Alpha-Synuclein ELISA

- **Reliable detection of alpha-synuclein in CSF**
- **Simple procedure using ready-to-use reagents**
- **Fully automated processing**

Technical data

Coating	Monoclonal anti-alpha synuclein antibody
Calibration	Quantitative, in picograms per milliliter (pg/ml) 7 calibrators between 0 and 6000 pg/ml, lot dependent
Sample dilution	CSF, 25 µl; undiluted
Reagents	Ready for use, with the exception of the calibrators and controls (lyophilised) and the wash buffer (10 x); colour-coded solutions
Test procedure	180 min / 30 min / 30 min (sample/conjugate/substrate incubation), room temperature, fully automatable
Measurement	450 nm, reference wavelength between 620 nm and 650 nm
Kit format	96 single break-off wells; kit includes all necessary reagents
Order no.	**EQ 6545-9601-L**

Clinical significance

Alpha-synuclein is a cytoplasmic protein, which is concentrated in the presynaptic terminals. Its physiological function is not yet completely understood, but several studies suggest an involvement in vesicle trafficking and neurotransmitter release.

Alpha-synuclein is associated with the development of synucleinopathies. These neurodegenerative diseases include Parkinson's disease, dementia with Lewy bodies and system atrophy. In synucleinopathies around 90 % of alpha-synuclein is phosphorylated at the serine at position 129 (pS129), compared to only 4 % of alpha-synuclein in healthy brain tissue. The phosphorylation reduces the solubility of the protein and leads to it aggregating into so-called Lewy bodies. The degree of phosphorylation correlates with the severity of the disease. Alpha-synuclein aggregations also occur in other neurodegenerative diseases, for example in around 60 % of patients with Alzheimer's disease.

Soluble, monomeric alpha-synuclein can be detected in cerebrospinal fluid (CSF). Up to now, however, there was no standardised immunological test system for quantification available. Cut-offs of the protein concentration in CSF for the diagnosis of alpha-synuclein-associated diseases have not yet been established. Clinical studies show that the majority of patients with synucleinopathies have a reduced concentration compared to healthy persons, Alzheimer's patients and patients with other neurodegenerative diseases. In contrast, the alpha-synuclein concentration in the CSF of Alzheimer's patients is significantly higher than in patients with synucleinopathies and in healthy controls. This could be specific for Alzheimer's disease. In CSF diagnostics of alpha-synuclein it should be taken into account that the protein also occurs in peripheral blood and up to 20 % of lumbar punctures are contaminated with blood.

It is debated that in synucleinopathies soluble alpha-synuclein oligomers form first and subsequently aggregate and form deposits. Higher concentrations of these oligomers have been measured in the CSF of Parkinson's patients than in control patients and patients with other neurodegenerative diseases. The ratio of alpha-synuclein oligomers to monomeric total alpha-synuclein allows discrimination of patients with synucleinopathies from Alzheimer's patients and controls without neurodegenerative diseases.

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Diagnostic application

Clinical studies show that the majority of patients with Parkinson's disease have a reduced concentration of alpha-synuclein compared to healthy persons and patients with other neurodegenerative diseases. The EUROIMMUN Alpha-Synuclein ELISA allows reliable quantification of soluble, monomeric total alpha-synuclein in CSF and enables the use of alpha-synuclein as a research parameter. The relevance of this biomarker as a diagnostic parameter needs to be validated in clinical studies.

The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest clearly detectable alpha-synuclein concentration. The lower detection limit of the Alpha-Synuclein ELISA is 19 pg/ml.

Linearity

The linearity was determined by diluting 3 native CSF samples (1957 - 2844 pg/ml) with sample buffer in linear 10% steps. The mean concordance with respect to the expected value was 95% (72 - 109% with a mean correlation coefficient of r = 0.992).

Reproducibility

The reproducibility of the test was investigated by determining the intra-assay, inter-assay and inter-lot coefficients of variation using 3 samples for each.

Nr.	Intra-assay variation n = 20		Nr.	Inter-assay variation n = 10 x 2		Nr.	Inter-lot variation n = 3 x 2 x 2	
	Mean value (pg/ml)	CV (%)		Mean value (pg/ml)	CV (%)		Mean value (pg/ml)	CV (%)
1	1022	4.9	4	612	3.0	7	583	3.8
2	2045	4.9	5	1630	4.5	8	1623	5.5
3	3327	6.9	6	4590	3.6	9	4565	3.7

Literature

1. Goedert M, et al. **The Synucleinopathies: Twenty Years On.** J Parkinsons Dis (2017), 7(s1): 53-71.
2. Sancesario GM, et al. **How many biomarkers to discriminate neurodegenerative dementia?** Crit Rev Clin Lab Sci (2015), 52(6): 314-326.
3. Parnetti L, et al. **Value of cerebrospinal fluid α-synuclein species as biomarker in Parkinson's diagnosis and prognosis.** Biomark Med (2016), 10(1): 35-49.
4. Walker Z, et al. **Lewy body dementias.** Lancet (2015), 386(10004): 1683-1697.
5. Weil RS, et al. **Current concepts and controversies in the pathogenesis of Parkinson's disease dementia and Dementia with Lewy Bodies.** F1000Res (2017), 6: 1604.
6. Shi M, et al. **Cerebrospinal fluid α-synuclein contributes to the differential diagnosis of Alzheimer's disease.** Alzheimers Dement (2018).
7. Mollenhauer B, et al. **Investigating Synuclein Consortium of the Michael J. Fox Foundation for Parkinson's Research. A user's guide for α-synuclein biomarker studies in biological fluids: Perianalytical considerations.** Mov Disord (2017), 32(8): 1117-1130.

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