Elecsys β-Amyloid (1-42) CSF II

Materials provided

REF		Σ	SYSTEM
08821941190	08821941500	100	**cobas** e 402 **cobas** e 801

For reagents, refer to the "Reagents" section.

Materials required (but not provided)

REF	Description
08821976190	CalSet β-Amyloid (1-42) II, for 4 x 1.0 mL
08821968190	PreciControl β-Amyloid (1-42) II, for 6 x 1.0 mL
63.614.625	2.5 mL Low bind False bottom tube, Sarstedt (for cerebrospinal fluid (CSF) collection)
	General laboratory equipment
	cobas e analyzer

Additional materials for **cobas** e 402 and **cobas** e 801 analyzers:

REF	Description
06908799190	ProCell II M, 2 × 2 L system solution
04880293190	CleanCell M, 2 × 2 L measuring cell cleaning solution
07485409001	Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
06908853190	PreClean II M, 2 × 2 L wash solution
05694302001	AssayTip/AssayCup tray, 6 magazines × 6 magazine stacks × 105 assay tips and 105 assay cups, 3 wasteliners
07485425001	Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution / Elecsys SysClean for Liquid Flow Cleaning Detection Unit
07485433001	PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution / Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
11298500316	ISE Cleaning Solution / Elecsys SysClean, 5 × 100 mL system cleaning solution

Note

The measured β-amyloid (1-42) value in a given sample, determined with assays from different manufacturers, can vary due to differences in assay methods and reagent. Values determined on samples by different assay methods and on different **cobas** e platforms cannot be used interchangeably.

Please note that due to the sticky properties of the β-amyloid protein, the Elecsys assay cutoff provided in this document is only valid if the below described pre-analytical handling procedure (see section "Specimen collection and preparation") is strictly followed.

All analytical performance data were generated using frozen cerebrospinal fluid (CSF) material. A positive β-amyloid (1-42) result in CSF does not establish a diagnosis of Alzheimer's disease (AD) and should always be interpreted in conjunction with clinical information.

The cutoffs for the ratio pTau/Abeta42 (calculated based on results of the Elecsys Phospho-Tau (181P) CSF assay and the Elecsys β-Amyloid (1-42) CSF II assay) mentioned in the intended uses 2, 4, and 5 of this document differ from each other. For the intended uses 2 and 4, apply the amyloid cutoff. For intended use 5, apply the tau cutoff. The cutoff values are specified in the "Clinical performance data" section of this document.

System information

Short name	ACN (application code number)
AB42 2	10097

Intended use

Elecsys β-Amyloid (1-42) CSF II is an in vitro diagnostic immunoassay intended for the quantitative determination of the β-amyloid (1-42) protein concentration in human cerebrospinal fluid (CSF).

1. The Elecsys β-Amyloid (1-42) CSF II assay is intended to be used in adult subjects with cognitive impairment being evaluated for Alzheimer disease (AD) and other causes of cognitive impairment. Result above the cutoff is consistent with a negative amyloid positron emission tomography (PET) scan. Negative β-amyloid PET scans indicate sparse to no neuritic plaques and are inconsistent with a neuropathological diagnosis of AD at the time of image acquisition; a negative scan result reduces the likelihood that a patient's cognitive impairment is due to AD.

2. The Elecsys β-Amyloid (1-42) CSF II assay is intended to be used in combination with Elecsys Phospho-Tau (181P) CSF or Elecsys Total-Tau CSF assay as a ratio in adult subjects with cognitive impairment being evaluated for AD and other causes of cognitive impairment wherein a positive and negative CSF result are concordant with positive and negative amyloid Positron Emission Tomography (PET) scan result, respectively.
3. The Elecsys β-Amyloid (1-42) CSF II assay is intended to be used in combination with the Elecsys β-Amyloid (1-40) CSF assay as a ratio. The ratio aids in assessing amyloid pathology in adult subjects with cognitive impairment being evaluated for AD and other causes of cognitive impairment. A negative result, defined as Abeta42/Abeta40 ratio value above cutoff, is consistent with a negative amyloid result based on the PET scan. A positive result, defined as Abeta42/Abeta40 ratio value below cutoff, is consistent with a positive amyloid result based on the PET scan. The ratio of the Elecsys β-Amyloid (1–42) CSF II assay to the Elecsys β-Amyloid (1–40) CSF assay is an adjunct to other clinical diagnostic evaluations and aids the diagnosis of AD.
4. Elecsys β-Amyloid (1-42) CSF II assay is intended to be used alone or in combination with Elecsys Phospho-Tau (181P) CSF or Elecsys Total-Tau CSF assay as a ratio in adult subjects with mild cognitive impairment (MCI) as an aid to identify subjects who are at lower vs. higher risk of cognitive decline as defined by change in a clinical score within a 2 year period.
5. The Elecsys β-Amyloid (1-42) CSF II assay is intended to be used in combination with the Elecsys Phospho-Tau (181P) CSF assay as a ratio in adult subjects with cognitive impairment being evaluated for AD and other causes of cognitive impairment. A negative result, defined as pTau/Abeta42 ratio value below cutoff, is consistent with a negative tau result based on the tau PET scan. A positive result, defined as pTau/Abeta42 ratio value above cutoff, is consistent with a positive tau result based on the tau PET scan.

Limitations of use

- Elecsys β-Amyloid (1-42) CSF II assay is an adjunct to other clinical diagnostic evaluations.
- A positive Elecsys β-Amyloid (1-42) CSF II assay result and/or a positive Elecsys Phospho-Tau (181P) CSF or Elecsys Total-Tau CSF to Elecsys β-Amyloid (1-42) CSF II ratio result does not establish a diagnosis of AD or other cognitive disorder.
- A positive test result from the ratio of the Elecsys β-Amyloid (1–42) CSF II assay to the Elecsys β-Amyloid (1–40) CSF assay must always be interpreted in conjunction with other clinical information.
- The safety and effectiveness of the Elecsys β-Amyloid (1-42) CSF II assay have not been established for monitoring responses to therapies.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas** e immunoassay analyzers.

Summary

The Elecsys β-Amyloid (1-42) CSF II assay is designed to detect the β-amyloid (1-42) peptide, a small, 4 kDa protein of about 40 amino acids that is formed following proteolytic cleavage of a transmembrane protein known as amyloid precursor protein (APP). Cleavage of APP occurs via 2 events: cleavage by β-secretase within the extracellular domain and cleavage by γ-secretase in the transmembrane region. Due to its hydrophobic nature, the β-amyloid (1-42) peptide has the property to form aggregates and oligomers. Oligomers of higher order form fibrils that accumulate into β-amyloid plaques.[1] A Clinical relevance of β-amyloid (1-42) peptide deposition in the brain as 1 of the 2 hallmarks of AD, besides neurofibrillary tangles, can be detected by several methods: (a) histopathological staining of β-amyloid (1-42) deposits in post mortem brain tissue; (b) use of radiolabeled tracers that bind to β-amyloid deposits in the brain and can then be detected in vivo using PET scan; (c) measuring the β-amyloid 42 level in CSF because lower titers in CSF are believed to reflect accumulation of this molecule in the brain.[2, 3]

Pathological changes in the β-amyloid metabolism are the earliest alterations during AD development known so far that can be utilized diagnostically. They are reflected by the decrease in the CSF concentrations of β-amyloid (1-42) as well as by the increase in the brain uptake of the specific tracers on the β-amyloid PET.[4] Current clinical diagnostic criteria for AD require a patient to have dementia before a diagnosis of AD can be made, and are largely based on the exclusion of other disorders. No clinical method is available for identifying prodromal AD in patients with MCI, as such individuals have only mild disturbances in episodic memory.[5]

Numerous studies show that while CSF β-Amyloid (1-42) levels decrease to around half the level in controls, CSF tTau and CSF pTau 181 levels increase around 2-3 fold in mild-moderate AD patients compared to age-matched controls.[6, 7] CSF tTau has been shown to reflect the intensity of the neuronal and axonal damage and degeneration. High CSF tTau is also associated with a faster progression from MCI to AD.[8]
CSF pTau 181 levels are also associated with a faster progression from MCI to AD with more rapid cognitive decline in AD patients[9] as well as in very mild AD dementia cases. CSF pTau and CSF tTau biomarkers have the highest power when used in combination with CSF β-Amyloid (1-42) for detecting the likely progression of subjects with MCI to AD.[10]

The diagnostic accuracy of amyloid pathology can be improved by calculating the ratio of Aβ42 to Aβ40 (Aβ42/Aβ40), with Aβ42 reflecting aggregation-prone species and Aβ40 serving as a stable indicator of total Aβ production.[11, 12] The Aβ42/Aβ40 ratio reduces inter-individual variability in CSF Aβ42 levels[11, 12, 13, 14] and helps correct for differences in CSF dynamics and pre-analytical conditions that may affect both peptides.[12, 13, 14, 15] CSF Aβ42 and the Aβ42/Aβ40 ratio are markedly reduced in subjects with AD and are among the AD biomarkers that become abnormal, at the earliest, during the clinical course of the disease.[14, 15] In AD and mild cognitive impairment (MCI), no major change in the CSF levels of Aβ40 has been detected. The Aβ42/Aβ40 ratio shows high concordance with amyloid PET imaging,[16] and across multiple cohorts and platforms, this concordance is higher than for Aβ42 alone.[11, 12, 13, 14, 15] Additionally, the Aβ42/Aβ40 ratio is highly concordant with PET in the early stages of the disease, including in subjects with subjective cognitive decline (SCD) and MCI.[15] Importantly, individuals who are CSF-positive but PET-negative often convert to PET-positive within a few years. This phenomenon highlights the sensitivity of CSF biomarkers in detecting early amyloid pathology.[11]

The extent and distribution of tau pathology can provide insights into diagnosis, disease severity and stage.[17, 18] Tau PET is an imaging technique that uses a radiotracer to detect and visualize tau protein deposits in the brain. While tau PET has potential for use in patients across the AD patient journey, its use in clinical routine is limited due to cost and accessibility constraints. Additionally, the variability in tau pathology patterns makes it challenging to establish a standardized interpretation of tau PET results[19]. The utilization of fluid biomarkers to identify tau pathology has the potential to address some of the

limitations of PET imaging and enhance the diagnostic capabilities in the context of AD. Recent studies also show that the CSF pTau/Abeta42 ratio correlates with both amyloid and tau PET measures, highlighting the versatility of CSF biomarkers in reflecting amyloid and tau pathologies in the brain, increasing their diagnostic value.[20]

The use of biomarkers to diagnose AD was included in the diagnostic criteria established by consensus research for AD, mild cognitive impairment (MCI), and preclinical AD, proposed by the National Institute on Aging (NIA) and the Alzheimer's Association.[21, 22] The use of CSF biomarkers in the Alzheimer's disease diagnostic work-up was accounted for in subsequent revisions[23] and International Working Group (IWG) recommendations.[24]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- First incubation: 30 µL of sample, a biotinylated monoclonal ß-amyloid (1-42)-specific antibody (21F12), and a monoclonal ß-amyloid (1-42)-specific antibody (3D6) labeled with a ruthenium complex[a)] react to form a sandwich complex.
- Second incubation: After streptavidin-coated microparticles have been added, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell, where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission, which is measured by a photomultiplier.
- Results are determined via a calibration curve that is instrument-specifically generated by 2-point calibration and a leading calibration curve provided via **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents

The **cobas** e pack is labeled as AB42 2.

M	Streptavidin-coated microparticles, 1 bottle, 6.1 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1	Anti-β-amyloid (1-42)-Ab~biotin, 1 bottle, 6.8 mL: Biotinylated monoclonal anti-β-amyloid (1-42) antibody 21F12 (mouse) 2.0 mg/L; phosphate buffer approx. 100 mmol/L, pH 7.2; preservative.
R2	Anti-β-amyloid (1-42)-Ab~Ru(bpy), 1 bottle, 6.8 mL: Monoclonal anti-β-amyloid antibody 3D6 (mouse) labeled with ruthenium complex 1.75 mg/L; phosphate buffer approx. 100 mmol/L, pH 7.2; preservative.

Warnings and precautions

For in vitro diagnostic use for laboratory professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste

Warning: Handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards

Apply all relevant local disposal regulations to determine safe disposal.

The Safety Data Sheet is available for professional users on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.

Prevention:

P261	Avoid breathing mist or vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Hazardous components:

- 2-methyl-2H-isothiazol-3-one hydrochloride

Product safety labeling follows EU GHS guidance.

Contact phone for all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators, and controls).

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Calibration

Traceability: This method has been standardized against the 3 certified reference materials (CRMs), ERM®-DA480/IFCC, ERM®-DA481/IFCC and ERM®-DA482/IFCC.

The predefined leading calibration curve is adapted to the analyzer using the relevant calibrators.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e., not more than 24 hours after the **cobas** e pack was registered on the analyzer).

The calibration interval may be extended based on acceptable calibration verification values determined by the laboratory.

Renewed calibration is recommended as follows:

- every 12 weeks when using the same reagent lot
- every 28 days when using the same reagent kit on the analyzer
- as required, such as when quality control findings are outside the defined limits

Quality control

For routine quality control procedures, use PreciControl β-Amyloid (1-42) II or other suitable controls.

It is recommended to run the controls for the various concentration ranges individually at least once every 24 hours when the test is in use, once per **cobas** e pack, and following each calibration.

Special care needs to be taken to ensure that the accuracy and precision of the testing stays within acceptable limits. Besides meeting the PreciControl β-Amyloid (1-42) II target ranges provided, the user needs to ensure that the systematic bias with respect to the assigned target value is within ± 10 %, the intermediate precision CV is ≤ 10 %, and the maximal total error is within ± 26.5 % (TE = |bias| + 1.65*CV). It is recommended to use quality control rule software.

For those users who are not familiar with the special QC setup and application, detailed information is available in the brochure **"Guidance: Statistical Quality Control Rule Implementation"** (in English language), which is available via **navifyportal.roche.com**. This brochure explains, e.g., how to check if the maximal total error is within the allowed range based on the local QC results, besides other useful information.

Adjust the limits and control intervals based on the laboratory's individual requirements. If values fall outside the limits, each laboratory is advised to establish corrective measures.

Follow the applicable government regulations and local guidelines.

If necessary, repeat sample measurement.

Specimen collection and preparation

For CSF sample collection and measurement, follow the steps listed below.

The technical notes are an essential part of the instructions and must be read thoroughly before completing each step.

Steps	Technical notes
1. **Perform a lumbar puncture (LP)** using gravity drip collection method.	Avoid the use of syringes or tubings. Perform the LP before noon.

2. **Do not use the first 2 mL of CSF for measuring AD biomarkers on an Elecsys analyzer.**	None
3. **Subsequently collect at least 2.5 mL of CSF directly into the CSF tube** REF 63.614.625 (Sarstedt) for measuring AD biomarkers (note: 2.5 mL filling volume corresponds to filling up to the mark on the tube).	Visually inspect each sample for hemolysis. Do not use CSF samples that appear reddish for measuring AD biomarkers on an Elecsys analyzer. Instead, collect additional clear (non-hemolytic) CSF in a new CSF tube. If required, collection of CSF for other purposes can follow thereafter.
4. Do not process the CSF sample before transport to the measuring site (i.e. no mixing/inverting, no tube transfers, no aliquoting, and no centrifugation until measurement).	Keep the sample at 2-8 °C or -15 to -25 °C during transport and storage up to the time of measurement. Samples can be stored at -15 to -25 °C for up to 8 weeks or at 2-8 °C for up to 14 days. If transport and storage at -15 to -25 °C or 2-8 °C is not feasible, the sample can be transported/stored at room temperature (20-25 °C). If this is the case, measurement is to be performed within 5 days after sample draw. Note that samples cannot be frozen at -80 °C.
5. For samples stored at -15 to -25 °C, thaw samples for 30 minutes at room temperature on a roller mixer.	Only 1 freeze/thaw is acceptable
6. Measurement on the **cobas** e systems: Directly place the CSF sample tube on the analyzer for measurement. To prevent evaporation, open the sample tube only immediately before measurement.	None

Stability of CSF samples: 8 weeks at -15 to -25 °C (1 freeze/thaw cycle), 14 days at 2-8 °C, and 5 days at 20-25 °C.

Do not use hemolyzed CSF samples that are visibly colored red.

Centrifuge samples containing precipitates before performing the assay.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, analyze and measure samples and calibrators on the analyzers within 2 hours.

Always keep samples, controls, and calibrators capped if not in use.

Test procedure

For optimum performance of the assay, follow the instructions given in this document for the corresponding analyzer. For analyzer-specific assay instructions, refer to the corresponding User Guide.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas** e pack on the reagent manager.

Avoid foam formation.

The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas** e pack.

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via **cobas** link.

Calculation

The analyzer automatically calculates the analyte concentration of each sample in pg/mL.

Limitations and interferences

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations, and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 0.51 µmol/L or ≤ 0.03 mg/dL
Hemoglobin	≤ 0.0031 mmol/L or ≤ 5 mg/dL
Intralipid	≤ 10 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 4 IU/mL
IgG	≤ 0.02 g/dL

IgA	≤ 0.002 g/dL
IgM	≤ 0.0005 g/dL
Albumin	≤ 0.05 g/dL

Criterion: recovery within ± 48 pg/mL of initial value for samples ≤ 480 pg/mL and within ± 10 % of initial value for samples > 480 pg/mL.

There is no high-dose hook effect at β-amyloid (1-42) concentrations up to 6000 pg/mL.

Pharmaceutical substances

In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

Commonly used pharmaceuticals

Pharmaceutical	Concentration tested mg/L
Acetaminophen	156
Acetylcysteine	150
Acetylsalicylic acid	30
Ampicillin-Na	75
Ascorbic acid	52.5
Cefoxitin	750
Cyclosporine	1.8
Doxycycline	18
Heparin	1100 IU/L
Ibuprofen	219
Itraconazole	0.06
Levodopa	7.5
Methyldopa	22.5
Metronidazole	123
Phenylbutazone	107
Rifampicin	48
Theophylline	60

Special drugs

In addition, the following 15 special drugs were tested. No interference with the assay was found.

Drug	Concentration tested mg/L
Atorvastatin	0.75
Clopidogrel	0.3
Digoxin	0.039
Donepezil	30
Escitalopram	0.192
Esomeprazole	6.9
Furosemide	15.9
Galantamine	250
Hydrochlorothiazide	1.13
Lisinopril	0.246
Memantine	0.117
Metformin	12
Metoprolol	1.5
Rivastigmine	45
Simvastatin	1.68

Criterion: recovery within ± 48 pg/mL of initial value for samples ≤ 480 pg/mL and within ± 10 % of initial value for samples > 480 pg/mL.

Drug interferences are measured based on recommendations given in the CLSI guidelines EP07 and EP37 and in other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

In rare cases, interference caused by extremely high titers of antibodies to analyte-specific antibodies, streptavidin, or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, always assess the results in conjunction with the patient's medical history, clinical examination, and other findings.

Limits and ranges

Measuring range

150-2500 pg/mL (defined by the Limit of Quantitation and the maximum of the leading calibration curve). Values below the Limit of Quantitation are reported as < 150 pg/mL. Values above the measuring range are reported as > 2500 pg/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection, and Limit of Quantitation

Limit of Blank = 50 pg/mL

Limit of Detection = 100 pg/mL

Limit of Quantitation = 150 pg/mL

The Limit of Blank, the Limit of Detection, and the Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th-percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low-concentration samples. The Limit of Detection corresponds to the lowest analyte concentration that can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 30 %.

Specific performance data

Representative performance data on the analyzers is given below. Results obtained in individual laboratories may differ from the representative performance data.

Analytical specificity

The test is highly specific for human β-amyloid (1-42).

The following potential cross-reactivity was found.[25]

Cross-reactant	Concentration tested pg/mL	Cross-reactivity %
β-Amyloid (1-38)	10000	< 0.9
β-Amyloid (1-40)	10000	< 1.6

Clinical performance data

Each laboratory is advised to investigate the transferability of the expected values to its own patient population and, if necessary, to determine its own reference ranges.

Notes

1. Part of the clinical performance data were generated using the first generation Elecsys β-Amyloid (1-42) CSF assay (REF 06986811190) that highly correlates with Elecsys β-Amyloid (1-42) CSF II assay. In an internal method comparison study (n = 103) the observed Pearson's correlation coefficient was 0.999. The Elecsys β-Amyloid (1-42) CSF II assay was re-standardized leading to systematic differences between the first and second version. These differences were taken into account by the definition of the clinical decision thresholds.
2. Since the BioFINDER 1 study used a different pre-analytical handling procedure compared to that described in the "Specimen collection and preparation" section, an adjustment factor was determined in the RD002842 study to adjust the cutoffs for the new pre-analytical protocol measured with the Elecsys β-Amyloid (1-42) CSF II assay. The adjustment factor was generated by bridging between the BioFINDER pre-analytical handling procedure, measured with the first generation Elecsys β-Amyloid (1-42) CSF assay, and the new pre-analytical procedure, measured with the Elecsys β-Amyloid (1-42) CSF II assay.

3. All clinical performance data were generated using a previous version of the Elecsys β-Amyloid (1-40) CSF assay, that highly correlates with the Elecsys β-Amyloid (1-40) CSF assay. In an internal method comparison study (n = 171), the observed Pearson's correlation coefficient was 0.996. The Elecsys β-Amyloid (1-40) CSF assay was re-standardized leading to systematic differences. Hence, the transfer of data from the previous version of the Elecsys β-Amyloid (1-40) CSF assay to Elecsys β-Amyloid (1-40) CSF assay required a conversion factor to ensure data consistency across assay versions. The conversion factor was derived from the method comparison study.

Concordance with amyloid PET visual read

Concordance with PET visual read was assessed in a retrospective study (Roche study RD002145) based on samples from the BioFINDER cohort.[26]The primary analysis population consisted of 277 mild cognitive symptoms (MCS) patients for whom banked CSF samples and PET scan results were available (PET tracer: [18F]-Flutemetamol). Of the 277 patients, 120 had subjective cognitive decline (SCD), 153 MCI and for 4 patients no assignment was available. The average age was 70 years (range 59-80 years), 42 %/ 58 % of patients were female/male and 45 %/ 54 % of patients were ApoE4 carriers/non-carriers. The median (1.48*Median absolute deviation) of the Elecsys markers at baseline was as follows: Abeta42, 1048 (593) pg/mL measured with the first generation Elecsys β-Amyloid (1-42) CSF; pTau, 20.0 (9.4) pg/mL; tTau 240 (100) pg/mL. The amyloid PET scans were read independently by 3 trained readers and majority voting was used to rate an image as positive or negative, resulting in 110 (40 %) positive, and 167 (60 %) negative amyloid PET reads. The cutoffs for Abeta42 and the ratios pTau/Abeta42 and tTau/Abeta42 were established based on the amyloid PET visual read.

The agreement rates for the Elecsys CSF markers with amyloid PET visual read were as follows:

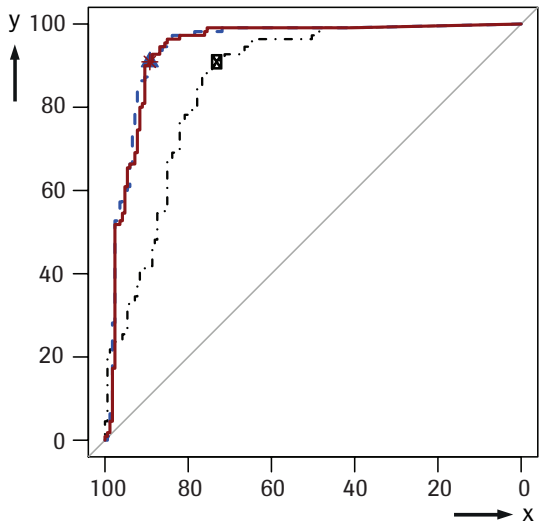
Agreement rates [%] (95 % CI[A)])			
	Abeta42	pTau/Abeta42	tTau/Abeta42
PPA[B)]	90.9 (83.9, 95.6)	90.9 (83.9, 95.6)	90.9 (83.9, 95.6)
NPA[C)]	72.5 (65.0, 79.1)	89.2 (83.5, 93.5)	89.2 (83.5, 93.5)
OPA[D)]	79.8 (74.6, 84.4)	89.9 (85.7, 93.2)	89.9 (85.7, 93.2)

A) CI = confidence interval

B) PPA = Positive percentage agreement ("sensitivity")

C) NPA = Negative percentage agreement ("specificity")

D) OPA = Overall percentage agreement



x: NPA (Specificity) (%) y: PPA (Sensitivity) (%)

Abeta42 ratio — tTau/Abeta42 ratio — pTau/Abeta42

Figure: Receiver-operating characteristic curves for Abeta42 and ratios pTau/Abeta42 and tTau/Abeta42 with outcome amyloid PET. The Circle/Triangle/asterisk denotes PPA and NPA at the cutoffs for the 3 biomarkers, respectively; Abeta42, AUC: 86.5 % (82.3 %, 90.7 %); pTau/Abeta42, AUC: 94.4 % (91.5 %, 97.3 %); tTau/Abeta42, AUC: 94.0 % (91.0 %, 97.0 %)

The clinical performance of the Abeta42/Abeta40 ratio with amyloid PET visual read as a reference method was assessed in the prospective Apollo study[27] (Roche study RD002678) based on samples from the Swedish BioFINDER-2 study (NCT03174938).[28] The Apollo study enrolled subjects from BioFINDER-2 cohorts C and C2 at Swedish memory clinics. The detailed inclusion and exclusion criteria for BioFINDER-2 are publicly available.[28]

All participants of the BioFINDER-2 study underwent detailed clinical assessment, including cognitive testing and lumbar puncture for CSF collection. Based on this assessment, subjects were assigned into different cohorts. All subjects assigned to cohorts C or C2 (SCD/MCI) underwent an amyloid PET scan. All SCD/MCI subjects who underwent clinical assessment, lumbar puncture, and an amyloid PET scan as part of BioFINDER2 (cohorts C or C2) were included

in the Apollo study. Subject inclusion began at the start of the study and ended when the minimum sample size requirement was fulfilled. The Apollo study included two main categories of subjects: the initial visit subject and subjects from cohorts C or C2 who were included at baseline, returned for their year-2 follow-up appointment, and remained SCD/MCI following cognitive re-assessment (RD002678).

The original cutoff < 0.058 for Abeta42/Abeta40 was based on previously published data.[29] The cutoff was determined and cross-validated in samples from the BioFINDER (n = 172; 85 cognitively unimpaired [CU] and 87 with mild cognitive impairment [MCI]), ADNI[30] (n = 318; 54 CU and 264 MCI), and UCSF (n = 260; 55 CU, 22 MCI, 37 AD dementia, and 146 with various non-AD disorders) cohorts, using visual amyloid PET as an endpoint. All clinical diagnosis groups within each cohort comprised both PET-positive and PET-negative subjects (figure 1 in Leuzy et al., 2023). In the BioFINDER cohort, this cutoff had PPA 93.97 % [90.45, 96.98], NPA 83.92 % [79.60, 88.23] and OPA 88.33 % [85.24, 91.19] (table S1 in Leuzy et al., 2023). The value 0.058 was adjusted for the fresh routine-use pre-analytical procedure (described in the "Specimen collection and preparation" section) and the updated version II of the Abeta42 assay, using results of the pre-analytical bridging study RD002842. The adjusted value was rounded to 3 significant digits: 0.052.
This cutoff was verified in the analysis population described in the Apollo study.[27] The analysis population consisted of participants with subjective cognitive decline (n = 41) or mild cognitive impairment (n = 31), and 19 study participants with unknown sub-classification, for whom amyloid PET scan results were previously generated under the Swedish BioFINDER-2 study (PET tracer: 18F-flutemetamol).
The amyloid PET scans were read independently by 3 trained readers, and majority voting was used to rate an image as positive or negative, resulting in 40 (44 %) positive and 51 (56 %) negative amyloid PET reads. The average age was 70.3 years (range: 43–90 years), 38 patients (42 %) were female, 53 patients (58 %) were male. The median values (1.48 × the median absolute deviation) of the Elecsys markers at baseline were as follows: Elecsys β-Amyloid (1–42) CSF II assay: 906 (404) pg/mL; Elecsys β-Amyloid (1–40) CSF assay: 10318 (2217) pg/mL.

The agreement rates for the ratio of the Elecsys β-Amyloid (1-42) CSF II assay to the Elecsys β-Amyloid (1-40) CSF assay with amyloid PET visual read were as follows:

Agreement rates [%] (95 % CI[A])	
	Abeta42/Abeta40
PPA[B]	0.950 (0.835, 0.986)
NPA[C]	0.824 (0.697, 0.904)
OPA[D]	0.879 (0.796, 0.931)

A) CI = confidence interval

B) PPA = Positive percentage agreement ("sensitivity")

C) NPA = Negative percentage agreement ("specificity")

D) OPA = Overall percentage agreement

Identification of patients at risk of cognitive decline

The ability of the single marker Abeta42 as well as the biomarker ratios pTau/Abeta42 or tTau/Abeta42 to identify patients at higher vs. lower risk of cognitive decline as defined by change in a clinical score within a 2-year period was assessed in a retrospective study (Roche study RD002530) based on samples from the ADNI1/GO/2 studies.[30] The primary analysis population included a total of 619 patients from the early (EMCI, 277) and late mild cognitive impairment (LMCI, 342) cohorts with baseline Elecsys CSF measurements available. For each of these patients also baseline assessments of the clinical scores Clinical Dementia Rating – Sum of Boxes (CDR-SB) and Mini-Mental State Examination (MMSE) were available. The average age of the 619 subjects was 72 years (range 54-91 years), 41 %/59 % were female/male, the average education time was 16 years (range 6-20 years) and 51 %/39 %/11 % carried 0/1/2 ApoE4 alleles. The averages (standard deviation, SD) of clinical scores were as follows: CDR-SB, 1.5 (0.9) at baseline, 2.3 (2.1) at 2-year follow-up; MMSE, 27.7 (1.8) at baseline, 26.6 (3.3) at 2-year follow-up. The median (1.48*Median absolute deviation) of the Elecsys CSF marker concentrations at baseline were as follows: Abeta42, 838 (410) pg/mL; pTau, 24.0 (12.0) pg/mL; tTau, 257 (107) pg/mL.

The ability of the biomarkers to separate patients at lower vs. higher risk of cognitive decline (as measured by change in CDR-SB or MMSE) within 2 years was assessed using linear mixed-effects models. The models were adjusted for age, sex, education time and baseline value of the respective clinical score.

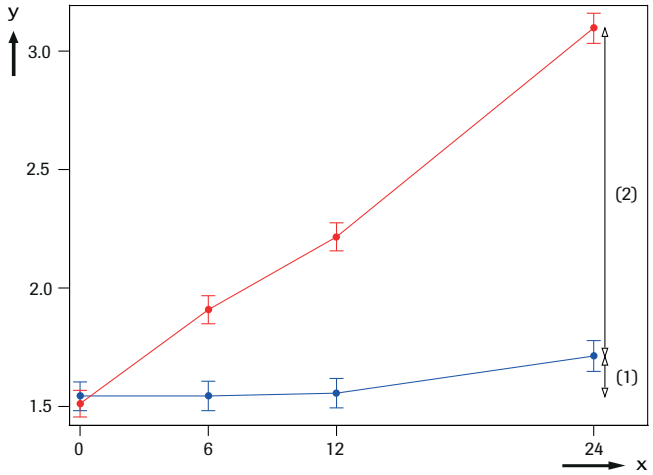
Due to different preanalytic handling procedures between BIOFINDER and ADNI, a bridging study RD002475 was used to adjust the cut offs from Biofinder to ADNI based on optimization for concordance with amyloid PET.

Using these cutoffs for cognitive decline analysis, the model-based average change in clinical scores (CDR-SB; MMSE) between baseline and 2 years in the biomarker-negative group (effect (1)) and the difference in change in clinical scores between biomarker-positive and -negative groups (effect (2)) were as follows:

Clinical score	Biomarker	Effect (1) Estimate (95 % CI)	Effect (2) Estimate (95 % CI)
CDR-SB	Abeta42	0.31 (0.16, 0.46)	1.10 (0.89, 1.31)
	pTau/Abeta42	0.17 (0.02, 0.32)	1.42 (1.21, 1.62)
	tTau/Abeta42	0.21 (0.07, 0.35)	1.41 (1.20, 1.62)
MMSE	Abeta42	-0.25 (-0.53, 0.04)	-1.79 (-2.19, -1.40)
	pTau/Abeta42	-0.08 (-0.36, 0.20)	-2.17 (-2.56, -1.77)
	tTau/Abeta42	-0.13 (-0.40, 0.14)	-2.19 (-2.58, -1.79)

All 3 biomarkers separated patients at lower vs. higher risk of cognitive decline within 2 years. The ratios showed superior performance compared to the single marker Abeta42. For instance, change in CDR-SB and MMSE over 2 years between the biomarker-positive and -negative groups according to pTau/Abeta42 or tTau/Abeta42 ratio differed by more than 1 and -2.5 units (lower confidence limit of effect (2)), respectively. Biomarker ratio-negative patients did not show a change in CDR-SB and MMSE over 2 years more than 0.5 and -0.5 (upper confidence limit of effect (1)), respectively. These results did not change after additional adjustment for ApoE4 genotype (number of E4 alleles).

Model-based time course plot for change in CDR-SB over 2 years for pTau/Abeta42 ratio-based classification (without adjustment for ApoE4 genotype):



x: Visit y: CDR-SB

Figure: Model-derived average and standard error of CDR-SB in pTau/Abeta42 ratio-positive (red) and –negative (blue) groups over follow-up time (x-axis; visit time point in months). Effects (1) and (2) as described above are symbolized by arrows.

Concordance with tau PET visual read

The concordance of the Elecsys CSF pTau/Abeta42 ratio with tau PET visual read as a reference method was assessed in a retrospective analysis using 2 independent cohorts.[31]
Cutoff determination was performed in a subset of 133 subjects from the ADNI-2/3[30] cohort, in subjects who had a clinical diagnosis of mild cognitive impairment (MCI) (n = 104; 78.2 %) or dementia due to AD (n = 29; 21.8 %), available Elecsys Phospho-Tau (181P) CSF assay and Elecsys β-Amyloid (1-42) CSF II assay measurements, and available tau PET scans. Tau PET scans were performed with [18F]-flortaucipir (FTP; Tauvid™, Eli Lilly, Utrecht, the Netherlands) and interpreted using the FTP visual-read methodology (tau-positive if visual read = AD+ or AD++, and tau-negative if visual read = AD–). In this cohort, 52 subjects (39.1 %) were tau-PET-positive and 81 (60.9 %) were tau-PET-negative. The average age was 70.9 years (range: 55.3-89.2 years), 56 patients (42.9 %) were female and 76 patients (57.1 %) were male; 63 (47.4 %) were APOE4 carriers and 56 (42.1 %) were non-carriers. APOE4 status was missing for 14 patients (10.5 %). For better comparability, CSF biomarker measurements of ADNI-2/3 were adjusted for the fresh routine-use pre-analytical procedure (described in the “Specimen collection and preparation” section) and the updated version II of the Abeta42 assay using results of the pre-analytical bridging study RD002842 published in Blennow et al., 2022.[32] The median values (1.48 × the median absolute deviation) of the Elecsys markers were as follows: Elecsys Phospho-Tau (181P) assay: 24.9 (11.2) pg/mL; Elecsys β-Amyloid (1–42) CSF II assay: 802.0 (453) pg/mL. A cutoff of 0.037 was selected as the best compromise between PPA and NPA. In the ADNI-2/3 cohort, this cutoff had PPA 88.5 % (77.0, 94.6), NPA 82.7 % (73.1, 89.4), and OPA 85.0 % (77.9, 90.0) (table 3 in Smith et al., 2025).

The cutoff was validated in an independent subset of 62 subjects from the Swedish BioFINDER-2 study (NCT03174938) assigned to either cohort C (SCD and MCI) or cohort D (AD dementia). The detailed inclusion and exclusion criteria for BioFINDER-2 are publicly available.[28] The analysis population consisted of subjects with MCI (n = 36; 58.1 %) and dementia due to AD (n = 26; 41.9 %), for whom Elecsys Phospho-Tau (181P) CSF assay and Elecsys β-Amyloid (1-42) CSF II assay measurements and tau PET scans were available (PET tracer: [18F]-RO948). The FTP visual read methodology was used for both tracers because [18F]-RO948 is structurally similar to FTP.[33, 34, 35] The tau PET status of the study subjects was derived based on the FTP visual read methodology performed by a single reader, resulting in 35 positive (56.5 %) and 27 negative (43.5 %) tau PET visual reads. The average age was 75.1 years (range: 54.5-87.4 years), 29 patients (46.8 %) were female and 33 patients (53.2 %) were male; 33 (53.2 %) were APOE4 carriers and 29 (46.8 %) were non-carriers. The median values (1.48 × the median absolute deviation) of the Elecsys markers were as follows: Elecsys Phospho-Tau (181P) CSF assay: 26.4 (13.9) pg/mL; Elecsys β-Amyloid (1–42) CSF II assay: 733.0 (307) pg/mL.

The agreement rates for the ratio of the Elecsys Phospho-Tau (181P) CSF assay to the Elecsys β-Amyloid (1-42) CSF II assay with tau PET visual read were as follows:

	Agreement rates (%) (95 % CI)
Positive percentage agreement (PPA, sensitivity)	85.7 (70.6, 93.7)
Negative percentage agreement (NPA, specificity)	70.4 (51.5, 84.1)
Overall percentage agreement	79.0 (67.4, 87.3)

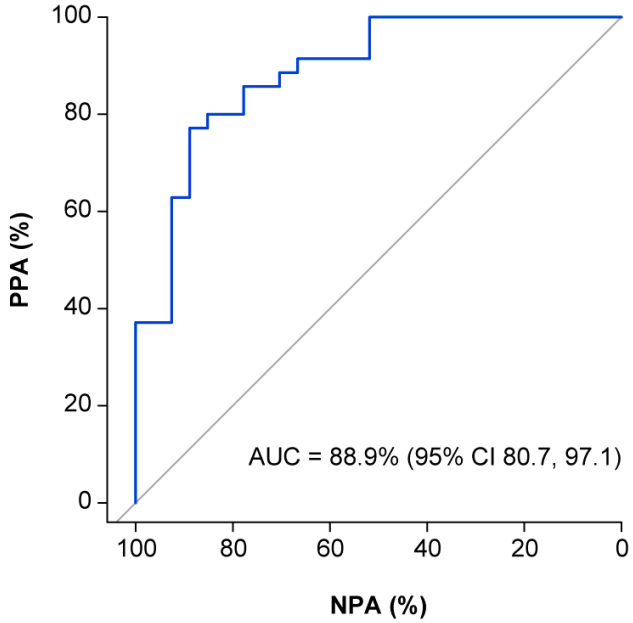


Figure: Receiver-operating characteristic (ROC) analysis using the tau PET visual read endpoint in the BioFINDER-2 cohort. AUC = area under the ROC curve, CI = confidence interval, NPA = negative percent agreement, PET = positron emission tomography, PPA = positive percent agreement

Cutoffs for PET concordance and cognitive decline

As the BioFINDER study used a different pre-analytical handling procedure compared to that described in the section “Specimen collection and preparation”, an adjustment factor was determined in study RD002842 to adjust the cut offs for the new preanalytical protocol measured with the Elecsys β-Amyloid (1-42) CSF II assay. The adjustment factor was generated by bridging between the Biofinder preanalytical handling procedure, measured with Elecsys β-Amyloid (1-42) CSF first generation and the new preanalytical procedure measured with the Elecsys β-Amyloid (1-42) CSF II assay.

The cutoffs for **amyloid PET** concordance and cognitive decline using the pre-analytical procedure described in the section “Specimen collection and preparation” are shown below:

If Abeta42 ≤ 1030 pg/mL: test result positive

If Abeta42 > 1030 pg/mL: test result negative

If pTau/Abeta42 ratio* > 0.023: test result positive

If pTau/Abeta42 ratio* ≤ 0.023: test result negative

*The ratio should be rounded to 4 decimal places before comparing against 0.023. If the concentration of one of the analytes is outside the measuring range, the following rules apply:
In cases Abeta42 < 150 pg/mL, Abeta42 > 2500 pg/mL, pTau > 120 pg/mL, pTau < 8 pg/mL, the value should be set to the respective limit of the measuring range and the ratio should be calculated.

If tTau/Abeta42 ratio* > 0.28: test result positive

If tTau/Abeta42 ratio* ≤ 0.28: test result negative

*The ratio should be rounded to 3 decimal places before comparing against 0.28. If the concentration of one of the analytes is outside the measuring range, the following rules apply:
In cases Abeta42 < 150 pg/mL, Abeta42 > 2500 pg/mL, tTau > 1300 pg/mL, tTau < 80 pg/mL, the value should be set to the respective limit of the measuring range and the ratio should be calculated.

Since the previous version of the Elecsys β-Amyloid (1-40) CSF assay was used in the study, an adjustment factor was determined to adjust the cutoff to the Elecsys β-Amyloid (1-40) CSF assay (refer to the “Clinical performance data” section).

The new derived cutoff for **amyloid PET** concordance using the pre-analytical procedure described in the "Specimen collection and preparation" section of the Elecsys β-Amyloid (1-40) CSF assay Method Sheet (REF 10282142190) is shown below:

If Abeta42/Abeta40 ratio* ≥ 0.097: test result negative
If Abeta42/Abeta40 ratio* < 0.097: test result positive
* Round the ratio to 4 decimal places before comparing against 0.097. If the concentration of 1 of the analytes is outside the measuring range, the following rules apply: If Abeta42 < 150 pg/mL, Abeta42 > 2500 pg/mL, Abeta40 < 750 pg/mL, Abeta40 > 20000 pg/mL, set the value to the corresponding limit of the measuring range and calculate the ratio.

The new derived cutoff for tau PET concordance is shown below:

If pTau/Abeta42 ratio* > 0.037: test result positive
If pTau/Abeta42 ratio* ≤ 0.037: test result negative
* Round the ratio to 4 decimal places before comparing against 0.037. If the concentration of 1 of the analytes is outside the measuring range, the following rules apply: If Abeta42 < 150 pg/mL, Abeta42 > 2500 pg/mL, pTau > 120 pg/mL, pTau < 8 pg/mL, set the value to the corresponding limit of the measuring range and calculate the ratio.

Precision

Precision was determined using Elecsys reagents, samples, and controls based on a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day, in duplicate each, for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas** e 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pg/mL	SD pg/mL	CV %	SD pg/mL	CV %
Human CSF 1	157	1.53	1.0	2.44	1.6
Human CSF 2	200	1.83	0.9	3.17	1.6
Human CSF 3	764	15.2	2.0	21.3	2.8
Human CSF 4	973	20.9	2.1	27.2	2.8
Human CSF 5	1042	21.2	2.0	30.1	2.9
Human CSF 6	1186	22.5	1.9	32.6	2.7
Human CSF 7	1243	27.7	2.2	37.3	3.0
Human CSF 8	2124	84.3	4.0	126	5.9
Human CSF 9	2290	21.6	0.9	28.6	1.2
PC[A)] β-Amyloid (1-42) 1	616	4.86	0.8	6.76	1.1
PC β-Amyloid (1-42) 2	1689	12.6	0.7	18.2	1.1

A) PC = PreciControl

Method comparison

A comparison of the Elecsys β-Amyloid (1-42) CSF II assay, REF 08821941190 (**cobas** e 402 analyzer; y), with the Elecsys β-Amyloid (1-42) CSF II assay, REF 08821941190 (**cobas** e 801 analyzer; x), gave the following correlation (pg/mL):

Number of samples measured: 133

Passing/Bablok[36]	Linear regression
y = 1.04x - 6.70	y = 1.03x -1.85
τ = 0.982	r = 0.999

The sample concentrations were between 168 and 2464 pg/mL.

Additional information

For further information, refer to the User Guide for the corresponding analyzer, to the corresponding application sheets, and to the Method Sheets of all necessary components.

A point (period/stop) is always used in the English version of a Method Sheet as the decimal separator to mark the boundary between the integral and the fractional parts of a decimal numeral. The translated Method Sheets use decimal commas. Labels only use the decimal point as separator. Separators for thousands are not used.

Report any serious incident that has occurred in relation to the device to the manufacturer and the competent authority of the member state in which the user and/or patient is established.

Symbols

In addition to the ISO 15223-1 standard, Roche Diagnostics uses the following symbols and signs:

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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Changelog

For this document version only:

Due to technical reasons, changes that have been made since the last version of this document are listed in the following table instead of indicated by change bars in the margin.

Section headers are indicated in bold letters.

In addition to the changes listed in the table below, this method sheet version contains several editorial and layout updates.

Section	Current version	Previous version
Note	**Note**	**Please note**

Section	Current version	Previous version
	All analytical performance data were generated using frozen cerebrospinal fluid (CSF) material. Section updated	All performance data were generated using frozen cerebrospinal fluid (CSF) material.
Intended use	Section updated	
Summary	Section updated	
Quality control	Follow the applicable government regulations and local guidelines.	Follow the applicable government regulations and local guidelines for quality control.
Warnings and precautions	**Warnings and precautions**	**Precautions and warnings**
Warnings and precautions	laboratory	health care
Warnings and precautions	Hazardous components: • 2-methyl-2H-isothiazol-3-one hydrochloride	
Calibration	every	after
Quality control	Use PreciControl β-Amyloid (1-42) II or other suitable controls for routine quality control procedures.	For quality control, use PreciControl β-Amyloid (1-42) II.
Test procedure	**Test procedure**	**Reagent handling** **Assay**
Limitations and interferences	**Limitations and interferences**	**Limitations - interference**
Specific performance data	**Clinical performance data** Each laboratory is advised to investigate the transferability of the expected values to its own patient population and, if necessary, to determine its own reference ranges. Section updated	**Clinical performance** Each laboratory should investigate the transferability of the expected values to its own patient population.
Additional information	A point (period/stop) is always used in the English version of a Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. The translated Method Sheets use decimal commas. Labels only use the decimal point as separator. Separators for thousands are not used.	A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the boundary between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.
References	New references: 11-36	
Symbols	Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:	Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):