 25 ml	5X Wash buffer containing PBS, protein and Proclin 300.
5X IP buffer <b>IP B 5X</b>	1 x 15 ml	IP buffer containing HEPES, NaCl and detergents. Ready to use.
Elution buffer <b>IP EB</b>	1 x 40 ml	Elution buffer containing PBS and detergent. Ready to use.
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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 2:** Recommended Products.

Component	Manufacturer	Order Number <i>[x] = [1]: 6x IP, [2]: 24x IP</i>
Immuno Beads total Tau	Roboscreen GmbH	847-060100010[x]
Immuno Beads p50-Tau	Roboscreen GmbH	847-060100020[x]
Immuno Beads brain derived Tau	Roboscreen GmbH	847-060100030[x]
Immuno Beads Beta-Amyloid	Roboscreen GmbH	847-060100040[x]

## 8 Preparation of components

### 8.1 1X Wash buffer solution

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

**Table 3:** Preparation of 1X Wash buffer solution.

Number IPs	Volume 1X Wash buffer solution needed	Volume <b>IP WB 5X</b>	Volume deionized water
1	5 ml	1 ml	4 ml
6	25 ml	5 ml	20 ml
12	50 ml	10 ml	40 ml
24	100 ml	20 ml	80 ml

## 8.2 5X IP buffer + Protease Inhibitor Cocktail

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

**Table 4:** Preparation of 5X IP buffer + Protease Inhibitor Cocktail.

Number IPs	Volume 5X IP buffer + Protease Inhibitor Cocktail needed	Volume <b>IP B 5X</b>	Number of cOplete, Mini, EDTA-free Protease inhibitor cocktail tablets
1 - 19	2 ml	2 ml	1
20 - 24	4 ml	4 ml	2

## 9 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 shelf life as indicated on the kit box while retaining its endurance and stability.

Prepared kit components have the following expiry dates:

**Table 5:** Storage and expiry date of components.

Component	Preparation step	Expiry date
1X Wash buffer solution	<b>IP WB 5X</b> diluted 1:5 with deionized water	At $6 \pm 4^\circ\text{C}$ up to 1 week.
<b>IP B 5X</b> + Protease inhibitor cocktail	<i>cOplete, Mini, EDTA-free Protease inhibitor cocktail tablet</i> dissolved in <b>IP B 5X</b>	At $5 \pm 3^\circ\text{C}$ up to 2 weeks. At $-20 \pm 5^\circ\text{C}$ 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 use of 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 cell free samples such as blood plasma or serum and CSF 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 10 min at 10,000 x g at room temperature in a fixed angle rotor.

## 12 Immunoprecipitation procedure

1. *Per IP reaction* transfer 20 µl of magnetic Immuno Beads of choice to a 1.5 ml Low Protein Binding Microcentrifuge Tube.
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. In the following order add 200 µl deionized water, 100 µl 5X IP buffer with protease inhibitor cocktail and 200 µl sample to the beads (Table 6).
5. Incubate reaction tube at RT for 60 min in the rotation mixer under continuous vigorous agitation<sup>1</sup>. Ensure that the liquid is in contact with the beads.

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<sup>1</sup> 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

## Immunoprecipitation procedure

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**Table 6:** Example for the composition of an IP reaction<sup>2</sup>.

Total IP volume	Immuno Beads	Volume deionized water	IP B 5X <sup>+</sup> Protease inhibitor cocktail	Sample e.g. Plasma
0.5 ml	20 µl	200 µl	100 µl	200 µl

6. After incubation, shortly spin down the reaction tube by centrifuge to remove bead / liquid residues from the lid.
7. Place the reaction tube into the magnetic separation rack and incubate for 2 min to immobilize beads.
8. Carefully remove supernatant to avoid loss of beads.

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### NOTE

It is possible to use the supernatant for a second subsequent IP reaction. For example, if tau protein was precipitated from the sample in the first IP reaction, Beta-amyloid can be precipitated in a subsequent IP reaction. For this purpose, the corresponding beads must be prepared (see steps 1-3). Then add the supernatant of the first IP to the beads and continue with step 5.

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9. 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.
10. Repeat the wash step (9.) two more times with 1X Wash buffer solution.

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<sup>2</sup> The composition of the IP reaction was tested and optimized. The ratio of Immuno Bead amount to sample specified here corresponds to the optimum.

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. In this case, the user needs to test out which bead amount and which volume of sample and water correspond to the optimum. The 5X IP Buffer always accounts for one fifth of the total IP reaction volume.

11. Wash the beads with 1X with Elution buffer as described (9.) – Be careful to use 1X Elution buffer instead of 1X Wash buffer solution.
12. After washing add the desired volume of elution buffer to the beads and carefully resuspend the beads in elution buffer using a pipette.

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**NOTE**

For 200 µl sample 20 to 100 µl elution buffer are recommended, depending on the desired concentration and following analysis method

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13. Heat the resuspended beads to  $99\pm 1$  °C for 10 min using a thermomixer.
14. Cool reaction tube quickly for 2 min on ice or in a cooling rack and shortly spin down by centrifuge.
15. Place reaction tube into magnetic separation rack for 2 min and carefully remove the eluate from beads.

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**NOTE**

The eluate can now be measured in the system of choice.

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## 13 References

**Morgado B, et al 2023.** Two-step blood-based Alzheimer's Disease bio-marker immunoassay for Amyloid- $\beta$  and phospho Tau 181. Poster 368, AD/PD 2023, Gothenburg.

**Klafki HW, et al. 2020.** Development and Technical Validation of an Immunoassay for the Detection of APP<sub>669-711</sub> (Ab<sub>3-40</sub>) in Biological Samples. *Int J Mol Sci.* Sep 8; 21(18):6564. doi: 10.3390/ijms21186564. PMID: 32911706. PMCID: PMC7555726.

**Shahpasand-Kroner H, et al. 2018.** A two-step immunoassay for the simultaneous assessment of A $\beta$ 38, A $\beta$ 40 and A $\beta$ 42 in human blood plasma supports the A $\beta$ 42/A $\beta$ 40 ratio as a promising biomarker candidate of Alzheimer's disease. *Alzheimers Res Ther.* 2018 Dec 8;10(1):121. doi: 10.1186/s13195-018-0448-x. PMID: 30526652. PMCID: PMC6286509.

