ROBOSCREEN DIAGNOSTICS

Instructions for Use

hSYN total ELISA

Enzyme immunoassay for quantitative determination of α-Synuclein in human CSF.

Rev. 5/2020

For research use only

Order Number:

847-0108000103	96 reactions

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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 22 ± 3 °C
SD	Standard deviation
TMB	Tetramethylbenzidine

CONTENTS

1. Introduction

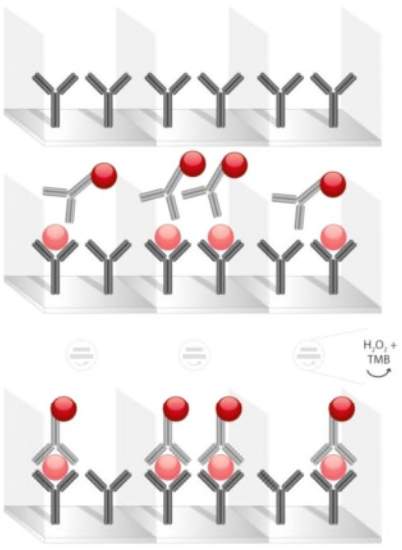
The hSYN total ELISA is a sensitive sandwich ELISA using monoclonal antibodies specific to human α-Synuclein for capturing as well as HRP conjugated for detection.

Using recombinant α-Synuclein standards with range between 600 and 50 pg/ml quantification of α-Synuclein content in biological samples is rendered possible. Quality controls are included for lab reproduceability and evaluation of the assay performed.

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

For testing of human CSF samples this sandwich-ELISA is designed as homogenous protocol with incubation of sample and HRP detection antibody at same time onto ELISA plate.

Test principle



1. Ready-to-use: Capture antibody coated on well plate
2. Binding of α-Synuclein 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, to human α-Synuclein, 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 standards lyophilized for semi-quantification of target in unknown samples. Preservative: Proclin 300. Concentration is calculated per mL.	6 x 3 natural tubes with coloured caps: - red 600 pg - natural 400 pg - violet 300 pg - yellow 150 pg - green 100 pg - blue 50 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 **20X HRP conjugate**	HRP conjuagted anti-α-Synuclein antibody 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 withlow content of α-synuclein. Preservative: Proclin 300.	3 x natural tubes with orange caps
D9 **TMB staining solution**	Staining solution in transparent bottle containing TMB in buffered solution, 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.	ON REQUEST.
Sealing tapes	Sealing tapes for covering plates/strips	2 tapes

3. Preparation of the components

Ready to use reagents:

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

General reconstitution of reagents:

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

Volumes of D2 and deionized water for preparing volumes of wash solution:

Volume of wash solution	Volume of 10X wash buffer D2	Volume of deionized 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 **0.25 ml** of **D6** to each tube of standards **D3.1 – D3.6** and **1 ml** of **D6** to each positive controls **D7** and **D8** and vortex each tubes for 2-3 s.

☞ Dilution of standards with D5 in relation of 1:4 according to protocol in (5) dues to final concentration per mL how described in (2).

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 strips used	Volume of assay buffer D6	Volume of 20X D5 HRP conjugate
1-4	3.8 ml	0.2 ml
5-8	7.6 ml	0.4 ml
9-12	11.4 ml	0.6 ml

4. Storage conditions and shelf life of the components

Store all reagents of the detection kit at 6 ± 4 °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 6 ± 4 °C.
Wash buffer	1X wash solution.	2 weeks at 22 ± 3 °C.
D3.1-D3.6, D7 and D8	Diluted standards and controls.	Working day at 6 ± 4 °C.
D5	Diluted HRP conjugate.	24 h at 6 ± 4 °C.

5. Immunoassay

Sample preparation

- Allow samples to reach 22 ± 3 °C before use. For samples stored frozen keep them at 22 ± 3 °C at least 30 min before pipetting onto plate.
- Mix samples before use by vortexing for 6-10 s.
- For human CSF samples **¼ dilution** directly in the plate is recommended to avoid possibly adsorption of synuclein onto e.g. tubes.
- For blood, plasma or serum samples, respectively, an alternative incubation protocol (see below) and use of blocking solution to inhibit unspecific reactions are recommended.

Reconstitution of reagents

- Standards **D3.1 – D3.6** and controls **D7** and **D8** should be reconstituted before starting the test.

Immunassay procedure for testing of human CSF samples

Step	Protocol	comments
1.	• Pipet 75 µl of **1:20 diluted** HRP conjugate **D5** per well onto the plate. • Pipet 25 µl of standards **D3.1-D3.6**, quality controls **D7** + **D8** and blank **D4** onto the plate and mix 2 – 3 times using the pipet . • Pipet 25 µl of samples onto the plate and mix 2 – 3 times using pipet.	A multichannel pipet can be used for pipetting of conjugate. Inverse pipetting is recommended. For pipetting of standards, controls and samples inverse pipetting is recommended. Avoid to use the same tip for the duplicate.
2.	• Cover the strips with sealing tape and incubate at **6 ± 4°C** for **22 ± 2 h**.	Incubation time.
3.	• Remove cover and wash **9** times with 300 µl wash solution manually or using plate washer.	Washing of plate using preparated wash solution.

☞ Staining should be performed immediately after washing step within 5 min

☞ For blood, plasma or serum samples, respectively, an alternative protocol should be use

Immunoassay procedure for blood, plasma and serum samples

Step	Protocol	comments
1.	• Pipet 75 µl of **D6** containg 10% blocking solution per well onto the plate. • Pipet 25 µl of standards **D3.1-D3.6**, quality controls **D7** + **D8** and blank **D4** onto the plate and mix 2 – 3 times using the pipet. • Pipet 25 µl of samples onto the plate and mix 2 – 3 times using pipet.	A multichannel pipet can be used for pipetting of dilution buffer. Inverse pipetting is recommended. For pipetting of standards, controls and samples inverse pipetting is recommended. Avoid to use the same tip for the duplicate.
2.	• Cover the strips with sealing tape and incubate at **6 ± 4°C** for **22 ± 2 h**.	Incubation time.
3.	• Remove cover and wash 5 times with 300 µl wash solution manually or using plate washer.	Washing of plate using preparated wash solution.
4.	• Pipet 100 µl of **D5** diluted 1:20 in assay buffer **D6**.	A multichannel pipet can be used for pipetting of conjugate.
5.	• Cover the strips with sealing tape and incubate at **22± 4 °C** for **1.5 h**.	Incubation time.
6.	• Remove cover and wash 5 times with 300 µl wash solution manually or using plate washer.	Washing of plate using preparated wash solution.

☞ Staining should be performed immediately after washing step 3 within 5 min

Staining procedure

Step	TMB staining protocol	comments
1.	• Pipet 100 µl of staining solution **D9** per well onto the plate.	A multichannel pipet can be used for pipetting of conjugate. Inverse pipetting is recommended.
2.	• Incubate for **15 mi**n in the **dark** at **22 ± 3 °C**.	Incubation time.
3.	• Terminate staining by pipetting of 150 µl stop solution **D10** per well onto the plate.	A multichannel pipet can be used for pipetting of conjugate. Inverse pipetting is recommended.

Reading of absorbance using TMB-Kit

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

Quality criteria of the assay

- $OD_{450/620\ nm}$ value of the negative control **D4** (blank) should be **< 0.2**.
- Concentration of positive controls **D7** and **D8** should be **inside range** corresponding to batch **specific certificate**.
- $OD_{450/620\ nm}$ of the standard **D3.1** with 600 pg/ml should be **> 2.0**.
- For each case calculate $\mathbf{r^2}$ of the calibration curve that should be **> 0,99**.

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/ml.

Dilution factor of **4** has to be included for estimation of real concentration of α-Synuclein within **samples** and controls **D7** and **D8** after exponentiation.

An automated method performed by common reader software could be also used for quantification, 4 or 5 parameter logistics or logit-log methods are recommended.

The calibration curve typically shows a linear part between a plateau for highest standard **D3.1** (600 pg/ml) and plateau for lowest standard **D3.6** (50 pg/ml).

7. Warranty notice/ Additional general remarks

During the warranty period the **hSYN total 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 **hSYN total 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.

In case of in vitro diagnosis each clinical laboratory should analytically validate using a full validation protocol as recommended by the DIN ISO 15189 (see Anderson et al. 2015).

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!

10. Notes