



Instructions for Use

hSYN total ELISA

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



Rev. 7/2026

For research use only

Order Number:

847-0108000103

96 reactions

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Subject to change!

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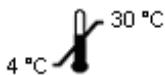
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For easy reference and orientation, the IFU uses the following warning and information symbols, as well as the shown methodology:

	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:

CSF	Cerebrospinal fluid
CV	Coefficient of variation
ELISA	Enzyme-linked immunosorbent assay
GLP	Good Laboratory Practice
HRP	Horseradish peroxidase
OD	Optical density
RT	Room temperature (18-25°C)
TMB	Tetramethylbenzidine

CONTENTS

	Page
1. Introduction	5
2. Kit components	6
3. Preparation of the components	7
4. Storage conditions and shelf life of the components	8
5. Immunoassay	9
6. Interpretation of the results	11
7. Warranty notice/Additional general remarks	12
8. General comments	12
9. References	12

1. Introduction

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

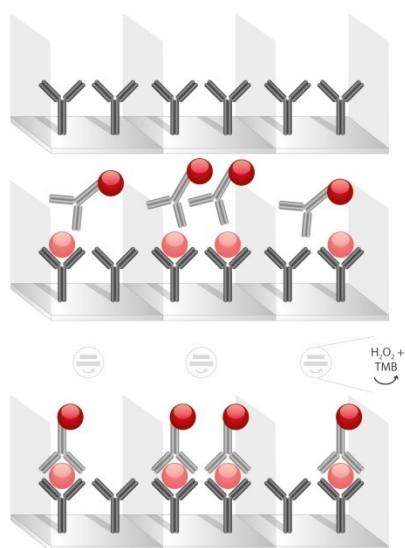
Using recombinant α -Synuclein standards with a range between 600 and 50 pg/ml, quantification of α -Synuclein content in biological samples is rendered possible. Quality controls are included to ensure lab reproducibility and the evaluation of the assay performed.

The evidence of bound HRP immuno complexes is shown by TMB/H₂O₂ staining.



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

Test principle



1. Ready-to-use: Capture antibody coated on a 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/H₂O₂ 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 conjugated anti- α -Synuclein antibody concentrate. Preservative: Proclin 300.	3 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 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.	2 x 1 ml
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 inverting and dilute **D2** with deionized water 1:10 as described below before performing the washing steps 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 :

For CSF protocol add **0.25 ml** of **D6** to each tube of standards **D3.1 – D3.6** and **1 ml** of **D6** to each positive control **D7** and **D8**, and vortex each tube for 2-3 s.

For serum/plasma protocol add **75 µl** of **D6** to each tube of standards **D3.1 – D3.6** and **150 µl** of **D6** to each positive control **D7** and **D8**, and vortex each tube for 2-3 s.



Dilution of standards with D5 or D6-BL in a ratio of 1:4 according to the protocol in (5) c to final concentration per mL as described in (2).

D5 HRP conjugate:

For CSF protocol 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 the 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

For serum/plasma protocol dilute **D5** HRP conjugate **1:5** 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 the number of strips used:

Number of strips used	Volume of assay buffer D6	Volume of 20X D5 HRP conjugate
1-4	3.2 ml	0.8 ml
5-8	6.4 ml	1.6 ml
9-12	9.6 ml	2.4 ml

D6-BL:

For serum/plasma protocol add 1 ml **Blocking solution** to 9 ml **D6** result in D6-BL for first step of the protocol.

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.
D6-BL	D6 with blocking solution.	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 for at least 30 min before pipetting onto the plate.
- Mix samples before use by vortexing for 6-10 s.
- For human CSF samples $\frac{1}{4}$ **dilution** directly in the plate is recommended to avoid possible adsorption of synuclein onto, e.g. tubes.
- For human serum/plasma samples **1:2 – 1:5** predilution of samples using D6 is recommended.

Reconstitution of reagents

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

Immunoassay procedure for testing of human CSF samples

Step	Protocol	comments
1.	• Pipet <u>75 μl</u> of 1:20 diluted HRP conjugate D5 per well onto the plate. • Pipet <u>25 μl</u> of standards D3.1-D3.6, quality controls D7 + D8, and blank D4 onto the plate and mix 2 – 3 times using the pipette. • Pipette <u>25 μl</u> of samples onto the plate and mix 2 – 3 times using pipet.	A multichannel pipette can be used for pipetting of conjugate. Inverse pipetting is recommended. For pipetting of standards, controls, and samples inverse pipetting is recommended. Avoid using 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 <u>300 μl</u> wash solution manually or using a plate washer.	Washing of plate using prepared wash solution.



Staining should be performed immediately after washing step within 5 min

Immunassay procedure for testing of human serum/plasma samples

Step	Protocol	comments
1.	- Pipet <u>75 μl</u> of D6-BL per well onto the plate. - Pipet <u>25 μl</u> of standards D3.1-D3.6, quality controls D7 + D8, and blank D4 onto the plate and mix 2 – 3 times using the pipette. - Pipette <u>25 μl</u> of pre-diluted samples onto the plate and mix 2 – 3 times using pipet.	A multichannel pipette can be used for pipetting of conjugate. Inverse pipetting is recommended. For pipetting of standards, controls, and samples inverse pipetting is recommended. Avoid using the same tip for the duplicate.
2.	Cover the strips with sealing tape and incubate at 6 $\pm$ 4°C for 22 $\pm$ 2 h.	Incubation time.
3.	Remove cover and wash 5 times with <u>300 μl</u> wash solution manually or using a plate washer.	Washing of plate using prepared wash solution.
4.	Pipet 100 μl of 1:5 diluted HRP-conjugate onto the plate .	A multichannel pipette can be used for pipetting of conjugate. Inverse pipetting is recommended.
5.	Cover the strips with sealing tape and incubate at room temperature for 90 $\pm$ 5 min.	Incubation time.
6.	Remove cover and wash 5 times with 300 μl wash solution manually or using a plate washer.	Washing of plate using prepared wash solution.

Staining procedure

Step	TMB staining protocol	comments
1.	Pipet <u>100 μl</u> of staining solution D9 per well onto the plate.	A multichannel pipette can be used for pipetting of conjugate. Inverse pipetting is recommended.
2.	Incubate for 15 min in the dark at 22 $\pm$ 3 °C.	Incubation time.
3.	Terminate staining by pipetting <u>150 μl</u> stop solution D10 per well onto the plate.	A multichannel pipette can be used for pipetting of conjugate. Inverse pipetting is recommended.

Reading of absorbance using TMB-Kit

Mix the plate with the 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 wavelength 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 the 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 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 the 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.



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

An automated method performed by common reader software could also be 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 the highest standard **D3.1** (600 pg/ml) and a plateau for the 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 in violation of the general GLP guidelines and the manufacturer's recommendations, the right to claim under guarantee has expired.

8. General comments

All contents of the **hSYN total ELISA** are produced under the guidelines of quality control according 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. References

Lerche S, Zimmermann M, Roeben B, Wurster I, Fries FL, Deuschle C, **Waniek K, Lachmann I**, Jakobi M, Joos TO, Knorpp T, Schneiderhan-Marra N, Brockmann K. [Inflammatory CSF profiles and longitudinal development of cognitive decline in sporadic and GBA-associated PD](https://doi.org/10.1038/s41531-023-00476-2). npj Parkinsons Dis. 9, 38 (2023). <https://doi.org/10.1038/s41531-023-00476-2>

Wurster I, Quadalti C, Rossi M, Hauser AK, Deuschle C, Schulte C, **Waniek K, Lachmann I**, la Fougere C, Doppler K, Gasser T, Bender B, Parchi P, Brockmann K. [Linking the phenotype of SNCA Triplication with PET-MRI imaging pattern and alpha-synuclein CSF seeding](https://doi.org/10.1038/s41531-022-00379-8). NPJ Parkinsons Dis. 2022 Sep 15;8(1):117. doi: 10.1038/s41531-022-00379-8.

Lerche S, Zimmermann M, Wurster I, Roeben B, Fries FL, Deuschle C, **Waniek K, Lachmann I**, Gasser T, Jakobi M, Joos TO, Schneiderhan-Marra N, Brockmann K. [CSF and Serum Levels of Inflammatory Markers in PD: Sparse Correlation, Sex Differences and Association With Neurodegenerative Biomarkers](https://doi.org/10.3389/fneur.2022.834580). Front Neurol. 2022 Feb 25;13:834580. doi:10.3389/fneur.2022.834580

Brockmann K, Quadalti C, Lerche S, Rossi M, Wurster I, Baiardi S, Roeben B, Mammana A, Zimmermann M, Hauser AK, Deuschle C, Schulte C, **Waniek K, Lachmann I**, Sjödin S, Brinkmalm A, Blennow K, Zetterberg H, Gasser T, Parchi P. [Association between CSF alpha-synuclein seeding activity and genetic status in Parkinson's disease and dementia with Lewy bodies](https://doi.org/10.1186/s40478-021-01276-6). Acta Neuropathol Commun. 2021 Oct 30;9(1):175. doi: 10.1186/s40478-021-01276-6

Lerche S, Schulte C, Wurster I, Machetanz G, Roeben B, Zimmermann M, Deuschle C, Hauser AK, Böhringer J, Krägeloh-Mann I, **Waniek K, Lachmann I**, Petterson XT, Chiang R, Park H, Wang B, Liepelt-Scarfone I, Maetzler W, Galasko D, Scherzer CR, Gasser T, Mielke MM, Hutten SJ, Mollenhauer B, Sardi SP, Berg D, Brockmann K. [The Mutation Matters: CSF Profiles of GCase, Sphingolipids, \$\alpha\$ -Synuclein in PDGBA](#). Mov Disord. 2021 Feb 6. doi: 10.1002/mds.28472

Bousiges O, Philippi N, Lavaux T, Perret-Liaudet A, **Lachmann I**, Schaeffer-Agalède C, Anthony P, Botzung A, Rauch L, Jung B, de Sousa PL, Demuynck C, Martin-Hunyadi C, Cretin B, Blanc F. [Differential diagnostic value of total alpha-synuclein assay in the cerebrospinal fluid between Alzheimer's disease and dementia with Lewy bodies from the prodromal stage](#). Alzheimers Res Ther. 2020 Sep 29;12(1):120. doi: 10.1186/s13195-020-00684-5. PMID: 32993772

Lerche S, Wurster I, Roeben B, Zimmermann M, Riebenbauer B, Deuschle C, Hauser AK, Schulte C, Berg D, Maetzler W, **Waniek K, Lachmann I**, Liepelt-Scarfone I, Gasser T, Brockmann K. [Parkinson's Disease: Glucocerebrosidase 1 Mutation Severity Is Associated with CSF Alpha-Synuclein Profiles](#). Mov Disord. 2020 Mar;35(3):495-499. doi: 10.1002/mds.27884

Wurster I, Lerche S, Hauser AK, Schulte C, **Lachmann I**, Beschorner R, Neumann M, Brockmann K. [Do longitudinal cerebrospinal fluid profiles correspond to postmortem brain pathology in LRRK2 Parkinson's disease?](#) Eur J Neurol. 2020 Jan;27(1):e5-e6. doi: 10.1111/ene.14060

Lerche S, Machetanz G, Wurster I, Roeben B, Zimmermann M, Pilotto A, Preische O, Stransky E, Deuschle C, Hauser AK, Schulte C, **Lachmann I, Waniek K**, Gasser T, Berg D, Maetzler W, Brockmann K. [Dementia with lewy bodies: GBA1 mutations are associated with cerebrospinal fluid alpha-synuclein profile](#). Mov Disord. 2019 Jul;34(7):1069-1073. doi: 10.1002/mds.27731

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