ROBOSCREEN DIAGNOSTICS

Instructions for Use

Human α-Synuclein PATHO ELISA

Enzyme immunoassay for the quantitative determination of β-sheet oligomers of human α-Synuclein in biological samples.

Rev. 21/2022

For research use only

Order Number:

847-0104000108	96 reactions

This documentation describes the state at the time of publishing. It needs not necessarily agree with future versions. Subject to change!

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Abbreviations:

CSF	cerebrospinal fluid
ELISA	Enzyme-linked immunosorbent assay
GLP	good laboratory praxis
HRP	Horseradish peroxidase
mab	monoclonal antibody
OD	optical density
RT	room temperature 18 – 25°C
SD	Standard deviation
TMB	Tetramethylbenzidine

CONTENTS

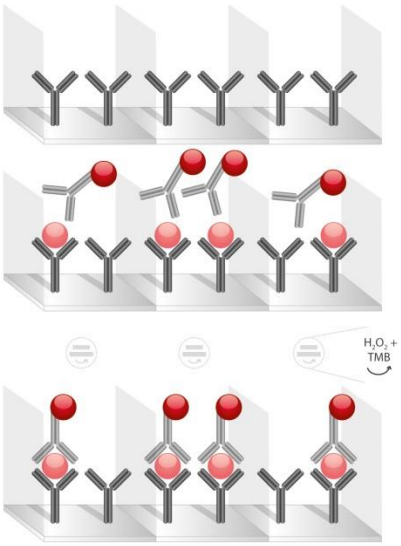
1. Introduction

The Human α-synuclein PATHO ELISA bases on a sensitive sandwich ELISA using mab 5G4 for capturing of β-sheet oligomers of human α-synuclein (Kovacs et al. 2012 & 2014).

α-synuclein β-sheet oligomers captured onto the plate are detected by another α-synuclein specific mab to amino acids 119-126 HRP conjugated. Synthetic aggregated α-synuclein standards in a range of 250 – 5 pg/ml are used for semi-quantification of unknown samples.

The evidence of bound HRP immuno complexes is shown by TMB/H_2O_2 staining or luminescence staining.

Test principle



1. Ready-to-use: Capture antibody 5G4 coated on well plate
2. Binding of α-Synuclein β-sheet oligomers by capture antibody and incubation of HRP-conjugated anti-α-Synuclein antibody
3. Direct detection using HRP-conjugated antibody and TMB/H_2O_2 staining

2. Kit components

Short name	Type/content	Quantity
D1 **Immunostrips**	Immunostrips coated with monoclonal mouse capture antibody 5G4 specific for β-sheet oligomers of α-synuclein aggregates, stabilized, ready-to-use.	12 x 8 wells
D2 **10X wash buffer**	Wash buffer (10X concentrate) in a transparent bottle, containing Na-Merthiolat as preservative.	1 x natural bottle with natural cap - 100 ml
D3.1 - D3.6 **Standards**	α-Synuclein aggregate standards lyophilized for semi-quantification of target in unknown samples. Preservative: Proclin 300.	6 x 3 natural tubes with coloured caps: - red 250 pg - natural 100 pg - violet 50 pg - yellow 25 pg - green 10 pg - blue 5 pg
D4 **Negative control (blank)**	Negative control in tubes with white caps containing negative control – ready to use. Preservative: Proclin 300.	1 natural tube with white cap - 1 ml
D5 **HRP conjugate**	HRP conjuagted anti-α-Synuclein antibody to amino acids 119-126 concentrate. Preservative: Proclin 300.	1 x brown tube with black cap – 1.0 ml
D6 **Assay buffer**	Assay buffer in transparent bottle containing buffered saline solution with BSA, ready to use. Preservative: Proclin 300.	1 x natural bottle with natural cap - 50 ml
D7 **Positive control HIGH**	Positive control lyophilized with high content of α-synuclein. Preservative: Proclin 300.	3 x natural tubes with brown caps
D8 **Positive control LOW**	Positive control lyophilized with low content of α-synuclein. Preservative: Proclin 300.	3 x natural tubes with orange caps
D9 **Staining solution**	TMB substrate solution in brown bottle Ready to use.	1 x brown bottle with brown cap - 20 ml
D10 **Stop solution**	Stop solution in transparent bottle containing 1 M sulphuric acid, ready-to-use.	1 x natural bottle with natural cap - 25 ml
Blocking solution	10X blocking solution for reduction of unspecific reactions to mabs of the assay.	1x brown tube with red cap - 1 ml
Sealing tapes	Sealing tapes for covering plates/strips	3 tapes

3. Preparation of the components

Ready to use reagents:
Immunostrips **D1**, negative control **D4** (blank), assay buffer **D6,** staining solution **D9** and Stop solution **D10** are ready to use reagents.

General reconstitution of reagents:
*Wash solution from **D2** 10X wash buffer:*

Mix 10X wash buffer **D2** by 2-3 x inversing and dilute **D2** with distilled water 1:10 as described below before performing washing step of the immunoassay.

Volumes of D2 and de-ionized water for preparing volumes of wash solution

Volume of wash solution	**Volume of 10X wash buffer D2**	**Volume of de-ionized water**
100 ml	10 ml	90 ml
200 ml	20 ml	180 ml
300 ml	30 ml	270 ml
400 ml	40 ml	360 ml
500 ml	50 ml	450 ml
1000 ml	100 ml	900 ml

Reconstitution of reagents for Immunoassay:

Standards and positive controls :

Add **0.5 ml** of **D6** to each tube of standards **D3.1 – D3.6** and positive controls **D7** and **D8** and vortex each tube for 2-3 s.

D5 HRP conjugate:

Dilute **D5** HRP conjugate **1:20** with assay buffer **D6** as described below and vortex it for 3-5 s.

Volumes of **D5** and **D6** for preparing different volumes of conjugate solution depending on number of strips used:

Number of immuno strips	Volume of 20X D5 HRP conjugate	Volume of assay buffer D6
1-4	0.1 ml	1.9 ml
5-8	0.2 ml	3.8 ml
9-12	0.4 ml	7.6 ml

4. Storage conditions and shelf life of the components

Store all reagents of the detection kit at 2-10°C. The guaranteed shelf life of the reagents is documented on labels. Reconstituted reagents have a shelf life as follows:

Short name	Reagent	Shelf life
D1	Coated immunostrips after closing alloy bag.	4 weeks at 2-10°C.
Wash buffer	1X wash solution.	2 weeks at RT.
D3.1-D3.6, D7 and D8	Diluted standards and controls.	Working day at 2-10°C.
D5	Diluted HRP conjugate.	24 h at 2-10°C.

5. Immunoassay

Sample preparation

- Allow samples to reach room temperature before use.
- Mix samples before their use by vortexing for 6-10 s.
- *Recommendation*: Human CSF samples should be used undiluted.
- *Recommendation*: Human plasma/serum samples should be diluted 1:2 by means of assay buffer D6 containing blocking solution (see below).
  → It is recommended to mix samples with D6/ blocking solution in separate tubes or plates.

Reconstitution of reagents

- Standards **D3.1 – D3.6** and controls **D7** and **D8** should be reconstituted before starting the test.
- Blocking solution should be diluted 1:10 using assay buffer **D6** before testing
  (e.g. 0.5 ml Blocking solution + 4.5 ml D6)

Immunoassay procedure

Step	Protocol	comments
1.	• Pipet 50 µl of **1:20** diluted **D5** in each well. Mix 2-3 x using pipet. *afterwards* • Pipet 50 µl of standards **D3.1-D3.6**, quality controls **D7** + **D8**, blank **D4** and **samples** into the respective wells of the plate. → Human CSF samples undiluted → Human plasma/serum samples 1:2 diluted with D6 containing blocking solution (e.g. 25 µl plasma/serum + 25 µl D6/ blocking solution) *the sequence of pipetting steps can be reversed* • Mix thoroughly e.g. by pipette at least 5 times	Pipetting of 1:20 diluted D5 HRP conjugate and standards, controls and samples.
2.	• Cover the strips with sealing tape and incubate at **2-10°C** for **18±2 h**	Incubation time.
3.	• Remove cover and wash **5** times with 300 µl wash buffer manually or using plate washer.	Washing of plate.

Staining procedure

Step	TMB staining	comments
7.	• Pipet 100 µl of staining solution.	Pipetting of staining solution.
8.	• Incubate for **30 min** in the **dark** at **RT**.	Incubation time.
9.	• Terminate staining by pipetting of 150 µl stop solution **D10** per well.	Termination of TMB reaction procedure.

Reading of absorbance

Mix plate with shaker of the reader for 3-5 s and let it settle down for 5 s and then read the O.D. at 450 nm and 620 nm as reference wave length using the microplate reader within 10 minutes after termination of the reaction.

☞ In high concentrated standards or samples staining components could precipitate some time after termination. In this case additional mixing before reading is recommended.

6. Interpretation of the results

Value of the negative control D4 (blank) should be < 0.2 (450/620 nm). Concentration of standards D3.1-D3.6 and positive controls D7 and D8 should be inside range corresponding to batch specific certificate.

Calculation of unknown α-Synuclein concentration

Use logarithmic values (LN) of standard OD's and standard concentrations for plotting them onto x-axis (OD) and y-axis (concentration) of a linear diagram or for using them in linear regression analysis to estimate concentration of each sample. Logarithmic values of measured sample OD's have to be used for this regression analysis followed by exponentiation to calculate concentration in pg/ml or ng/l.

Dilution factor of 2 has to be included for estimation of real concentration of β-sheet oligomers of α-Synuclein within human plasma/serum samples after exponentiation.

An automated method performed by common reader software could be also used for quantification, 4 parameter logistics or logit-log methods are recommended.

7. Warranty notice/ Additional general remarks

During the warranty period Human α-Synuclein PATHO ELISA allows precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired.

8. General comments

All contents of the Human α-Synuclein PATHO ELISA are produced under the guidelines of quality control accordingly to the DIN EN ISO 13485 requirements.

The use of this test is allowed for research and development testing only.

9. Symbols


Note.

Cat.-No.

No. of tests.


Store at:


Read instructions before use!


Use by:


Manufacturer


Keep away from heat or direct sun light.


Caution!