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User's Manual

Amyloid-beta (1-40) CSF ELISA

REF **DEIA-NS0621-1**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Enzyme Immunoassay for the quantitative determination of human amyloid-beta (1-40) in human CSF.

General Description

In 2015, the number of dementia patients worldwide was estimated at almost 47 million. Assuming an ongoing lack of sufficient preventive and curative treatments, this is expected to double every 20 years. Alzheimer's Disease accounts for roughly 60-70% of all dementia cases. Both prevalence and incidence increase with age. Prevalence is around 1-3% in those aged 65-69, and more than 30% in those aged 90 or older.

The first case of Alzheimer's Disease was defined and reported in 1907 by German scientist Dr. Alois Alzheimer. He described two hallmarks of the Disease - the plaques and tangles in the brains of Alzheimer's patients. These plaques consist mainly of amyloid-beta (Aβ) peptides. Amyloid-beta peptide isoforms are produced during normal cell metabolism by β- and γ-secretase from the amyloid precursor protein (APP) and are secreted into the CSF. APP is an integral membrane protein that consists of 695, 751 or 770 amino acids. Many different Aβ isoforms have been shown to exist. In 1995, a dominant and differential deposition of distinct amyloid-beta peptides, Aβ (N3pE) was found in senile plaques. However, the most abundant species in plaques is amyloid-beta (1-42), which decreases to approximately 50% in AD patients compared to an age-matched control group in CSF.

The development of the Disease is characterized by three stages, as defined by the US National Institute on Aging workgroups. A preclinical stage of Alzheimer's Disease, the mild cognitive impairment (MCI) stage due to AD, and the dementia stage due to AD. Amyloidosis occurs as early as the preclinical stage. The first cognitive deficits can manifest themselves in MCI stage, while in the dementia stage patients are unable to do any work or daily chores. The concentration of amyloid-beta (1-42) is therefore recognized as a useful biomarker (in combination with other biomarkers such as Tau and Phospho-Tau) in diagnosing Alzheimer's Disease. Moreover, a number of independent studies showed the ratio of amyloid-beta (1-42) to amyloid-beta (1-40) to be a superior diagnostic marker for Alzheimer's Disease.

As more and more laboratories are offering CSF testing, there is a high need of standardization of the CSF biomarkers, resulting in several commutability studies to assess international reference material.

In summary: Studies over the last 20 years have shown that the determination of 4 CSF biomarkers, namely Amyloid-β (1-42), Amyloid-β (1-40), TAU total and phospho-TAU in combination are a good tool to help in the diagnosis of Alzheimer's disease. The discriminatory power for the combination of the Amyloid-β biomarkers for example has surpassed the 90% sensitivity and specificity level. Furthermore, the CSF measurement of Amyloid-β peptides and forming the Amyloid-β42/40 ratio is well correlated to Amyloid-β PET scan results.

Principles of Testing

This kit uses a monoclonal antibody directed at the C-terminus of the amyloid-beta (1-40) peptide coated onto the surface area of the microtiter plate. The presence of the amyloid-beta (1-40) peptide is detected by the concomitant binding of the amyloid-beta peptide to the antibody that is bound to the surface of the microtiter

plate and the binding of a monoclonal antibody (clone 82E1) directed at the N-terminus of the amyloid-beta (1-40) peptide. The binding of the monoclonal antibody clone 82E1 is detected via a conjugated horseradish peroxidase using the chromogenic substrate Tetramethylbenzidine (TMB). The concentration of the amyloid-beta (1-40) is proportional to the obtained optical density.

Reagents And Materials Provided

1. MTP Microtiter Plate, 1×12×8, Coated with C-terminal specific mouse monoclonal antibody.

2. CAL A-F LYO Standard A-F, lyophilized, 3×6×1.0 mL, 0; 118; 235; 470; 940; 1880 pg/mL. Contains: Amyloid-beta (1-40) and stabilizers.

3. CONTROL 1 LYO / CONTROL 2 LYO Control 1+2, lyophilized. Contains: Amyloid-beta (1-40) and stabilizers. Concentrations / acceptable ranges see QC certificate.

4. ENZCONJ CONC Enzyme Conjugate Concentrate (30×),1×0.5 mL, Contains: N-terminal specific mouse monoclonal antibody conjugated to HRP (clone: 82E1) and stabilizers.

5. SAMPLEDIL Sample Diluent, 1×100 mL, Ready to use. Contains: Buffer, BSA and stabilizers.

6. ASSAYBUF Assay Buffer, 1×20 mL, Ready to use. Green colored. Contains: Buffer, BSA and stabilizers.

7. TMB SUBS TMB Substrate Solution, 1×15 mL, Ready to use. Contains: TMB, buffer and stabilizers.

8. TMB STOP TMB Stop Solution, 1×15 mL, Ready to use. Contains: 1 M H_2SO_4

9. WASHBUF CONC Wash Buffer Concentrate (40×), 1×50 mL, Contains: Phosphate buffer, detergents and stabilizers.

Materials Required But Not Supplied

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 0-20 µL; 10-100 µL; 100-1000 µL
2. Orbital shaker (200-900 rpm) (e.g. EAS 2/4, SLT)
3. Vortex mixer
4. Wash bottle, automated or semi-automated microtiter plate washing system
5. Bidistilled or deionised water
6. Paper towels, pipette tips and timer
7. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
8. Tubes for sample dilution (disposable polypropylene tubes)
9. 8-Channel Micropipettor with reagent reservoirs

Storage

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the indicated expiry after the kit is opened. Make sure that the opened bag is tightly closed when stored at 2-8°C.

Specimen Collection And Preparation

The Alzheimer's Biomarker Standardization Initiative provides the following recommendations for the preanalytical and analytical aspects for AD biomarker testing in CSF.

Specimen collection

Each laboratory should use one kind of polypropylene tubes only to collect CSF. Glass or polystyrene tubes should in no circumstances be used. Tubes of the smallest volume should be used, and these should be filled to at least 50% of their volume. It is important to have carefully recorded and validated details concerning each stored sample so that any investigator when using these samples has a precise history of the sample. Centrifugation is only required for visually hemorrhagic samples. Centrifuge as soon as possible - within 2 hours of LP (on site or at nearest laboratory). Centrifugation speed has no effect; however it is recommend applying 2000 g for 10 minutes at room temperature.

Specimen storage

It is recommended to freeze samples and store at -80°C for long time storage (< 2 years).

It is recommended to limit the number of freeze /thaw cycles to a maximum of 2.

Keep away from heat or direct sunlight.

Reagent Preparation

1. Preparation of lyophilized or concentrated components

The contents of the kit for 96 determinations can be divided into 3 separate runs. The volumes stated below are for one run with all strips (96 determinations).

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
each	CAL A-F LYO CONTROL 1 LYO CONTROL 2 LYO	with 1.0 mL	SAMPLEDIL		Dissolve for 5-15 min. Mix without foaming.	RT (18-25°C)	Prepare freshly and use only once.
400 µL	ENZCONJ CONC	with 11.6 mL	ASSAYBUF	1:30	Prepare freshly and use only once. Mix without foaming.	RT (18-25°C)	Prepare freshly and use only once.
50 mL	WASHBUF CONC	ad 2000 mL	bidist. water	1:40	Mix vigorously.	2-8°C	4 weeks

2. Dilution of Samples

For the dilution of CSF it is important to pipette Sample Diluent first into a polypropylene tube and add the CSF directly into the Sample Diluent.

Dilution must occur in polypropylene (PP) tubes. This applies to automated processes also.

Relation (dilution factor): e.g.: 20 µL CSF + 380 µL **SAMPLEDIL (1:20)**

Assay Procedure

1. Pipette 100 µL of each Standard, Control and diluted patient sample into the respective wells of microtiter plate. Cover plate with lid.
2. Incubate microtiter plate for 120 min at RT (18-25°C) on an orbital shaker (500 rpm).
3. Discard incubation solution. Wash plate 5× with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel. Automatic microtiter plate washers should be adjusted to

overflow mode.

4. Pipette 100 µL of diluted Enzyme Conjugate in each well. Cover plate with lid.
5. Incubate microtiter plate for 60 min at RT (18-25°C) on an orbital shaker (500 rpm).
6. Discard incubation solution. Wash plate 5× with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel. Automatic microtiter plate washers should be adjusted to overflow mode.
7. Pipette 100 µL of TMB Substrate Solution into each well. Briefly mix contents by gently shaking the plate.
8. Incubate microtiter plate for 30 min at RT (18-25°C).
9. Stop the substrate reaction by adding 100 µL of TMB Stop Solution into each well. Briefly mix contents by gently shaking the plate.
10. Measure optical density with a photometer at 450 nm (Reference-wavelength: 600-650 nm) within 15 min after pipetting the Stop Solution.

Quality Control

The test results are only valid if the test has been performed according to the instructions. Moreover the user must adhere strictly to the rules of GLP (Good Laboratory Practice) or comparable standards/laws.

User and/or laboratory must have a validated system for obtaining diagnosis according to GLP. All kit controls must be within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. Participating in appropriate quality assessment trials is recommended.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

Calculation

The obtained OD of the standards (y-axis, linear) is plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

To calculate the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

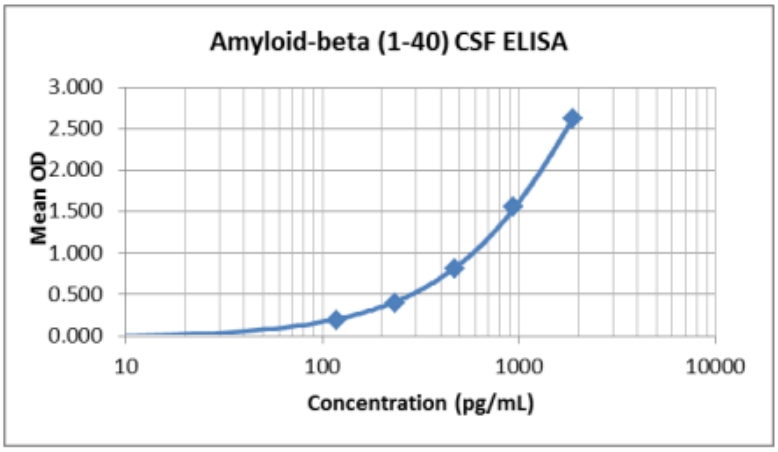
The concentration of the samples can be read from the standard curve. The initial dilution has to be taken into consideration when reading the results from the graph.

Results of samples of higher predilution have to be multiplied by the dilution factor. Samples with concentrations above the highest standard can be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Typical Standard Curve

Example: Do not use for calculation!

Standard	Amyloid-beta (1-40) (pg/mL)	OD_{mean}	OD/OD_{max} (%)
A	0	0.011	0.4
B	118	0.186	7.1
C	235	0.388	14.8
D	470	0.815	31.1
E	940	1.552	59.3
F	1880	2.619	100



Reference Values

In a clinical study, performed at two different labs in Germany, the following expected values were obtained for AD samples (= CSF samples from patients with early probable or possible Alzheimer's Disease (AD) or Mild Cognitive Impairment (MCI) of AD type) and Control samples (= CSF samples from patients without cognitive dysfunction). The studies included 115 AD samples and 88 Control samples from a European population.

		Amyloid-beta (1-40) (pg/mL)	Amyloid-beta (1-42) (pg/mL)	Amyloid-beta (1-42) /Amyloid-beta (1-40)
AD n=115	2.5% - percentile	7832	288	0.027
	Mean	15520	680	0.044
	97.5% - percentile	32144	2146	0.098
Control n=88	2.5% - percentile	6550	411	0.052
	Mean	14332	1389	0.100
	97.5% - percentile	23620	2451	0.120

It is recommended that each laboratory establishes its own range of normal values.

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

Precision

Intra-Assay: 3080 - 13506 (pg/mL), 1.8 - 4.5%. CV_{mean} (%): 2.6

Inter-Assay: 2257 - 19875 (pg/mL), 4.0 - 6.4%. CV_{mean} (%): 4.8

Inter-Lot: 2418 - 19407 (pg/mL), 2.7 - 6.3%. CV_{mean} (%): 4.8

Sensitivity

104 pg/mL

Specificity

Substance	Cross Reactivity (%)
Amyloid-beta (1-42)	0.84
Amyloid-beta (1-38)	0.01
Amyloid-beta (2-40)	1.29

Linearity

Range (pg/mL)	Range (%)	$Recovery_{mean}$ (%)
246 - 20562	1:32	94

Recovery

Range (pg/mL)	Range (%)	$Recovery_{mean}$ (%)
1907 - 15746	87 - 103	98

Precautions

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps must be followed strictly and in line with the instructions. Use calibrated pipettes and devices only.
2. Once the test has been initiated, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25°C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap vials that are not used. Do not reuse wells/tubes or reagents. Unused wells should be returned immediately to the resealed pouch including the desiccant.
4. It is advised to determine standards, controls and samples in duplicate in order to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting solutions in all wells.
7. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Automated washing might require adjustment of wash cycles. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Various types of shakers with different specifications are commercially available. Each laboratory is

encouraged to set their own optimal range.

9. Ensure the CSF samples are visually okay (e.g. samples with an erythrocyte count >500/µL should not be used without centrifugation).

Limitations

Specimen collection and storage have a significant effect on the test results.