ELISAs for Differential Dementia Diagnostics

Immunoassays setting new benchmarks in diagnosis of Alzheimer’s, Parkinson’s and Creutzfeldt-Jakob disease
Procedure of ELISAs

1. Ready-to-use: Capture antibody coated on well plate

2. Binding of target antigen by capture antibody

3. Direct detection using a HRP-conjugated species antibody
Recombinant proteins and the rapid generation of unique monoclonal antibodies for a variety of biomarkers are the bases for easy-to-handle and ultra-sensitive immunoassays encouraging an improved diagnosis of e.g. Alzheimer’s, Parkinson’s and Creutzfeldt-Jakob disease.

Trust in Analytik Jena's ELISAs and revolutionize your Differential Dementia Diagnostics

- **Multi parameter analysis** expanded diagnostic output
- **Certified for in vitro diagnostics** proven reliability
- **Lyophilized standards and controls** for convenience and precision of quantitative results
- **Fast and easy handling** clinical relevant results using daily protocols
- **Fast-processing** well suited for automation on relevant platforms in routine diagnostics
- **Flexible application depending on sample throughput** standardized ELISA format on 12 x 8 immuno strips
- **Independence of sample input material** applicable to CSF, serum or plasma
Alzheimer’s disease

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder that is the most common form of dementia. AD is affecting more than 36 million people worldwide and is most common in Western Europe. The prevalence of AD may triple by 2050 due to higher life expectancies. AD is associated with a progressive loss of neuronal function and subsequent gradual deterioration in cognition and behavior leading finally to death.

The brain pathology of AD patients is characterized by two types of pathological protein deposits. On the hand, extracellular amyloid plaques and on the other accumulation of intracellular neurofibrillary tangles (NFT), the major constituents of which are amyloid β protein and tau protein, respectively. Both biomarkers are generally accepted as early indicators of AD in humans.

Analytik Jena’s hTAU total and pTAU rel ELISA for quantification of human tau and a novel, non-phosphorylated tau fraction in CSF of patients with suspected Alzheimer’s disease enable a diagnostic quality that achieves exceptional results in terms of specificity and sensitivity. The hTau total ELISA is based on a monoclonal antibody immobilized on the immuno strip surface with specific binding affinity for amino acids 160-180 of tau protein isoforms. The pTAU rel ELISA utilizes a monoclonal antibody specific for the non-phosphorylated TPP sequences (position 175, 181 and 231) of the tau protein. After binding the antigens from human CSF, detection is conducted by a HRP-conjugated secondary antibody specific for tau protein using the chromogenic substrate tetramethylbenzidine (TMB). The lyophilized standards and controls included ensure clinical relevant results with high precision and accuracy. Both ELISAs are CE-IVD certified and particularly suitable for automation on corresponding platforms allowing convenient routine diagnostics of CSF samples. The standardized protocols to be performed within 4 - 5 h are optimally adapted and recommended to be used in conjunction with IBL Internationals (Hamburg, Germany) Amyloid-β CSF ELISAs.
hTAU total ELISA

- CE-IVD certified ELISA for Alzheimer’s disease diagnostics using quantification of tau protein in human CSF
- Standardized protocols optimally adapted and recommended to be used in conjunction with Amyloid-β CSF ELISAs (IBL International)
- 6-point standard curve using lyophilized standards and controls
- Exceptional diagnostic quality – outstanding clinical sensitivity and specificity
- Fast and easy handling: clinical relevant results within 4 h

Product specifications

<table>
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<tr>
<th>Target</th>
<th>Human tau total</th>
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<td>Detection time</td>
<td>4 h</td>
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<td>Storage/Stability</td>
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<tr>
<td>Performance feature</td>
<td>CE-IVD</td>
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Performance assessment

Total tau protein in the CSF in cases with Alzheimer’s disease (AD) and mild cognitive impairments (MCI), as well as a control group was determined using the hTAU total ELISA. The level of total tau was significantly higher in patients group compared to the control population (p<0.001), which resulted in reasonable sensitivities and specificities.

Order information

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<th>Quantity</th>
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<td>847-0108000101*</td>
<td>96 reactions</td>
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* Distributor: IBL International GmbH, Hamburg/Germany

References

The analyzes were performed in the Lab for Clinical Neurochemistry and Neurochemical Dementia Diagnostics, Department of Psychiatry and Psychotherapy, Universitätsklinikum Erlangen, Erlangen, Germany (Head: Prof. Dr. med. Piotr Lewczuk).
pTAU rel ELISA

- CE-IVD Kits for analyzing a novel non-phosphorylated fraction of tau protein in human CSF of patients with suspected Alzheimer’s disease
- Diagnostic quality that achieves exceptional results in terms of specificity and sensitivity
- Standardized protocols including 6-point standard curve
- Flexible application depending on sample throughput: standardized ELISA format on 12 x 8 immuno strips
- Fast and easy handling: clinical relevant results within 5 h

Product specifications

<table>
<thead>
<tr>
<th>Target</th>
<th>Non-phosphorylated tau fraction</th>
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Performance assessment

In human CSF, correlates of neuropathologic disorders defining Alzheimer’s disease are measurable. Currently relevant parameters are β-amyloid 1-42, total tau and phospho-tau (P-tau181). The novel pTAU rel ELISA is an alternative to the previously used P-tau181. Non-phosphorylated tau fraction in the CSF in cases with Alzheimer’s disease (AD) and mild cognitive impairments (MCI), as well as a control group was determined using the pTAU rel ELISA. The level of non-phosphorylated tau fraction was significantly higher in patients group compared to the control population (p<0.001). Furthermore the assay is characterized by outstanding clinical sensitivity and specificity.

Verification of highly significant differences of tau protein concentration in CSF samples of patients with AD and MCI (summarized as AD, n=58) as well as control group (n=42).

Clinical sensitivity 95 % (n=58)
Clinical specificity 98 % (n=42)
Linearity 100 - 1000 pg/ml
Precision
  - Intra-Assay 5.2 %
  - Inter-Assay 7.5 %
  - Inter-Lot 7.9 %
Cutoff 78 pg/ml
Area ROC curve 0.98
Youden-Index 0.92
Significance <0.0001

Order information

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* Distributor: IBL International GmbH, Hamburg/Germany

Related products

- hTAU total ELISA
- BetaPrion® HUMAN ELISA

References

The analyzes were performed in the Lab for Clinical Neurochemistry and Neurochemical Dementia Diagnostics, Department of Psychiatry and Psychotherapy, Universitätsklinikum Erlangen, Erlangen, Germany (Head: Prof. Dr. med. Piotr Lewczuk).
TAU aggregate ELISA

- Worldwide unique ELISA for analyzing aggregated tau in e.g. human and mouse brain tissue
- 6-point standard curve using lyophilized standards and controls
- Easy handling using an overnight procedure
- Optimized storage stability of in-house-produced antibodies for preservation of specific and exceptionally well characterized binding capabilities
- Flexible application depending on sample throughput: standardized ELISA format on 12 x 8 immuno strips

Product specifications

<table>
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<tr>
<th>Target</th>
<th>Aggregated tau</th>
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</table>
| Starting material | Human brain tissue
|                  | Biological samples from animal and cell models |
| Detection time  | 24 h           |
| Storage/Stability | 12 months at 4 °C |

Performance assessment

Tau protein is mainly expressed in neurons, where it binds and stabilizes microtubules. In Alzheimer’s disease and other tauopathies, tau protein has a reduced affinity toward microtubules. As a consequence, tau protein detaches from microtubules and eventually aggregates into β-sheet-containing filaments.

Order information

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[x] = Detection substrate [4] ECL; [0] TMB

Significant differences of concentration of tau aggregates measured by TAU aggregate ELISA in nerve tissue samples from Alzheimer’s disease and neurological control patients.

Development of tau aggregation during aging found by means of TAU aggregate ELISA in P301L mice as animal model for Alzheimer’s disease.

References

Material and analyzes were kindly provided by PhD Max Holzer, Paul-Flechsig-Institute in Leipzig/Germany.

Related products

<table>
<thead>
<tr>
<th>hTAU total ELISA</th>
<th>pTAU rel ELISA</th>
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<tbody>
<tr>
<td>Page 6</td>
<td>Page 7</td>
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Controls

<table>
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<th>AD</th>
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<tbody>
<tr>
<td>P: postnatal day, CR: Cortex, HB: Hindbrain</td>
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</table>

aggregated tau in ng/mg total protein
Parkinson’s disease

Parkinson’s disease is pathologically characterized by the presence of Lewy bodies in the subcortical regions of the brain, which are composed of aggregated and phosphorylated α-synuclein. The detection of pathologically relevant formations of α-synuclein, currently discussed as promising surrogate markers for Parkinson’s disease and dementia with Lewy bodies, in body fluids (CSF and plasma), can provide additional manifestations for accompanying diagnostics.

Due to the frequent co-occurrence of Lewy body pathology with Alzheimer’s disease (AD) pathology, it is crucial to differentiate it from pure AD forms. It is therefore most desirable to make use of it not only in autopsies, but also for early clinical diagnosis, while the patient could still potentially benefit from the result.

Analytik Jena provides improved ELISAs for detection of total human α-synuclein, the HUMAN α-Synuclein MONO ELISA, and its pathological aggregates by use of the HUMAN α-Synuclein PATHO ELISA. Initial clinical trials provided evidence that combined detection of total α-synuclein and disease-specific α-synuclein is a promising diagnostic tool for the detection of Parkinson’s disease (Unterberger et al. 2014).

The high specificity of the Human α-Synuclein PATHO ELISA is down to the unique characteristics of Anti-human α-synuclein 5G4, monoclonal antibody, which has set new standards in the diagnostics of conditions such as Parkinson’s disease (Kovacs et al. 2012 and 2014). The novel assays not only deliver an impressively easy and fast handling procedure, but also improve the examination of α-synucleinopathies due to its extremely stable implemented standards and controls, independent of the sample input material.
Anti-human α-synuclein 5G4, monoclonal antibody

- 5G4 is the first monoclonal antibody worldwide that clearly recognizes β-sheet depending epitopes
- Specifically binds to pathological relevant α-synuclein oligomers in
- Neuropathology of Lewy body/Parkinson’s disease
- Optimized storage stability of in-house-produced antibodies for preservation of specific and exceptionally well characterized binding capabilities
- Highly purified monoclonal antibody (>95 %)

**Product specifications**

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Clone</td>
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<td>Reactivity</td>
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**Performance assessment**

The Anti-human α-synuclein 5G4 shows high specificity for the disease-specific forms of α-synuclein, including high molecular weight fraction of β-sheet rich oligomers, while no binding to primarily disordered oligomers or monomers is observed.

**References**


Feasibility study on verification of total α-synuclein in the CSF in cases with Lewy body pathology (LBP) and Alzheimer’s diseases (AD), as well as a control group using the Human α-Synuclein MONO ELISA (ECL detection). The level of LBD patients was dropped compared to AD patients and was significantly lower compared to control groups (p=0.016).

Quantification of total α-synuclein in CSF of patients (n=8) three times repeated performing duplicate measurements using the Human α-Synuclein MONO ELISA (TMB detection). The ELISA is characterized by an excellent precision with variations of 4.2 % between the different experiments. The correlation between repetitive experiments per patient was significant for all patient samples (R²= 0,97 (p<0.001)).

Product specifications

<table>
<thead>
<tr>
<th>Target</th>
<th>Total α-synuclein</th>
</tr>
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</table>
| Starting material       | • Serum, plasma and CSF  
                          | • Biological samples from animal and cell models |
| Detection time          | 24 h              |
| Sensitivity             | 25 pg/ml          |
| Storage/Stability       | 12 months at 4 °C |

Performance assessment

The quantification of α-synuclein, a presynaptic protein, in human CSF is used to differentiate e.g. Lewy body pathology (LBP) from other neurodegenerative pathologies.

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<td>96 reactions</td>
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[x] = Detection substrate [4] ECL; [0] TMB

Related products

HUMAN α-Synuclein PATHO ELISA

References

Feasibility study on verification of disease-associated α-synuclein in the CSF in cases with Lewy body pathology (LBP) and Alzheimer’s diseases (AD), as well as a control group using the Human α-Synuclein PATHO kit. The levels of disease-associated α-synuclein were - on average - higher in PDD/DLB than in other groups.

Quantification of disease-associated α-synuclein in CSF of patients (n=10) two times repeated performing duplicate measurements using the Human α-synuclein PATHO ELISA (TMB detection). The ELISA is characterized by a very good precision with variations < 10 % between the different experiments. The correlation between repetitive experiments per patient was significant for all patient samples (R²= 0.91 (p<0.001)).

Performance assessment
The quantification of α-synuclein, a presynaptic protein, in human CSF is used to differentiate e.g. Lewy body pathology (LBP) from other neurodegenerative pathologies. The detection of pathologically relevant formations of α-synuclein can provide additional manifestations for accompanying diagnostics.

HUMAN α-Synuclein PATHO ELISA

- ELISA for analyzing diseases-associated α-synuclein from human CSF, plasma or serum
- 6-point standard curve using lyophilized standards and controls
- Available for detection using absorption (TMB) or chemiluminescence (ECL)
- Application of worldwide unique, patented antibodies
- Standardized ELISA format on 12 x 8 immuno strips for flexible application depending on sample throughput

Product specifications

<table>
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<th>Feature</th>
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<td>Target</td>
<td>Pathological relevant α-synuclein oligomers</td>
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<td>Sensitivity</td>
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<tr>
<td>Storage/ Stability</td>
<td>12 months at 4 °C</td>
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Performance assessment

The quantification of α-synuclein, a presynaptic protein, in human CSF is used to differentiate e.g. Lewy body pathology (LBP) from other neurodegenerative pathologies. The detection of pathologically relevant formations of α-synuclein can provide additional manifestations for accompanying diagnostics.

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References
A third area of focus is on the differential diagnostics of rapid-onset Creutzfeldt-Jakob disease (CJD), which is caused by aggregation of cellular prion protein. Human prion disorders are rare, affecting one to two cases per million inhabitants.

The identification of this disease, which is extremely serious for the patient and which shot to prominence in the bovine spongiform encephalopathy crisis, by distinguishing it from forms of dementia such as Alzheimer’s disease is a major challenge in neurochemical diagnostics.

Due to the fact that atypical AD phenotypes can be presented with high levels of total tau protein and/or positive 14-3-3 protein in the CSF, reflecting intense neuronal degeneration similar to what is found in CJD. The current diagnostic criterion is unfortunately characterized by a diagnostic specificity of 71% for CJD. Ideally, an additional biomarker more closely related to the pathological process would be helpful in these cases.

A recent study showed that atypical cases of Alzheimer’s disease could be clearly distinguished from CJD via the detection of prion protein in CSF samples (Dorey et al (2015)). The BetaPrion HUMAN ELISA enables precisely this quantification of the biomarker and may be beneficial in clinical practice in addition to the current classic biomarkers.
ROC curves of CSF biomarkers for differential diagnosis of AD and CJD. The t-PrP value (cutoff: 263 µg/L) distinguishes with 82.0% sensitivity and specificity, whereas T-tau/t-PrP ratio discriminates with 98.6% sensitivity and 97.7% specificity.

BetaPrion® HUMAN ELISA

- Quantitative detection of human prion protein in human CSF as well as diverse biological samples
- Ultra-sensitive immunoassay using TMB substrate
- Optimized storage stability of in-house-produced antibodies for preservation of specific and exceptionally well characterized binding capabilities
- Fast and easy handling: clinical relevant results within 3-5 h
- Flexible application depending on sample throughput: standardized ELISA format on 12 x 8 immuno strips
- 6-point standard curve using lyophilized standards and controls

Performance assessment

Although typical forms of AD and CJD are clinically distinguishable, atypical AD phenotypes remain a diagnostic challenge. Current clinical diagnostic criteria for identifying CJD, 14-3-3 protein in cerebrospinal fluid (CSF), are characterized by a diagnostic specificity of only 71% for CJD. The relevance of determining the total prion protein (t-PrP) level in CSF for differential biological diagnosis was evaluated using the BetaPrion® Human ELISA.

Analysis of total cerebrospinal fluid prion protein in controls, AD and CJD populations. Typical AD indicates definite AD and portable AD; and CJD, definite CJD and portable CJD.

Misclassification rate of atypical AD phenotypes decreased from 43.5% (p14-3-3 results), to only 4.3% when calculating the ratio of: Tau total/(P-tau181 x t-PrP)

References
