Biomarker Profiling for Neurodegenerative Diseases

ELISAs, Antibodies, and Proteins

ELISAs, Antibodies, Proteins

analytikjena
An Endress+Hauser Company

Beyond the Current Status of Neurodegenerative Biomarkers

Monoclonal antibodies for well-established biomarkers such as human tau protein, ß-amyloid and α-synuclein are the bases for our easy-to-handle and ultra-sensitive immunoassays for neurodegenerative diseases. Their rapid generation for additional biomarkers such as p231-tau or non-phosphorylated tau protein, prion protein, and TDP43 encourages more effective drug development and the establishment of a more personalized medicine approach. Trust in Analytik Jena's toolbox for current and novel biomarkers and find out how small details can make a huge difference.

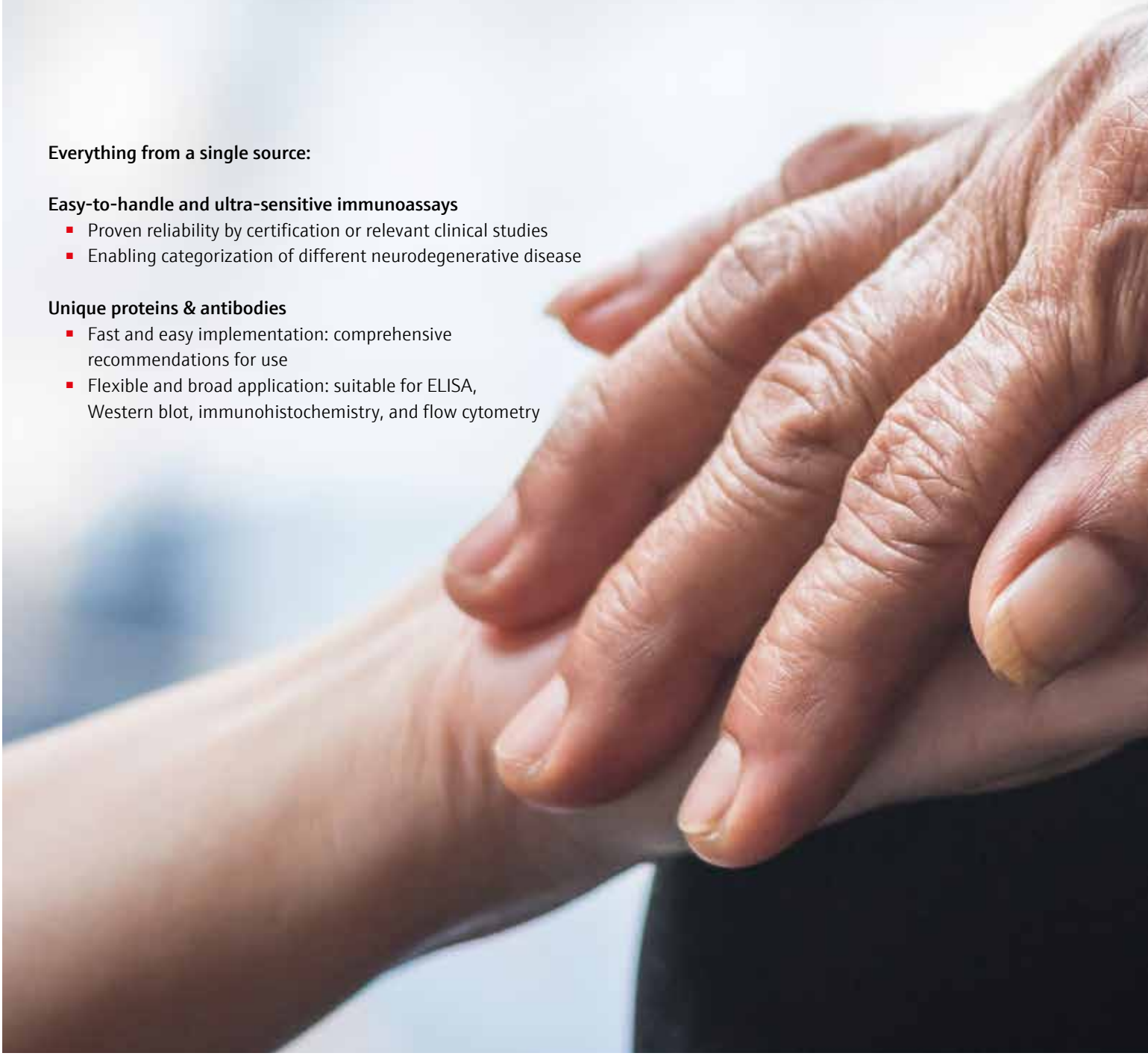
Everything from a single source:

Easy-to-handle and ultra-sensitive immunoassays

- Proven reliability by certification or relevant clinical studies
- Enabling categorization of different neurodegenerative disease

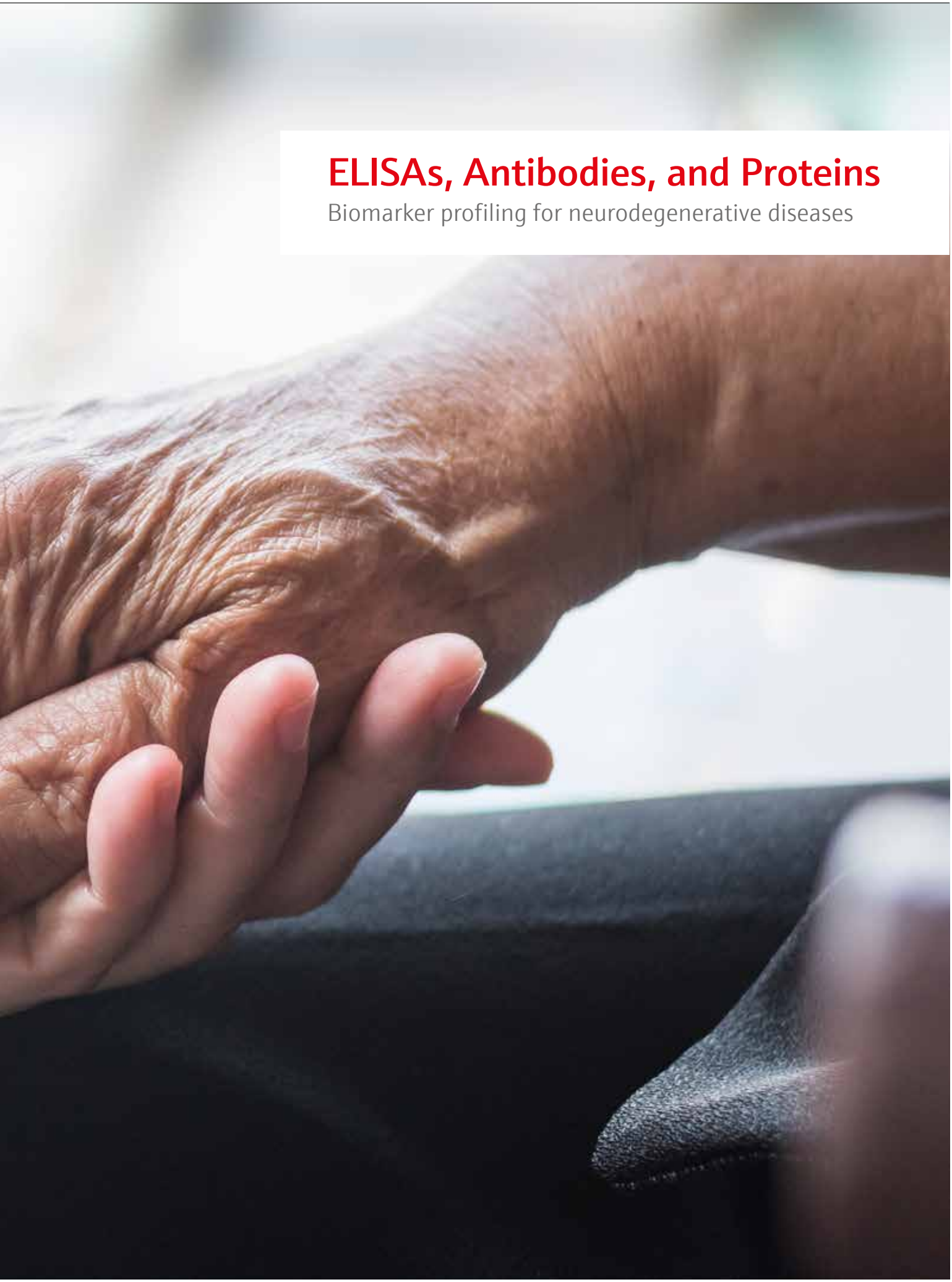
Unique proteins & antibodies

- Fast and easy implementation: comprehensive recommendations for use
- Flexible and broad application: suitable for ELISA, Western blot, immunohistochemistry, and flow cytometry



ELISAs, Antibodies, and Proteins

Biomarker profiling for neurodegenerative diseases



Biomarker Profiles for Neurodegenerative Disorders

Overview of selected biomarker behavior in varying neurodegenerative diseases and corresponding references

Biomarker	AD	FTD	PDD	DLB	MSA	CJD	ALS
t-tau	↑↑	↑	↑		-	↑↑↑	-
p-tau	↑	↑	-	-	-	-	-
non-p-tau	↑↑	↑	?	↑	?	↑↑↑	?
p231-tau	↑	?	?	?	?	?	?
A-beta 1-40	↓↑	-	-	-	-	-	-
A-beta 1-42	↓↓	↓	-	↓	-	↑↓	-
TDP43	-	↓↑	-	-	-	-	↑
t-PrP	↑	-	-	-	-	↓	-
t-α-syn	↓	-	↓	↓↓	↓	↑	-
β-sheet-α-syn	-	-	↑	↑	↑	-	-

AD Alzheimer's Disease
FTD Frontotemperaldementia
PDD Parkinson's Disease with Dementia
DLB Dementia with Lewy Bodies
MSA Multiple System Atrophy
CJD Creutzfeldt-Jakob-Disease
ALS Amytroph Lateralsclerosis

Nr.	Reference
1	Molinuevo et al. (2018) Acta Neuropathol. doi: 10.1007/s00401-018-1932-x
2	Lewczuk et al. (2017) J Alzheimers Dis. doi: 10.3233/JAD-160448
3	Calderón-Garciduenas et al. (2018) J Alzheimers Dis. doi: 10.3233/JAD-180853
4	Ermann et al. (2018) Ann. Clin. Transl. Neurol. doi: 10.1002/acn3.584
5	Eusebi et al. (2017) Movement Dis. Doi: 10.1002/mds.27110
6	Gossens et al. (2017) Alz. Res. & Therapy doi:10.1186/s13195-017-0275-5
7	Herrmann et al. (2017) Clin. Chim Acta doi: 10.1016/j.cca2017/01.010
8	Kovacs et al. (2012) A. Neuropath. Doi: 10.1007/s00401-012-0964-x
9	Meredith et al. (2013) PLOS One. Doi: 10371/journal.pone.0076523
10	Vallabh et al. (2018) bioRxiv. doi: 10.1101/295063
11	Villar-Piqué et al. (2018) Mol. Neurobiol. doi: 10.1007/s12035-018-1251-1
12	Rumeileh et al. (2017) Alzheimers Dis. doi: 10.3233/JAD-160740
13	Dorey et al. (2015) JAMA Neurol. doi:10.1001/jamaneurol.2014.4068
14	Villar-Piqué et al. (2018) Mol. Neurobiol. doi: 10.1007/s12035-018-1251-1
15	Llorens et al. (2018) Mol. Neurobiol. doi: 10.1007/s12035-018-1014-z
16	Fourier (2019) Anal. Bioanal. Chem. doi: 10.1007/s00216-018-1437-4
17	Unterberger et al. (2014) Clin. Neuropathol. doi: 10.5414/NP300796
18	Santos (2019) J. Neural. Transm. doi: 10.1007/s00702-019-01982-5
19	Kovacs et al. (2014) Neurobiol. Dis. doi: 10.1016/j.nbd.2014.05.020
20	Kovacs et al. (2014) Clin. Neuropathol. doi: 10.5414/NPP33328
21	Lerche et al. (2019) Neurol. Genet. Submitted
22	Calderón-Garcidueñas et al. (2016) J Alzheimers Dis. doi: 10.3233/JAD-160472

Tau protein & Beta-amyloid

An important role in neurodegenerative processes

Tau is a microtubule-associated protein comprised of six human isoforms predominantly located in the axons of neurons. Neuronal and/or glial inclusions of tau can be detected in several neurodegenerative diseases, or "tauopathies", including the prominent Alzheimer's disease (AD), which may be characterized by their tau isoform profile. The neurofibrillary tangles (NFT) characteristic of AD are composed primarily of hyperphosphorylated tau. In cerebrospinal fluid (CSF), decrease of amyloid beta 1-42 (Aβ42) and a low ratio of Aβ42 to amyloid beta 1-40 (Aβ42/Aβ40), together with an increase of both total tau protein (t-tau) and phosphorylated tau (p-tau), contribute to defining the "Alzheimer's signature." However, increased tau levels are found in other neurodegenerative diseases as well. These disorders include, inter alia, frontotemporal lobar degeneration (FTLD), Pick's disease, and corticobasal degeneration (CBD). In Creutzfeldt-Jakob disease (CJD), CSF t-tau levels are very high, whereas p-tau is close to normal, enabling no discrimination between AD and CJD. Several studies showed non-phosphorylated tau protein (non-p-tau) to be a potential biomarker for early detection of AD [3]. Furthermore, non-p-tau has been described to be a valuable tool for discrimination of AD from CJD [4].

The hTAU total ELISA detects all isoforms of tau protein and estimates total tau content. Additionally, the phosphoTAU ELISA identifies phosphorylated tau proteins. The non-pTAU ELISA utilizes a monoclonal antibody specific to the non-phosphorylated TPP sequences of the tau protein (positions T175 and T181). The portfolio is rounded out by the novel p231TAU ELISA.

Unique monoclonal antibodies against all isoforms of tau protein, phosphorylation, double-phosphorylation, and splicing forms as well as antibodies recognizing deposits of β-amyloid 1-42 in brains of Alzheimers's disease patients and transgenic mouse models are also available.

hTAU ELISA and phosphoTAU ELISA

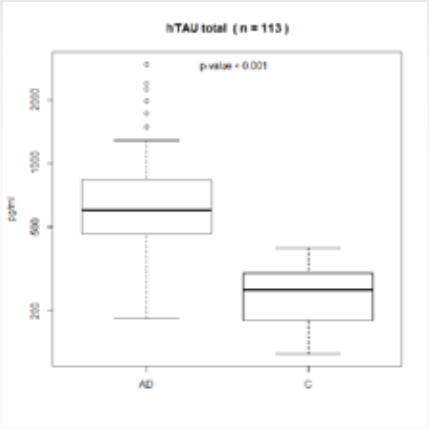
State-of-the art measurement – CE-IvD

ELISAs for supporting diagnosis of Alzheimer's disease

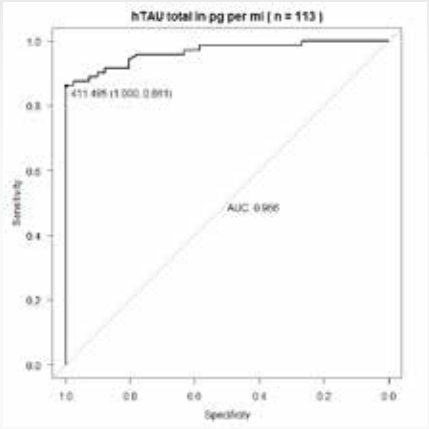
The hTAU and phosphor-Tau ELISA enable a diagnostic quality that achieves exceptional results in terms of specificity and sensitivity. The included lyophilized standards and controls ensure clinical relevant results with high precision and accuracy using a reproducible 6- point standard curve in a daily protocol. The standardized assays are optimally adapted and recommended to be used in conjunction with IBL Internationals/Tecans (Hamburg, Germany) Amyloid-β CSF ELISAs.

hTAU total & phosphor ELISA was used to assess the total tau and phosphor tau protein in CSF from patients with Alzheimer's disease (AD) from a control group.

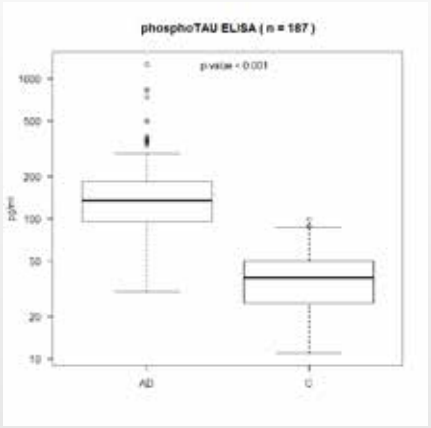
Clinical validation was kindly performed by Prof. Lewczuk, University Hospital Erlangen, Germany.



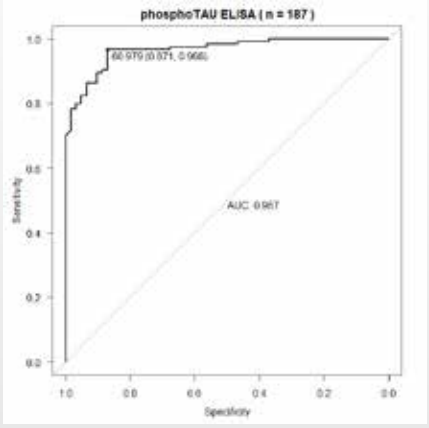
Verification using CSF from AD patients (n=72) as well as control patients (n=41) showed significant differences (p<0,001) of total tau.



Analysis of t-tau of AD patients (n=72) and control patients (n=41) using hTAU total ELISA. The test showed a sensitivity of 86 % and specificity of 100 %.



Measurements of CSF from AD patients (n=125) as well as control patients (n=62) showed significant differences (p<0,001) of p-tau between groups.



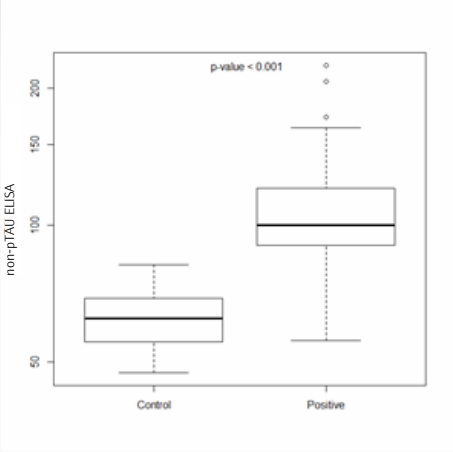
Analysis of p-tau of AD patients (n=125) and control patients (n=62) using phospho TAU ELISA showed a sensitivity of 86.5 % and specificity of 91 %.

non-pTAU ELISA

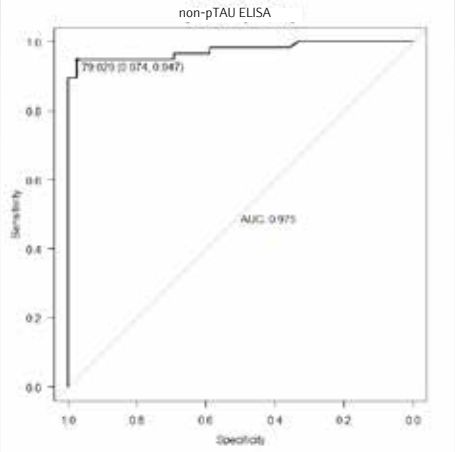
An outstanding biomarker – CE-IvD

Supporting companion and early diagnosis of AD

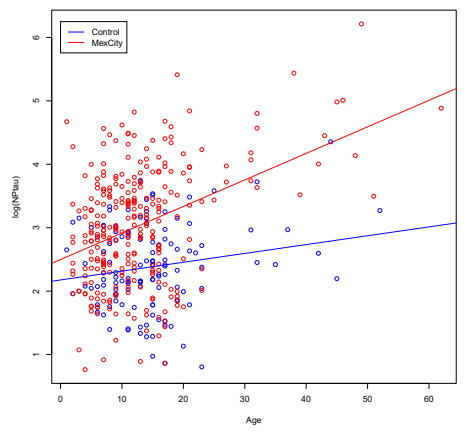
The non-pTAU ELISA enlarges the possibilities of companion diagnostics in order to support disease diagnosis, and was used to assess non-p-tau protein in CSF from patients with AD and mild cognitive impairment (MCI) from a control group [2]. Additionally it has recently been highlighted as a valuable tool for monitoring longitudinal changes during disease progression along with AD multianalyte classical CSF profile [3]. Furthermore, it was shown that non-p-tau quantification could serve as an excellent first discriminatory assay to distinguish AD from CJD [4].



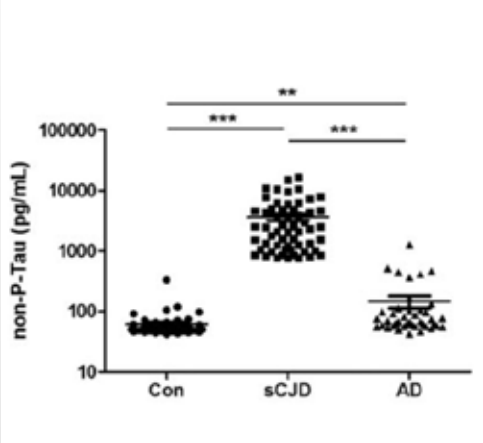
Verification using CSF of AD and MCI patients (n=57) and control patients (n=39) resulted in significant differences (p<0.001) between groups.



Analysis of non-p-tau of AD and MCI patients (n=57) and control patients (n=39) showed a sensitivity of 95 % and specificity of 97 %.



Non-p-tau tends to increase with age significantly faster among Mexico City young residents (n=354) exposed to fine particulate matter and ozone compared to controls (n=153) living in areas with less air pollution (p=0.0055).



Non-p-tau concentrations were increased in CJD (n=57; p<0.001) and AD (n=41; p<0.01) cases compared to controls, as well as in CJD compared to AD cases (p<0.001).

p231TAU ELISA and TAU aggregate ELISA

Additional markers for tauophaties

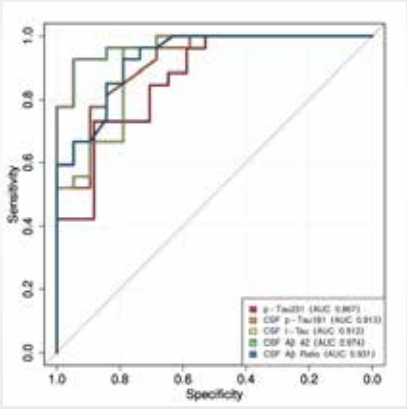
p231-tau in CSF

Phosphorylation of tau protein at threonine 231 has been shown to be characteristic in post-mortem brain tissue of patients with AD and it can be sensitively detected in CSF. Therefore, it may serve as a biomarker to support the diagnosis of AD.

In the study shown below CSF p231-tau was significantly higher in patients with dementia due to AD than in those with dementia due to other causes [18].

Comparison between AD-D and DC; p-values were calculated with Man-Whitney test or Fisher exact test for nominal variables.

Variables	Entire cohort	DC	AD-D	p-value*
No patients	106	19	27	-
t-tau, pg/ml (SD)	439.8 (272.9)	332.8 (163.0)	707.78 (273.35)	<0.0001
ptau181, pg/ml (SD)	57.47 (27.02)	45.79 (15.30)	83.96 (26.72)	<0.0001
ptau231, pg/ml (SD)	123.3 (49.6)	105.42 (37.40)	172.72 (52.24)	<0.0001

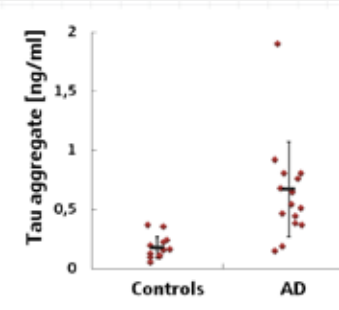


ROC Curve analysis of the three tau markers as discriminators of AD-D and DC individuals and Aβ1-42 as comparison.

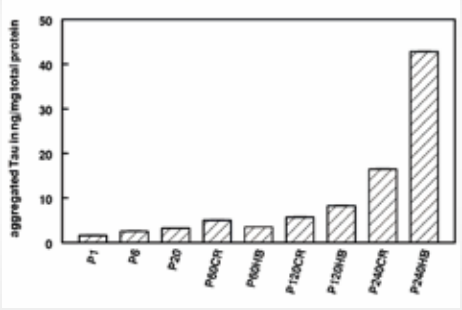
Evidence for aggregates related to tauopathies

Tau protein is mainly expressed in neurons, where it binds and stabilizes microtubules. In tauopathies, tau protein has a reduced affinity toward microtubules. Consequently, tau protein detaches from microtubules and aggregates into β-sheet containing filaments.

The TAU aggregate ELISA was used for examination of changes in tau aggregate concentration.
Material and analyses were kindly provided by Dr. Max Holzer, Paul-Flechsig-Institute, Leipzig, Germany.



Nerve tissue from patients with AD and control group were investigated and showed significant difference in tau aggregate concentration.



Development of tau aggregation during aging in P301L mica as an animal model for Alzheimer's disease.

Antibodies

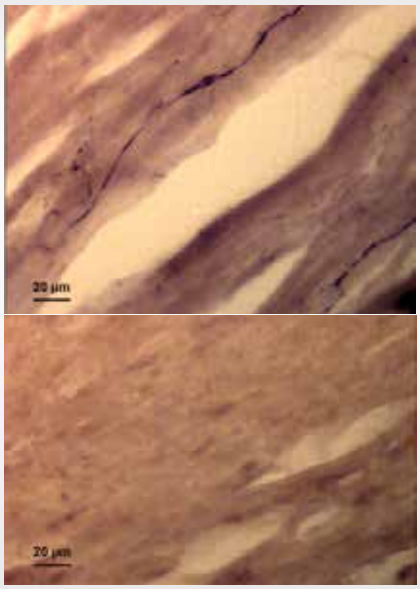
High-quality antibodies for tau-protein & β-amyloid

Addressing tau protein

Sensitive quantification of tau oligomers/aggregates in the presence of soluble, monomeric tau protein using Anti-human tau total 8F10, monoclonal antibody.

Results were kindly performed by Annemarie Mohring, Paul-Flechsig-Institute, Leipzig, Germany.

The specificity of 8F10 has been tested on brain tissue of tau knock-out mice and labels Alzheimer tau-pathology similar to the established antibody AT8.

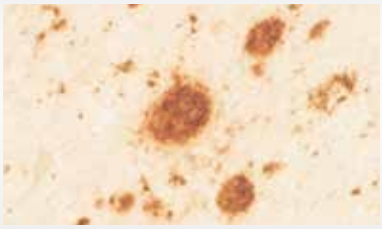


Tau protein immunohistochemistry of the heart muscle in a wildtype mouse (top) and a tau-knockout mouse with HRP-conjugated 8F10 (bottom) 1:500.

β-Amyloid protein in the AD detection

β-Amyloid protein is one of the most significant markers of Alzheimer's diseases and is used for diagnosis in CSF and pathological examinations post mortem.

β-amyloid analyses were kindly performed by Prof. Rossner, Paul-Flechsig-Insitute, Leipzig, Germany.



Detection of β-amyloid pathological related deposits in Cortex tissue of a patient suffering from Alzheimer's disease using Anti-human beta-amyloid 6D11

Highly specific reaction of Anti-human beta-amyloid 6D11 with β-amyloid of cerebral blood vessels in AD brain tissue.

Transactive Response DNA-binding protein 43

A potential candidate biomarker for FTLD

The transactive response region DNA-binding protein 43 (TDP43) binds both DNA and RNA and is involved in transcription and splicing. Under pathophysiological conditions, TDP43 accumulates in the cytoplasm and is hyperphosphorylated and/or ubiquitinated, and this is characteristic of the cytoplasmic inclusions observed in ALS and in many cases of frontotemporal labor degeneration syndrome (FTLD). Furthermore, TDP43 pathology is also detected in 20-50 % of AD patients, and appears to be associated with greater brain atrophy, memory loss, and cognitive impairment.

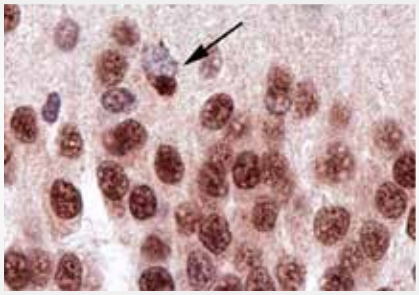
Several studies have been reported on CSF and plasma TDP43 in the context of ALS and FTLD, but research has been hindered by difficulties with detecting the protein. Overall, research suggests that blood-based TDP43 may have a role in neurodegenerative biomarkers and could be more useful than CSF TDP43. The monoclonal antibody Anti-human TDP43 2G10 has been identified to be a useful tool to confirm the specificity of TDP43 protein profiles.

Antibody

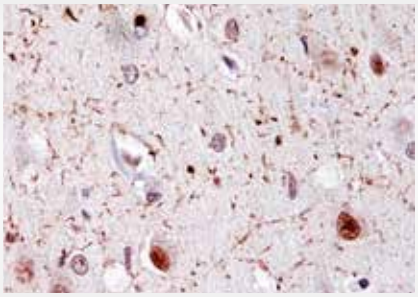
Highly specific to confirm TDP43 protein profiles

Detection of TDP43 in samples from FTD or AD patients

Application of Anti-human TDP43 2G10, monoclonal antibody for immunostaining. Results were kindly provided by Prof. Dr. Gabor G. Kovacs MUW (Vienna, Austria).



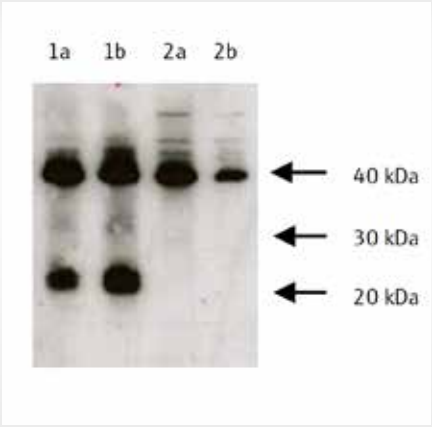
Prominent nuclear physiological nuclear immunostaining of TDP43 and a neuronal cytoplasmic inclusion body in a neuron lacking the physiological nuclear staining (arrow) using 2G10 antibody in a case with FTLD-TDP.



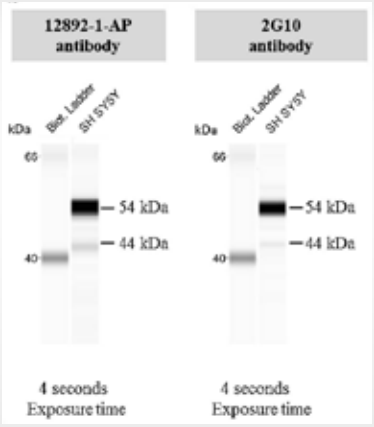
Fine neuropil threads in the hippocampus and neuronal cytoplasmic inclusions detected by 2G10 in an AD case with concomitant limbic TDP43 proteinopathy.

Reliable in Western blot analysis

Western blot analyses were kindly provided by Dr. Fourier, Hospital Bron (Lyon, France).Anti-human TDP43 2G10, monoclonal antibody was also used in comparison with one of the most popular anti-TDP 43 antibodies [16].



Cortex samples are prepared using SDS (a) and UREA (b) derived from FTD patient (1) or AD patient (2), respectively.



Identical Simple Western profiles obtained in SH SY5Y cell lysate using both rabbit polyclonal 12892-1-AP and mouse monoclonal 2G10 antibodies.

Prion Protein

A potential in vivo biomarker of cerebral prion pathology

Several human degenerative diseases appear as a result of misfolding and aggregation of proteins. The prototype central nervous system proteinopathy is CJD, in which neuronal prion protein (PrP) with high α-helical content switches into a stable structure rich in β-pleated sheets in a self-catalyzing process that eventually causes a plethora of neurological and psychiatric symptoms.

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 AD is a major challenge in neurochemical diagnostics.
This is because 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.

Recent studies have shown that atypical cases of AD can be clearly distinguished from CJD via the detection of prion protein in CSF samples [12], [13]. 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.

BetaPrion HUMAN ELISA

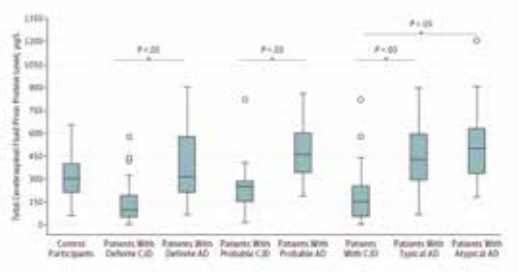
A promising tool: Prion protein quantification in CSF

Measurement of CSF t-PrP for distinguishing CJD from AD by detemination of Creutzfeld-Jakob factor

Neuronal injury may result in increased release of PrP from neurons into the CSF. The BetaPrion HUMAN ELISA is the only commercially available assay capable of reliably detecting t-PrP. The ultrasensitive assay, offering clinically relevant results in 3-5 hours, was used in addition to the determination of other relevant biomarkers such as t-tau and p-tau protein.
The relevance of t-PrP level in CSF for discriminating atypical AD phenotypes from CJD was evaluated including 232 patients.

The study provided evidence to combine CSF t-PrP with tau proteins into the so-called Creutzfeldt-Jakob factor (t-tau/(p-tau x t-PrP)) [13].

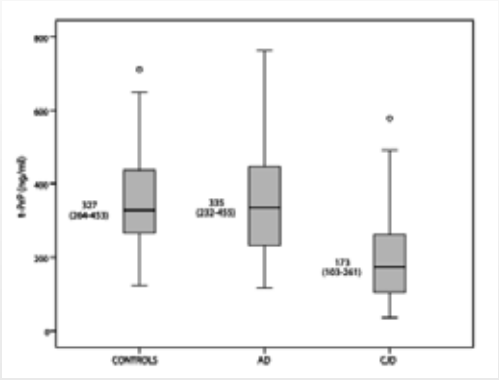
A second study that included 89 AD patients, 108 CJD patients as well as 33 controls, and focused on the diagnostic accuracy of a combined analysis of CSF t-PrP, t-tau, p-tau, and Aβ42 in discriminating CJD from AD with emphasis on atypical disease variants [12].



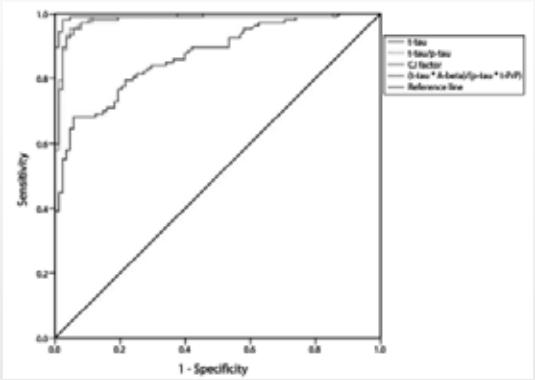
CSF t-PrP in control, AD and CJD populations. Typical AD indicates definite AD and portable AD; and CJD, definite CJD and portable CJD.

Parameters and Cutoff Values for Differential Diagnosis of CJD vs Typical AD	Patients Diagnosed as Having CJD, %	
	a-AD (n = 46)	CJD (n = 78)
p14-3-3 Test results (trace or positive)	43.5	96.2
T-tau, >1128 ng/L	65.2	91.3
T-tau:P-tau$_{181}$, >13.2	13.0	94.2
t-PrP, ≤263 µg/L	8.7	82.4
T-tau:t-PrP, >5.30	6.5	98.8
Creutzfeldt-Jakob factor, >0.054	4.3	95.7

The misclassification rate of atypical AD phenotypes decreased from 43.5 % when considering p14-3-3 results, to only 4.3 % when calculating the CJ factor.



CSF t-PrP in control, AD and CJD populations. CSF t-PrP levels were significantly lower in CJD compared to AD patients (p<0.001) and controls (p<0.001).



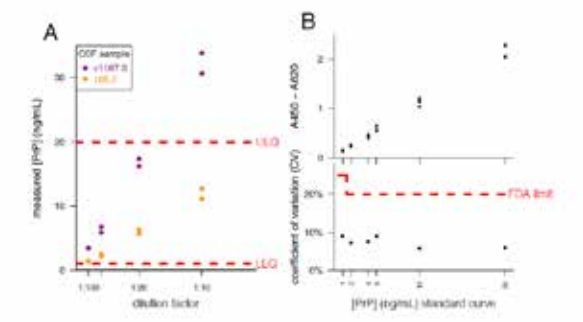
ROC curves illustrate sensitivity and specificity of various CSF biomarker combinations. The (t-tau x Aβ42)/(p-tau x t-PrP) ratio achieved the best accuracy, with 98.1 % sensitivity and 97.7 % specificity overall, and 96.2 % sensitivity and 95.5 % specificity for the "atypical" disease group.

Prion protein quantification for prion disease drug development

Clinical development of any PrP-reducing therapeutic will require an appropriate pharmacodynamics biomarker: a practical and robust method for quantifying PrP, reliably demonstrating its reduction in the CNS of a living patient.

The performance of the BetaPrion HUMAN ELISA has been investigated for this reason. The assays' precision, sensitivity, selectivity and reproducibility were analyzed taking 225 human CSF samples into account [10].

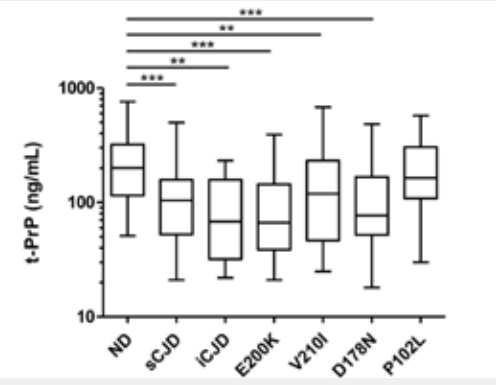
Experiment	Result
Within-plate reproducibility	CV = 11 %
Between-plate reproducibility	CV = 22 %
Sensitivity	LLOQ is 3-5 x blank signal
Selectivity	Non-reactive for mouse PrP, rat and monkey CSF, artificial and protease-digested CSF
Dilution linearity	Linear across two samples and five dilutions
Standard curve reproducibility	CV < 10 %



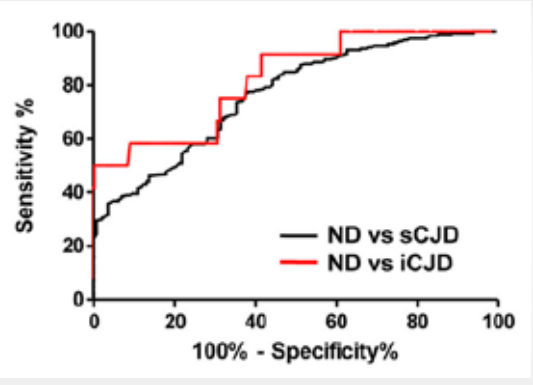
Consistent dilution linearity within the assay's dynamic range of 1-20 ng/ml PrP using two different samples measured in duplicate at each of four dilutions (left). Standard curve reproducibility (right).

Total prion protein in the spectrum of prion diseases

Data on comparative signatures of t-PrP across the spectrum of prion diseases, longitudinal changes during disease progression, and levels in pre-clinical cases were collected using BetaPrion HUMAN ELISA. The study included 561 CSF samples of CSF t-PrP in sporadic, iatrogenic, and genetic prion diseases in order to assess possible differences in t-PrP levels among diagnostic groups [14].



Analysis of CSF t-Prp concentrations in sporadic (sCJD), iatrogenic (iCJD) and genetic prion diseases associated with mutations in the PRNP gene. Boxes indicated 25th to 75th percentiles and whiskers minimum to maximum values. (*p<0.05, **p<0.01, ***p<0.001)



ROC curves for sporadic CJD (sCJD), iatrogenic (iCJD) vs ND group are shown. Area under the curve and 95 % confidence interval are: 0.76 (0.72-0.81) for sCJD (N=193) and 0.82 (0.70-0.94) for iCJD for (N=12).

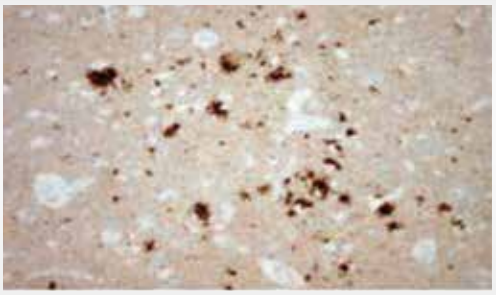
Antibodies & Proteins

Great variety of prion protein and prion protein antibodies

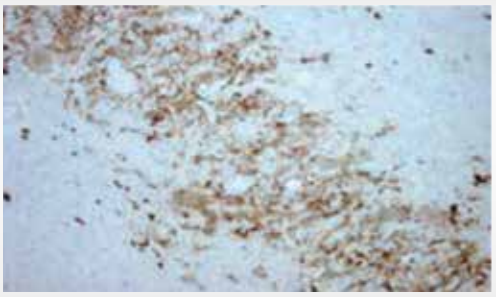
Immunopathology of CJD showing specific PrP^{sc} deposits

Immunohistochemical detection of plaque-like deposits in perivacuolar areas of cortical grey matter and deep nuclei in CJD diagnosis using anti-human prion protein 14D11, monoclonal antibody.

It detects fine synaptic accumulation in some nuclei such as the dentate nucleus of the cerebellum in classical CJD. Kindly performed by Dr. Navarro, Meixoeiro, Spain.



Immunohistochemical detection of plaque-like deposits in perivacuolar areas of cortical grey matter and deep nuclei in sections from a patient of CJD.

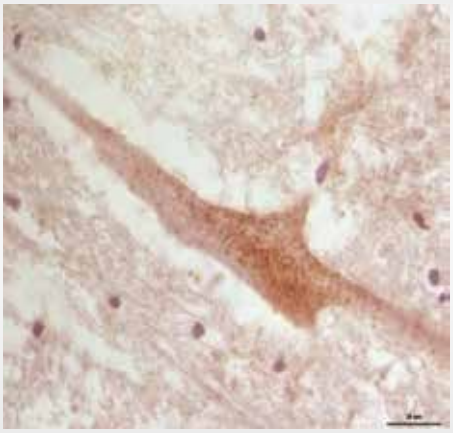


Immunohistochemical detection of fine synaptic accumulation in some nuclei such as the dentate nucleus of the cerebellum in sections from a patient with proven classic form of CJD.

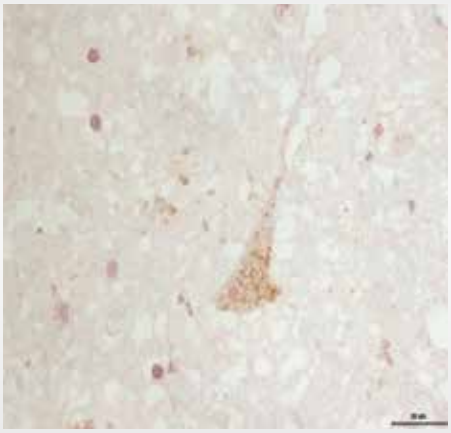
Confirmation of TSE in bovines and ovines made by detection of pathological changes in nerve tissue

Immunopathological confirmation of BSE in tested tissue from slaughtered animals is a common procedure according to OIE protocols and made by National Reference labs in Europe.

PrPsc deposits in sheep tested positive for Scrapie could be found in different nerve tissues as well as in peripheral areas and the spinal cord. Kindly performed by Dr. Hardt, Leipzig, Germany.



Detection of brown granular cytoplasmatic deposits in a neuronal cell in BSE positive Obex tissue using monoclonal antibody 14D11.



Detection of brown granular cytoplasmatic deposits in a neuronal cell using 14D11 in Scrapie-positive spinal cord tissue.

α-Synuclein

Focus on discrimination of total and disease-specific α-synuclein

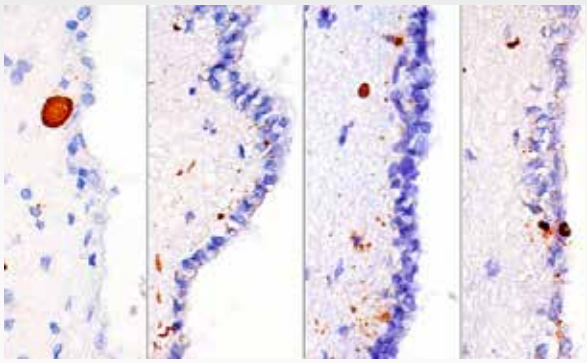
α-Synuclein is an abundant neuronal 140 amino acid protein, predominantly localized in the presynaptic terminals, and involved in vesicle fusion and neurotransmitter release. Aggregates of α-synuclein are the main components of Lewy bodies (LB), which are intracellular inclusions characteristics of certain neurodegenerative diseases collectively referred to as α-synucleinopathies. These include Parkinson's disease (PD), Parkinson's disease dementia (PDD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). However, α-synuclein aggregates are also found in approximately half of sporadic AD pathologies; consequently, it is crucial to differentiate it from pure AD forms. Via the hSYN total ELISA, Analytik Jena provides an improved ELISA for detection of total human α-synuclein. Additionally, the discrimination of total α-synuclein and disease-specific α-synuclein is of special interest for distinguishing between different patient groups. The Anti-Human α-Synuclein 5G4, monoclonal antibody strongly binds to the high molecular weight fraction of β-sheet rich oligomers, while no binding to primarily disordered oligomers or monomers was observed. This outstanding capability is used for the HUMAN α-synuclein PATHO ELISA suggesting a promising tool for PD.

Antibodies & Proteins

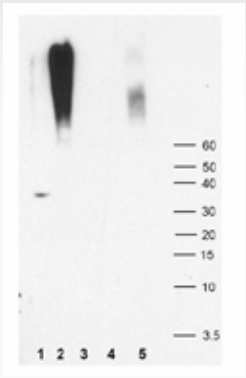
Worldwide unique antibody: Anti-human α-Synuclein 5G4

Monoclonal antibodies for total α-synuclein and disease-specific α-synuclein

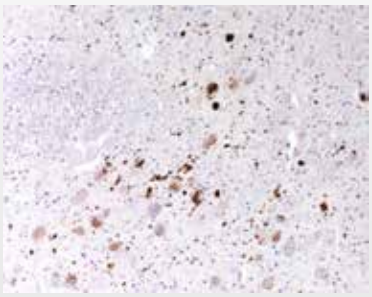
The portfolio includes antibodies for total, e.g. Anti-human α-synuclein 10D2,monoclonal as well as disease-specific α-synuclein. The anti-human α-synuclein 5G4, monoclonal antibody binds to pathologically relevant structures in neuropathology of Lewy body/Parkinson's disease and is capable of recognizing β-sheet-dependent epitope. The antibody was used in several techniques [8, 19, 20].



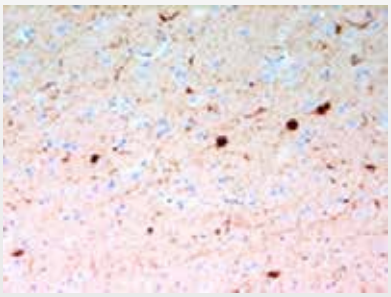
Light microscopic immunostaining patterns of α-synuclein using antibody 5G4. Tiny dots, thin neurites in the subependymal area, as well as tiny dots between ependymal cells and amorphous plaques.



Western blot data of reactivity of 5G4 antibody with α-synuclein monomer, fibrils and oligomers. Lane 1:monmers, lane 2: nitrated oligomers, lanes 3 and 4: oxidized oligomers, lane 5: fibrils.



For demonstration of the particular suitability of the antibody 5G4 for specific detection of pathologic relevant structures of LBD/PD immunohistological staining in brain tissue.



Immunostaining for alpha-synuclein using antibody 10D2 in the amygdala of an individual with Lewy body disease.

hSyn total ELISA

Convenient quantification of t-α-syn

Optimized for measurement from CSF

The quantification of α-synuclein, a presynaptic protein, in human CSF is used to differentiate Lewy Body Disease, for example, from other neurodegenerative pathologies. A feasibility study was performed to evaluate the significance and reliability of detection [17].
Furthermore, quantification of α-synuclein was performed using an in-house assay based on Mesoscale platform (UMG in comparison with hSYN total ELISA (AJ). Comparison to the hSYN total ELISA was kindly performed by Dr. Niels Kruse, University Medical Center Göttingen, Germany.

hSYN total ELISA was also used in the context of *GBA1* mutation and REM-sleep behaviour [21]. Severity of the type of *GBA1* mutation was associated with a younger age at onset and higher prevalence of REM-sleep-behavior disorder. Likewise, CSF levels of total alpha-synuclein were lowest in the DLB_{GBA} group with severe mutations compared to DLB_{GBA} patients with mild *GBA1* variants, $DLB_{wildtype}$ and healthy controls.

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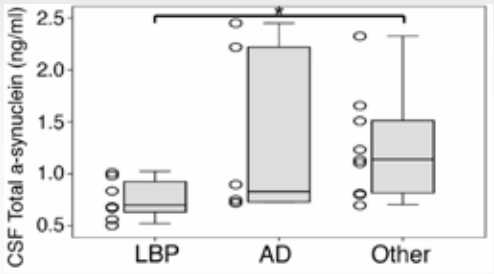
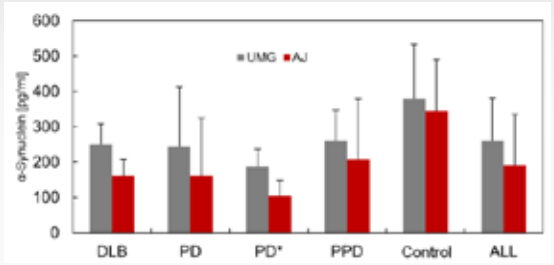
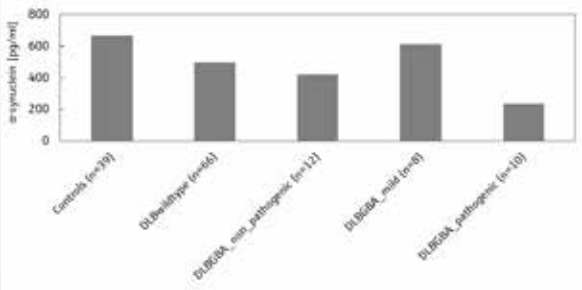


Verification of total α-synuclein in the CSF in cases with Lewy body pathology (LBP), AD, and a control group. The level in LBP patients dropped compared to AD patients and was significantly lower compared to control groups (p=0.016).



Means for α-synuclein concentration with standard deviation of the measurements per group and method (AJ and UMG) is shown. Further statistically comparison showed significant lower means for hSYN total ELISA than for the in-house assay ($p<0.05$), but data proved the hSYN total ELISA to be a promising tool enabling sharp distinction of DLB and PD (PD*: exclusion of one sample showing an extraordinary high value of α-synuclein level.



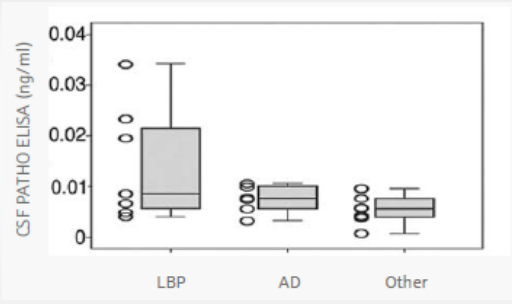
Similarly to PD, GBA1 mutations seem to promote alpha-synuclein-driven CSF profiles in DLB. This will allow patient stratification for specific alpha-synuclein-lowering compounds.

HUMAN α-Synuclein PATHO ELISA

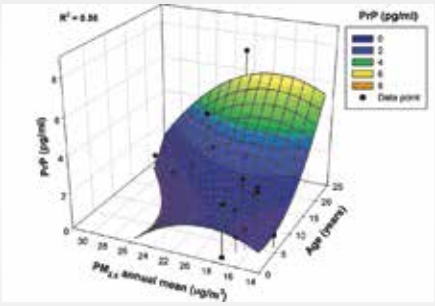
An outstanding biomarker

Accompanying diagnostic output

The HUMAN α-Synuclein PATHO ELISA allows detection of pathologically relevant formations of α-synuclein and can provide additional manifestations for accompanying diagnostics and prognosis. Firstly, a feasibility study was performed to evaluate pathological relevant α-synuclein measurements patients with different neurological disorders [17]. Secondly, oligomeric α-synuclein measurements were performed in young, highly polluted children [22].



Pathological relevant α-synuclein detection in the CSF from patients with Lewy body pathology (LBP), Alzheimer's diseases (AD), and from a control group. The level of disease-associated α-synuclein was - on average – higher in LBP than in other groups.



Application of HUMAN α-Synuclein PATHO ELISA showed significant correlation with TNF, IL10, and IL6 in Mexico City Metropolitan Area children. This reveals that processes of inflammation may be correlated with neurodegeneration in younger population.


kjena

Recommendations for Use: Fast and Easy Implementation of Antibodies

In general, the use of Analytik Jena's monoclonal antibodies is possible for all common immunochemical techniques as primary or secondary antibodies. Reactivity of monoclonal antibodies depends on the origin of samples and their pre-treatment as well as on reaction conditions. Follow the recommendations for use and possibilities given below for Analytik Jena's monoclonal antibodies as a guideline for application. Optimal reaction conditions must be tested by users within their own protocols.
For detection in immunochemical testing, secondary antibodies conjugated to enzymes, for example, are often used. Please check the instructions for the use of monoclonal antibodies regarding the isotype for the correct selection of anti-mouse-immunoglobulin antibody. It is recommended to use secondary antibody conjugates specific for IgG or IgM of mouse immunoglobulin and selected for minimum of cross-reactivity with other species.

General information

Monoclonal antibodies are usually delivered in PBS (pH 7.4) without additives. Common reaction buffers like TRIS, TBS, PBS, carbonate etc. with 10–50 mM and pH between 7 and 10 are applicable for all monoclonal antibodies. Additionally, all of the monoclonal antibodies are reactive in buffers and washing buffers with detergents such as Tween 20 or Triton X-100 with a concentration of 0.05–0.2 %. Bovine serum albumin in ELISA or immunoprecipitation or skim milk powder in Western blot, respectively, can be used in concentrations between 1–5 %. If special buffer conditions are necessary, follow the description in data sheet of each monoclonal antibody.

ELISA

Coating of monoclonal antibodies

- Dilute 5 µg/ml of monoclonal antibody and pipette 100 µl per well of 96 well ELISA plate with high binding capacity; incubate sealed plate at 2–10 °C overnight (minimum 12 hours)
- Wash 3x with 200–300 µl TRIS buffer/0.05 % Triton X-100
- Block wells with 200 µl 3 % BSA in washing buffer for 30 min
- Discard blocking solution

Incubation of antigen coated plates

- Coat ELISA plates with 0.1–1 µg/ml antigen as described above; for stronger blocking 5 % skim milk powder is recommended
- Dilute monoclonal antibody to 1 µg–0.1 ng in PBS containing 0.05 % Tween 20 and pipette 100 µl per well; incubate sealed plate at RT for 60–120 min
- Wash 3x with 200–300 µl TRIS buffer / 0.05 % Triton X-100
- Pipette 100 µl of secondary antibody HRP conjugated and diluted according to IFU; incubate sealed plate at RT for 30–60 min
- Wash 5x with 200–300 µl TRIS buffer / 0.05 % Triton X-100
- Pipette 100 µl of staining solution and incubate at RT in the dark for 15 min followed by stop using stop solution
- Measure OD at 450/620 nm

Sandwich-ELISA

- Dilute antigen in PBS containing 0.05 % Tween 20 and pipette 100 µl per well; incubate sealed plate at RT for 60–120 min or at 2–10 °C overnight
- Wash 3x with 200–300 µl TRIS buffer / 0.05 % Triton X-100
- Pipette 100 µl second antigen specific HRP conjugated antibody and dilute according to IFU; incubate sealed plate at RT for 60–120 min
- Wash 5x with 200–300 µl TRIS buffer / 0.05 % Triton X-100
- Pipette 100 µl of staining solution and incubate at RT in the dark for 15 min followed by stop using stop solution of the kit
- Measure OD at 450/620 nm

Immunohistochemistry

- Use of 4.5 % formaldehyde fixed tissue slides is recommended
- Incubate with citrate buffer at 95 °C for 20 min; rinse with water; incubate with concentrated formic acid at RT for 1 min; wash slides in water or antibody dilution buffer
- Dilute 0.1–2 µg antibody per ml in PBS pH 7.4 containing 3 % BSA and incubate tissue slides at RT for 30 min
- Wash slides 3x with TBS pH 7
- Incubate secondary antibody HRP conjugated and diluted according to IFU at RT for 30 min
- Wash slides 3x with TBS pH 7
- Staining tissue slides with DAB according to manufacturer's IFU

Western blot

- Block transferred antigen onto nitrocellulose with 5 % skim milk powder in TRIS buffer pH 10 containing 0.1 % Triton X-100 at RT for 1 hr
- Dilute 1–2 µg/ml monoclonal antibody in blocking buffer and incubate membrane in this solution at RT overnight
- Wash membrane 3x with TRIS buffer pH 10 containing 0.1 % Triton X-100
- Dilute anti-mouse Ig antibody HRP conjugated according to manufacturer's IFU in blocking buffer and incubate membrane in this solution at RT for 1–2 hours
- Wash membrane 5x with TRIS buffer pH 10 containing 0.1 % Triton X-100
- Incubate membrane in staining solution at RT and stop reaction during visual control using water

Immunoprecipitation

Bead preparation using magnetic beads (DynaBead M280 Streptavidin)

- Vortex 100 µl beads and place in 1.5 ml tube; collect beads using magnetic power for 3 min and discard supernatant
- Wash 3x with 500 µl PBS pH 7.4 containing 3 % BSA and 0.05 % Tween 20 by carefully pipetting; re-suspend beads with pipette into diluents; collect beads every time with magnetic power for 3 min

Coating with monoclonal antibody

- Pipette 20 µg of biotin-conjugated monoclonal antibody in PBS pH 7.4 containing 3 % BSA and 0.05 % Tween 20 onto 1 mg of DynaBead M280 streptavidin
- Incubate with shaking or inverting tubes at RT for 30 min
- Collect beads using magnetic power for 3 min and discard supernatant
- Wash 3x with 500 µl PBS pH 7.4 containing 3 % BSA and 0.05 % Tween 20 by carefully pipetting; re-suspend beads with pipette into diluents; collect beads every time with magnetic power for 3 min
- Aliquotation of beads in 0.2 mg and storing at 2–10 °C is possible now

Immunoprecipitation of antigen

- Pipette 100–1000 µl of sample, undiluted or diluted in PBS pH 7.4 containing 3 % BSA and 0.05 % Tween 20 onto 1 aliquot of monoclonal antibody-coated beads (0.2 mg).
- Incubate by rotation at 2–10 °C overnight
- Collect beads using magnetic power for 3 min and discard supernatant
- Wash 3x with 500 µl PBS pH 7.4 containing 3 % BSA and 0.05 % Tween 20 by carefully pipetting; re-suspend beads with pipet into diluents; collect beads every time by magnetic power for 3 min.
- If immunoprecipitation is successful, trapped antigen can be detected in SDS-PAGE followed by Western blot

Order Information

ELISAs for tau protein

	Quantity	Order number
hTAU total ELISA	96 reactions	847-0108000101
phosphoTAU ELISA	96 reactions	847-0108000104
non-pTAU ELISA	96 reactions	847-0108000102
p231TAU ELISA	96 reactions	847-0104000112
TAU aggregate ELISA	96 reactions	847-0104000116

Antibodies for tau protein & beta-amyloid

Klon	Reactivity	Order number
1E7	Phosphorylated tau 181 (T), PHF-tau	847-010200380[x]
8B11	Phosphorylated tau 181 (T), PHF-tau	847-010200390[x]
8D2	Phosphorylated tau 181 (T), PHF-tau	847-010200620[x]
10D3	Phosphorylated tau 181 (T), PHF-tau	847-010200610[x]
1F3	Phosphorylated tau 181 (S), PHF-tau, tau	847-010200320[x]
9C8	Phosphorylated tau 199 (S) and 202 (S), PHF-tau, tau	847-010200460[x]
10F8	Phosphorylated tau 202 (S), PHF-tau, tau	847-010200450[x]
2B11	Phosphorylated tau 231 (T), PHF-tau	847-010200350[x]
5G7	Phosphorylated tau 231 (T), PHF-tau	847-010200360[x]
9D8	Phosphorylated tau 231 (T), PHF-tau	847-010200370[x]
4C10	Phosphorylated tau 231 (T), PHF-tau	847-010200310[x]
3G3	Phosphorylated tau 231 (T) and 235 (S), PHF-tau	847-010200440[x]
4B5	PHF-tau, tau 441	847-010200480[x]
8F10	PHF-tau, tau 441	847-010200510[x]
12C2	PHF-tau, tau 441	847-010200520[x]
18B5	PHF-tau, tau 441	847-010200530[x]
7E5	All isoforms tau 441, CSF tau total	847-010200630[x]
2B6	Exon 3 human tau	847-010200640[x]
9E11	Exon 2 and 3 human tau	847-010200660[x]
polyclonal	Human tau total	847-010300100[x]
6D11	β-Amyloid	847-010200650[x]

[x] = quantity [1] 100 µg, [3] 0.1 mg

Antibody for TDP43

Klon	Reactivity	Order number
2G10	TDP43	847-010200740[x]

[x] = quantity [1] 100 µg, [3] 1 mg

ELISA for prion protein

	Quantity	Order number
BetaPrion HUMAN ELISA	96 reactions	847-0104000104

Antibodies for prion protein

Klon	Reactivity	Order number
5C4	Human, cattle, sheep, and deer	847-010200120[x]
1E2	Human and cattle	847-010200130[x]
6G3	Human, cattle, sheep, and deer	847-010200150[x]
5B9	Human and cattle	847-010200160[x]
6E2	Human and cattle	847-010200410[x]
7D5	Human and cattle	847-010200420[x]
5G11	Human and cattle	847-010200430[x]
14D11	Human, sheep, and cattle	847-0102001704*
4F7	Bovine and human prion protein, Pr^{Pres}	847-010200070[x]
1E5	Bovine and human prion protein, Pr^{Pres}	847-010200080[x]
3E7	Bovine, human, and ovine prion protein, Pr^{Pres}	847-010200090[x]
3B8	Bovine and ovine prion protein, Pr^{Pres}	847-010200100[x]
7B6	Bovine, human, sheep, and deer prion protein	847-010200110[x]
polyclonal	pAB R10 (sheep, human, cattle, deer, and mouse)	847-010300010[x]

[x] = quantity [1] 100 µg, [3] 0.1 mg
* = 0.05 mg

Prion protein

	Order number
Recombinant bovine prion protein	847-010100010[x]
Recombinant human prion protein	847-010100030[x]
Recombinant sheep prion protein	847-010100060[x]
Recombinant deer prion protein	847-010100070[x]
Recombinant murine prion protein	847-010100710[x]

[x] = quantity [1] 100 µg, [2] 0.5 mg, [3] 1 mg

ELISA for α-synuclein

	Quantity	Order number
hSYN total ELISA	96 reactions	847-0108000103
HUMAN α-Synuclein PATHO ELISA	96 reactions	847-0104000108

Antibodies for α-synuclein

Klon	Reactivity	Order number
10C3	Human α-synuclein	847-010200180[x]
5G4	β-sheet oligomers of human α-synuclein	847-010200400[x]
10D2	Human α-synuclein	847-010200470[x]
polyclonal	Human α-synuclein	847-010300090[x]

[x] = quantity [1] 100 µg, [3] 1 mg

Recombinant synclein

	Order number
Human α-synuclein, His-tagged	847-010100850[x]
Human α-synuclein	847-010100860[x]

[x] = quantity [1] 100 µg, [2] 0.5 mg, [3] 1 mg

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