Instructions for Use

Amyloid-beta (1-40) CSF ELISA

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

REF **RE59651**

Σ **96**

EU: IVD CE

I B L I N T E R N A T I O N A L G M B H

Flughafenstrasse 52a
D-22335 Hamburg, Germany

Phone: +49 (0)40-53 28 91-0
Fax: +49 (0)40-53 28 91-11

IBL@IBL-International.com
www.IBL-International.com

1. INTENDED USE

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

2. SUMMARY AND EXPLANATION

In 2010, the number of dementia patients worldwide was estimated at 36 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% 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 (see [1] and references therein). 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 [2]. 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 [9-11]. 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 (for example [3-5]) showed the ratio of amyloid-beta (1-42) to amyloid-beta (1-40) to be a superior diagnostic marker for Alzheimer's Disease.

3. TEST PRINCIPLE

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.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Make sure that everything is understood.
3. Should the kit package be severely damaged, please contact IBL or your supplier in writing, no later than one week after receiving the kit. Do not use damaged components in test runs. Instead, keep them safe for complaint-related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-website or directly from IBL on request.
7. Chemicals and reagents prepared or used must be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Professionals should guide the cleaning staff regarding potential hazards and handling.

9. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely. For this reason reagents should be treated as potential biohazards in use and for disposal.
10. Avoid contact with Stop solution. It may cause irritations and burns.

5. STORAGE AND STABILITY

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.

6. SPECIMEN COLLECTION AND STORAGE

The Alzheimer's Biomarker Standardization Initiative provides the following recommendations for the pre-analytical and analytical aspects for AD biomarker testing in CSF ([6]).

Specimen Collection

Lumbar puncture may be performed at the vertebral body L3-L5 with the patient either sitting or lying down. Use a small diameter (0.7 mm and 22 G), preferably atraumatic needle. A small-gauge needle will make a smaller hole in the dura, aiding healing, and an atraumatic needle will reduce the chance of blood contamination in the CSF.

Each laboratory should use one kind of polypropylene tubes only. **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 shipment

Samples may be sent by regular post as long as the duration of transport is less than 5 days.

Specimen Storage

It is recommended to freeze samples and store at -80°C for long time storage. It is recommended to limit the number of freeze /thaw cycles to a maximum of 1-2.

	Each laboratory should use the same polypropylene tubes. Glass or polystyrene tubes should in no circumstance be used for the collection, storage or dilution of CSF sample. All dilutions of CSF must be made using polypropylene tubes.
	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.
	Samples with an erythrocyte count >500/µL should not be used without centrifugation.

Storage:	-80 °C	Keep away from heat or direct sunlight.
Stability:	< 2 years	

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microtiter Plate** Coated with C-terminal specific mouse monoclonal antibody.
3 x 6 x 1.0 mL	CAL A-F LYO	**Standard A-F**, lyophilized 0; 118; 235; 470; 940; 1880 pg/mL Contains: Amyloid-beta (1-40) and stabilizers.
3 x 2 x 1.0 mL	CONTROL 1 LYO CONTROL 2 LYO	**Control 1+2**, lyophilized Contains: Amyloid-beta (1-40) and stabilizers. Concentrations / acceptable ranges see QC certificate.
1 x 0.5 mL	ENZCONJ CONC	**Enzyme Conjugate** Concentrate (30x) Contains: N-terminal specific mouse monoclonal antibody conjugated to HRP (clone: 82E1) and stabilizers.
1 x 100 mL	SAMPLEDIL	**Sample Diluent** Ready to use. Contains: Buffer, BSA and stabilizers.
1 x 20 mL	ASSAYBUF	**Assay Buffer** Ready to use. Green colored. Contains: Buffer, BSA and stabilizers.
1 x 15 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB, buffer and stabilizers.
1 x 15 mL	TMB STOP	**TMB Stop Solution** Ready to use. Contains: 1 M H_2SO_4.
1 x 50 mL	WASHBUF CONC	**Wash Buffer** Concentrate (40x) Contains: Phosphate buffer, detergents and stabilizers.

8. 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

9. PROCEDURE NOTES

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

10. PRE-TEST SETUP INSTRUCTIONS

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

10.1. Preparation of lyophilized or concentrated components

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

10.1. Predilution of samples

Patient sample	to be mixed	with	Relation	Remarks
CSF	generally	SAMPLEDIL	1:20	e.g.: 25 µL + 475 µL **Dilution must occur in polypropylene (PP) tubes. This applies to automated processes also.** 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.

11. TEST 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 **5x 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 **5x 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.

12. 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.

13. CALCULATION OF RESULTS

13.1. Determining the standard curve

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).

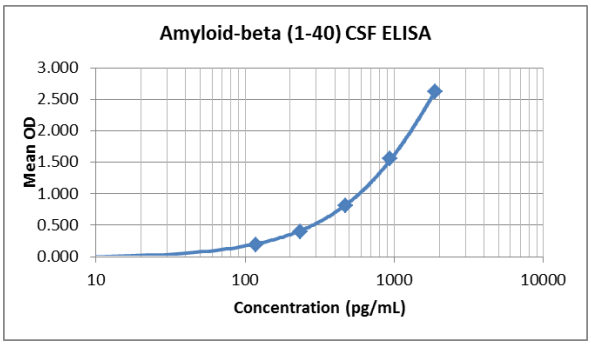
The concentration of the samples can be read from the standard curve. The initial dilution has to be taken into consideration when reading the results from the graph.

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 Calibration 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



14. EXPECTED VALUES

In a multicentric clinical (n=3) study 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).

		Amyloid-beta (1-40) (RE59651) (pg/mL)	Amyloid-beta (1-42) (RE59661) (pg/mL)	Amyloid-beta (1-42) /Amyloid-beta (1-40)
	5% - percentile	9206	252	0.024
	Mean	17043	645	0.038
AD n=119	95% - percentile	27055	1322	0.064
	5% - percentile	8256	514	0.055
	Mean	14401	1098	0.076
Control n=88	95% - percentile	22631	1779	0.088

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

15. LIMITATIONS OF THE PROCEDURE

Specimen collection and storage have a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

For cross-reactivities, see PERFORMANCE.

16. PERFORMANCE

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)	
	Amyloid-beta (1-42)	0.84	
	Amyloid-beta (1-38)	0.01	
	Amyloid-beta (2-40)	1.29	
Analytical Sensitivity (Limit of Detection)	104 pg/mL	Mean signal (Zero-Standard) + 3SD	
Cut-off	**Amyloid-beta (1-42) (RE59661)**	**Amyloid-beta (1-42) / Amyloid-beta (1-40)**	Each laboratory must establish its own cut-off values.
	650 pg/mL	**0.05**	
Clinical sensitivity	85%	98%	n=40
Clinical specificity	84%	91%	n=43
Precision	Range (pg/mL)	Range (%)	CV_{mean} (%)
Intra-Assay	3080 - 13506	1.8 - 4.5	2.6
Inter-Assay	2257 - 19875	4.0 - 6.4	4.8
Inter-Lot	2418 - 19407	2.7 - 6.3	4.8
Linearity	Range (pg/mL)	Range (%)	$Recovery_{mean}$ (%)
	246 - 20562	1:32	94
Recovery	Range (pg/mL)	Range (%)	$Recovery_{mean}$ (%)
	1907 - 15746	87 - 103	98
Method Comparison	IBL Assay = 0.90 x commercially available enzymatic assay + 28.78		r^2 = 0.94 n = 119

17. PRODUCT LITERATURE REFERENCES

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Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.


IBL International GmbH
Flughafenstr. 52A, 22335 Hamburg, Germany

Tel.: + 49 (0) 40 532891 -0 Fax: -11
E-MAIL: IBL@IBL-International.com
WEB: http://www.IBL-International.com