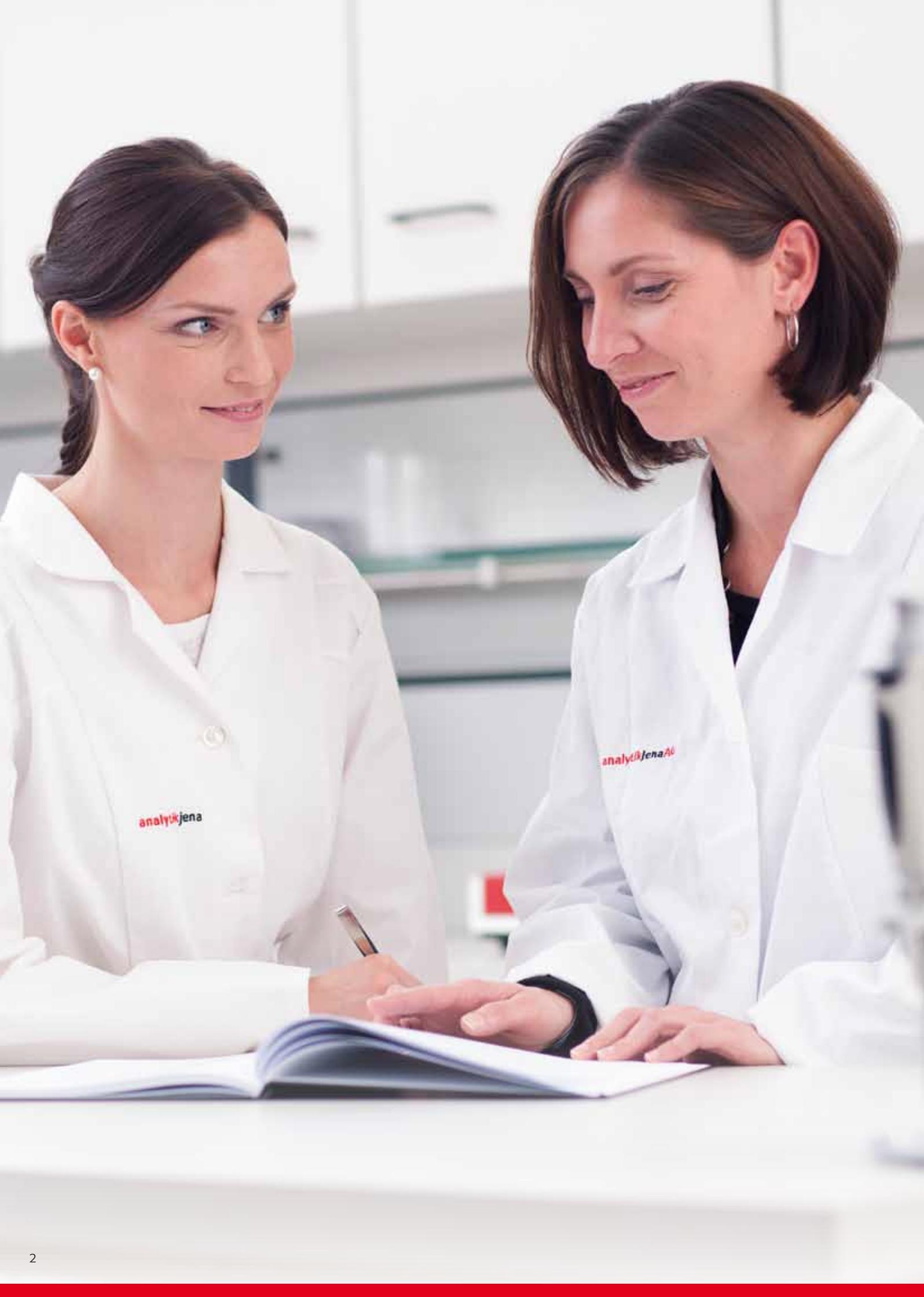


# Tools for Differential Dementia Diagnostics

Antibodies and proteins setting new benchmarks in research and development of Alzheimer's, Parkinson's and Creutzfeldt-Jakob disease







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** and **Research**



### **Efficient tools**

advanced application in Research & Development



### **Multi parameter analysis**

expanded diagnostic output



### **Fast and easy implementation**

comprehensive recommendations for use



### **Flexible and broad application**

suitable e.g. for ELISA , Western blot, Immunohistochemistry and FACS



# Recommendations for use – Fast and easy implementation according to your application



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 origin of samples and their pre-treatment as well as on reaction conditions. Follow the recommendations for use and possibilities given below of Analytik Jena's monoclonal antibodies as guideline for application. Optimal reaction conditions have to be tested by users within their own protocols.

For detection in immunochemical test secondary antibody conjugated to e.g. enzymes is often used. Please check instructions for use of monoclonal antibody regarding the isotype for right 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 pipet 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

## Sandwich-ELISA

- Dilute antigen in PBS containing 0.05 % Tween20 and pipet 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
- Pipet 100 µl second antigen specific HRP conjugated antibody and diluted 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
- Pipet 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

## Incubation of antigen coated plates

- Coat ELISA plates with 0.1 - 1 µg/ml antigen as described; for stronger blocking 5 % skim milk powder is recommended
- Dilute monoclonal antibody to 1 µg - 0.1 ng in PBS containing 0.05 % Tween20 and pipet 100 µl per well; incubate sealed plate at RT for 60 - 120 min
- Wash 3 x with 200 - 300 µl TRIS buffer/0.05 % Triton X-100
- Pipet 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
- Pipet 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

# 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 h
- 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 visually control using water

# Immunoprecipitation

## Immunoprecipitation of antigen

- Pipet 100 - 1000  $\mu$ l 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 3 x with 500  $\mu$ l 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 could be detected in SDS-PAGE followed by Western blot

## Beat preparation using magnetic beads (DynaBead M280 Streptavidin)

- Vortex 100  $\mu$ l beads and place in 1.5ml tube; collect beads using magnetic power for 3 min and discard supernatant
- Wash 3 x with 500  $\mu$ l 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

## Coating with monoclonal antibody

- Pipet 20  $\mu$ g 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 3 x with 500  $\mu$ l 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
- Aliquotation of beads in 0.2 mg and storing at 2–10 °C is possible now





*ikjena*

# Antibodies for human tau protein



- High-quality antibodies against all isoforms of tau protein, phosphorylation, double-phosphorylation and splicing forms
- Optimized storage stability of in-house produced antibodies for preservation of specific and exceptionally well characterized binding capabilities
- Highly purified monoclonal antibodies (> 95 %) and polyclonal antisera
- Broad application in different immunochemical techniques, e.g. ELISA and Western blot

## Product specifications

Antigen (Specificity)	Total tau, phosphorylated tau, exon 2 and 3
Host	Mouse and rabbit
Isotyp	IgM or IgG
Cross reactivity	Not known
Storage/Stability	12 months at -20 °C

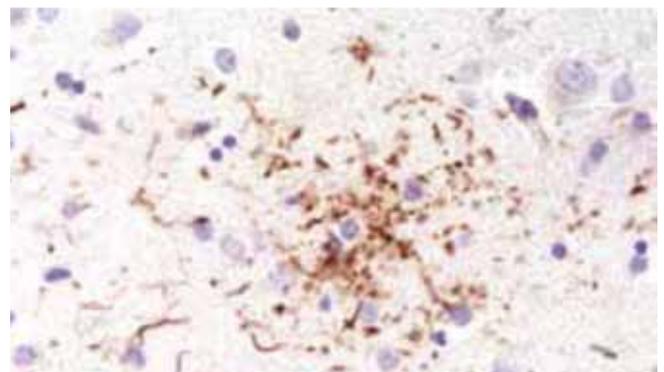
## Application sample

Application of Anti-human phospho-202 tau 10F8, monoclonal antibody to provide evidence of hyperphosphorylated tau in pathological structures such as plaque and tangles in the brain of patients suffering from Alzheimer's disease.

Detection of phosphorylation of tau protein at position 231 (T) is a potential biomarker for early diagnosis of Alzheimer's disease (Hampel et al (2004) Arch Gen Psychiatry 61(1):95-102).



▲ Specific detection of p202-tau in neocortex of AD brain using Anti-human phospho-202 tau 10F8.



▲ Specific detection of p231-tau in brain of an Alzheimer's disease patient using Anti-human phospho-231 tau 4C10.

## References

Wolf J, Jäger C, Lachmann I, Schönknecht P, Morawski M, Arendt T, Mothes T (2013) Tissue transglutaminase is not a biochemical marker for Alzheimer's disease. *Neurobiol Aging* 34:2495-98

## Order information

Order number	Product	Quantity	Antigen
847-0102003801	Anti-human phospho-181 tau 1E7, monoclonal	0.1 mg	Phosphorylated tau 181 (T), PHF-tau
847-0102003803		1.0 mg	
847-0102003901	Anti-human phospho-181 tau 8B11, monoclonal	0.1 mg	Phosphorylated tau 181 (T), PHF-tau
847-0102003903		1.0 mg	
847-0102006201	Anti-human phospho-181 tau 8D2, monoclonal	0.1 mg	Phosphorylated tau 181 (T), PHF-tau
847-0102006203		1.0 mg	
847-0102006101	Anti-human phospho-181 tau 10D3, monoclonal	0.1 mg	Phosphorylated tau 181 (T), PHF-tau
847-0102006103		1.0 mg	
847-0102003201	Anti-human phospho-199 tau 1F3, monoclonal	0.1 mg	Phosphorylated tau 199 (S), PHF-tau, tau
847-0102003203		1.0 mg	
847-0102004601	Anti-human phospho-199/202 tau 9C8, monoclonal	0.1 mg	Phosphorylated tau 199 (S) und 202 (S), PHF-tau, tau
847-0102004603		1.0 mg	
847-0102004501	Anti-human phospho-202 tau 10F8, monoclonal	0.1 mg	Phosphorylated tau 202 (S), PHF-tau, tau
847-0102004503		1.0 mg	
847-0102003501	Anti-human phospho-231 tau 2B11, monoclonal	0.1 mg	Phosphorylated tau 231 (T), PHF-tau
847-0102003503		1.0 mg	
847-0102003601	Anti-human phospho-231 tau 5G7, monoclonal	0.1 mg	Phosphorylated tau 231 (T), PHF-tau
847-0102003603		1.0 mg	
847-0102003701	Anti-human phospho-231 tau 9D8, monoclonal	0.1 mg	Phosphorylated tau 231 (T), PHF-tau
847-0102003703		1.0 mg	
847-0102003101	Anti-human phospho-231 tau 4C10, monoclonal	0.1 mg	Phosphorylated tau 231 (T), PHF-tau
847-0102003103		1.0 mg	
847-0102004401	Anti-human phospho-231/235 tau 3G3, monoclonal	0.1 mg	Phosphorylated tau 231 (T) and 235 (S), PHF-tau
847-0102004403		1.0 mg	
847-0102004801	Anti-human tau total 4B5, monoclonal	0.1 mg	PHF-tau, tau 441
847-0102004803		1.0 mg	
847-0102005101	Anti-human tau total 8F10, monoclonal	0.1 mg	PHF-tau, tau 441
847-0102005103		1.0 mg	
847-0102005201	Anti-human tau total 12C2, monoclonal	0.1 mg	PHF-tau, tau 441
847-0102005203		1.0 mg	
847-0102005301	Anti-human tau total 18B5, monoclonal	0.1 mg	PHF-tau, tau 441
847-0102005303		1.0 mg	
847-0102006301	Anti-human tau total 7E5, monoclonal	0.1 mg	All isoforms tau 441, CSF tau total
847-0102006303		1.0 mg	
847-0102006401	Anti-human tau E3 2B6, monoclonal	0.1 mg	Exon 3 human tau
847-0102006403		1.0 mg	
847-0102006601	Anti-human tau E2+E3 9E11, monoclonal	0.1 mg	Exon 2 and 3 human tau
847-0102006603		1.0 mg	
847-0103001001	Anti-human tau total pAB 64, polyclonal	0.1 ml	Human tau total
847-0103001003		1.0 ml	

# Antibody for $\beta$ -amyloid



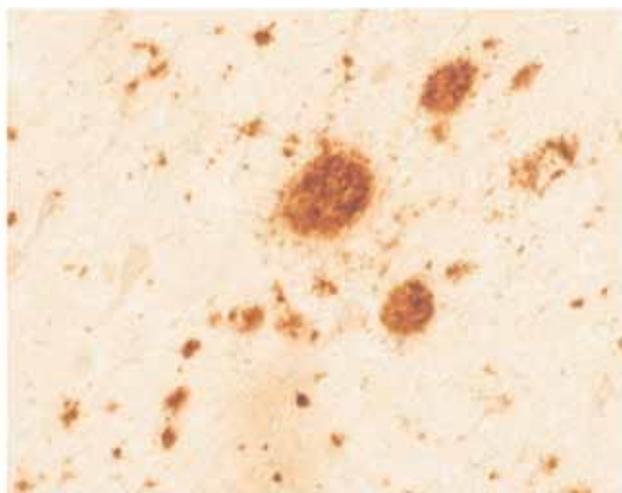
- Monoclonal antibody recognizing deposits of  $\beta$ -amyloid 1-42 in brains of Alzheimer's disease patients and transgenic mouse models
- Optimized storage stability of in-house produced antibodies for preservation of specific and exceptionally well characterized binding capabilities
- Highly purified (> 95 %)
- Broad application for ELISA, Western blot and IHC

## Product specifications

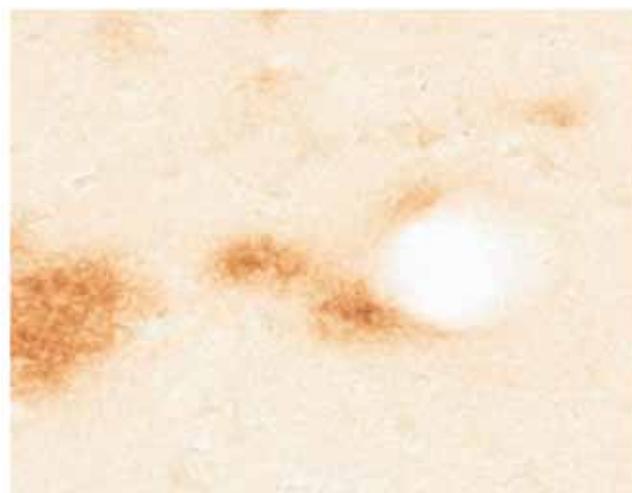
Antigen (Specificity)	$\beta$ -amyloid 1-42
Host	Mouse
Isotyp	IgM or IgG
Cross reactivity	Not known
Storage/Stability	12 months at -20 °C

## Application sample

$\beta$ -amyloid protein is one of the most significant markers of Alzheimer's disease and used for diagnosis in CSF and pathological examinations post mortem.



- ▲ Detection of  $\beta$ -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  $\beta$ -amyloid of cerebral blood vessels in AD brain tissue.

## References

The analyses were performed at the Paul-Flechsig-Institute for Brain Research, Leipzig, Germany (Prof. Dr. rer. nat. habil. Steffen Rossner).

## Order information

Order number	Product	Quantity	Antigen
847-0102006501	Anti-human beta-amyloid 6D11, monoclonal	0.1 mg	$\beta$ -amyloid
847-0102006503		1.0 mg	

# Antibodies for $\alpha$ -synuclein



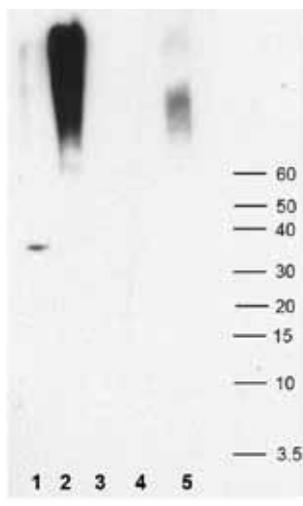
- Monoclonal antibodies for highly specific recognition of total  $\alpha$ -synuclein and disease-associated  $\alpha$ -synuclein
- Worldwide unique antibody 5G4 for detection of synucleopathies
- Optimized storage stability of in-house produced antibodies for preservation of specific and exceptionally well characterized binding capabilities
- Highly purified (> 95 %) monoclonal antibodies and polyclonal antisera
- Broad application for ELISA, Western blot and IHC

## Product specifications

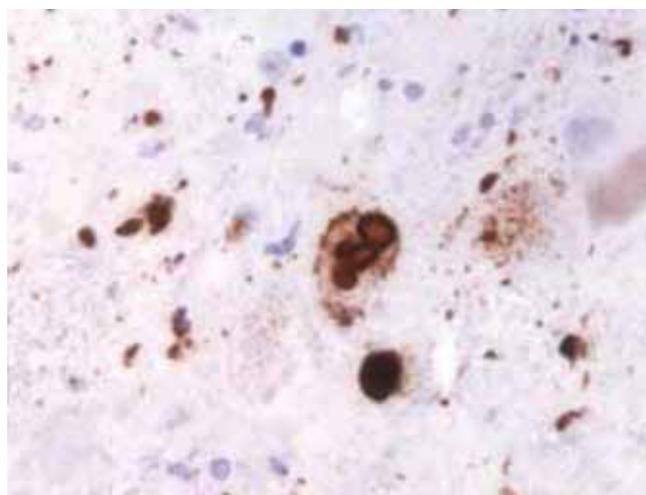
Antigen (Specificity)	$\alpha$ -Synuclein
Host	Mouse and rabbit
Isotyp	IgG
Cross reactivity	Negative for $\beta$ - and $\gamma$ -synuclein
Storage/Stability	12 months at -20 °C

## Application sample

Dementia with Lewy bodies, Parkinson's disease and Multiple system atrophy are characterized by deposition of diseases-associated  $\alpha$ -synuclein. The following western blot data and immunohistochemical staining represents the reactivity of 5G4 antibody to  $\beta$ -sheet rich  $\alpha$ -synuclein oligomers.



■ Anti-human  $\alpha$ -synuclein 5G4 preferentially binds to  $\beta$ -sheet oligomers. Lane1: monomers, lane 2: nitrated oligomers, lanes 3 and 4: oxidized oligomers, lane 5: fibrils



■ Specifically detection of pathologic related structures by Anti-human  $\alpha$ -synuclein 5G4, monoclonal antibody in the brain of patients with Parkinson's disease.

## References

- Kovacs GG, Wagner U, Dumont B, Pikkarainen M, Osman AA, Streichenberger N, Leisser I, Verchère J, Baron T, Alafuzoff I, Budka H, Perret-Liaudet A, Lachmann I (2012) An antibody with high reactivity for disease-associated  $\alpha$ -synuclein reveals extensive brain pathology. *Acta Neuropathol* 124 (1): 37-50
- Kovacs GG, Breydo L, Green R, Kis V, Puska G, Lőrincz P, Perju-Dumbrava L, Giera R, Pirker W, Lutz M, Lachmann I, Budka H, Uversky VN, Molnár K, László L (2014) Intracellular processing of disease-associated  $\alpha$ -synuclein in the human brain suggests prion-like cell-to-cell spread. *Neurobiol Dis* 69: 76-92
- Kovacs GG (2014) Clinical Neuropathology image 5-2014:  $\alpha$ -synuclein pathology in the ependyma in Parkinson's disease. *Clin Neuropathol* 33(5):328

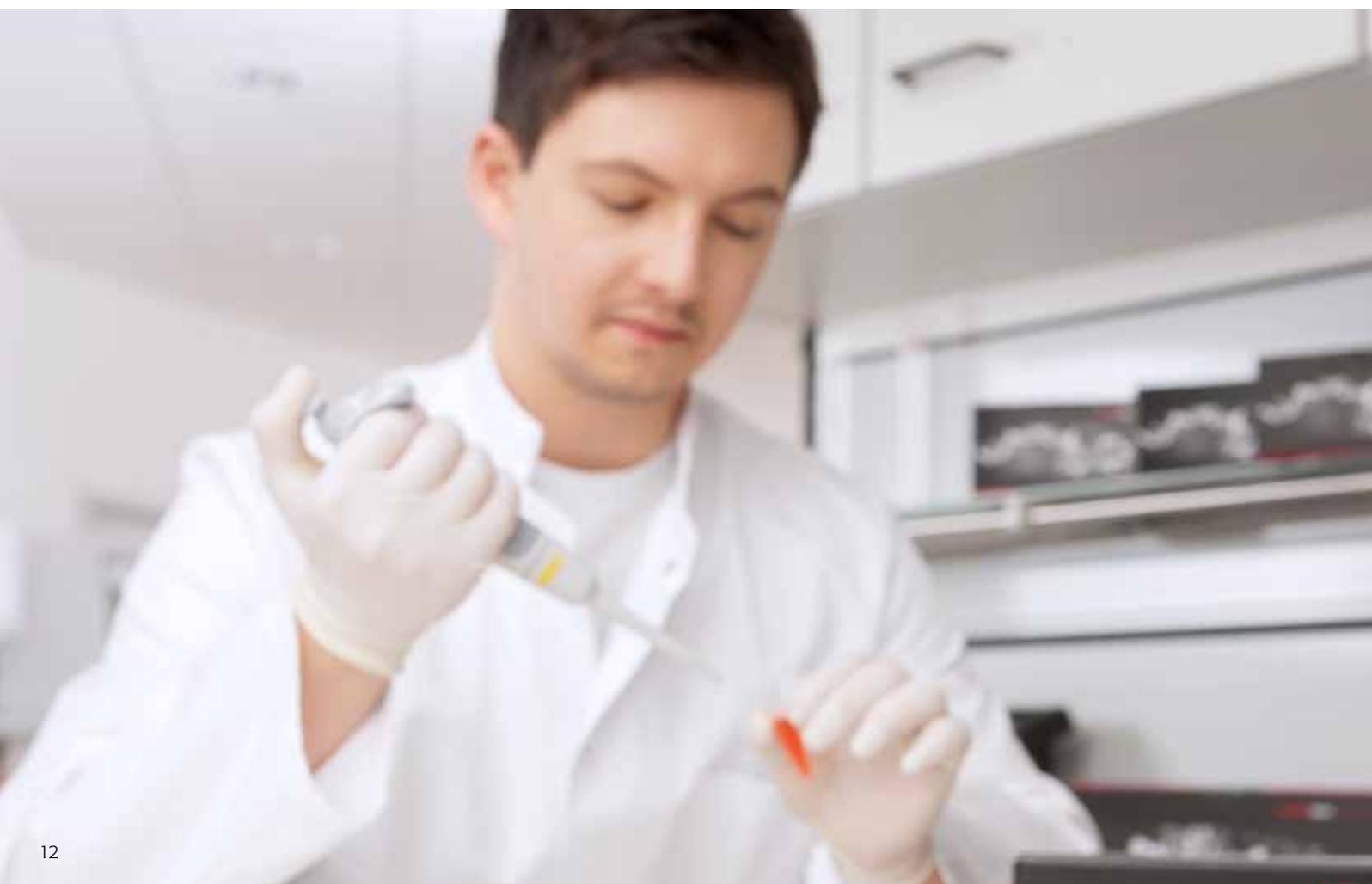
## Order information

Order number	Product	Quantity	Antigen
847-0102001801	Anti-human $\alpha$ -synuclein 10C3, monoclonal	0.1 mg	human $\alpha$ -synuclein
847-0102001803		1.0 mg	
847-0102004001	Anti-human $\alpha$ -synuclein 5G4, monoclonal	0.1 mg	$\beta$ -sheet oligomers of human $\alpha$ -synuclein
847-0102004003		1.0 mg	
847-0102004701	Anti-human $\alpha$ -synuclein 10D2, monoclonal	0.1 mg	human $\alpha$ -synuclein
847-0102004703		1.0 mg	
847-0103000901	Anti-human $\alpha$ -synuclein pAB 63, polyclonal	0.1 ml	human $\alpha$ -synuclein
847-0103000903		1.0 ml	

## Related products

Recombinant synuclein

Order number	Product	Quantity
847-0101008501	Human $\alpha$ -synuclein, His-tagged	0.1 mg
847-0101008502		0.5 mg
847-0101008503		1.0 mg
847-0101008601	Human $\alpha$ -synuclein	0.1 mg
847-0101008602		0.5 mg
847-0101008603		1.0 mg



# Antibodies for prion proteins



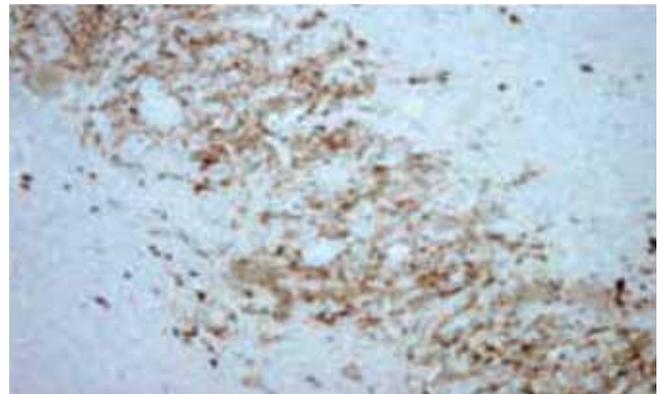
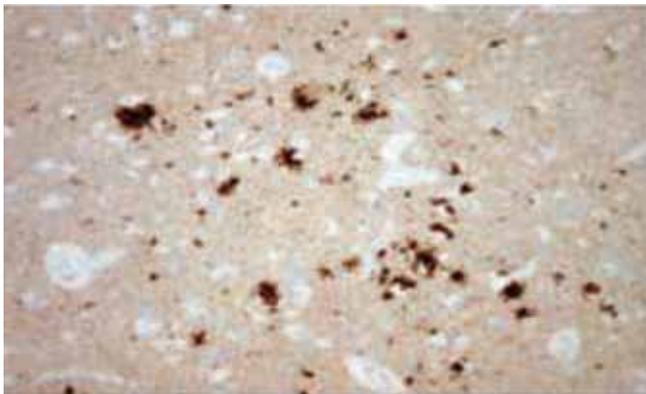
- Highly specific and well characterized monoclonal and polyclonal antibodies suitable for all common techniques
- Reactive to human, bovine, ovine, mouse and deer
- Optimized storage stability of in-house produced antibodies for preservation of specific and exceptionally well characterized binding capabilities
- Highly purified (> 95 %)
- Broad application for ELISA, Western blot and IHC

## Product specifications

Antigen (Specificity)	Prion protein
Host	Mouse
Isotyp	IgG
Cross reactivity	Not known
Storage/Stability	12 months at -20 °C

## Application sample

Immunohistochemical detection of the pathological form of prion protein in brain for confirmation of Creutzfeldt-Jacob disease diagnosis using the monoclonal antibody Anti-human prion protein 14D11.



- Immunohistochemical detection of plaque-like deposits in perivacuolar areas of cortical grey matter and deep nuclei [left] and fine synaptic accumulation in some nuclei such as the dentate nucleus of the cerebellum [right] in sections from a patient with proven classic form of CJD using 14D11 (1:3000).

## References

Dr. Carmen NAVARRO Department of Pathology and Neuropathology, Hospital Meixoeiro, Vigo 36 215 (Po), Spain

## Order information

Order number	Product	Quantity	Antigen
847-0102001201	Anti-human prion protein 5C4, monoclonal	0.1 mg	Human, cattle, sheep and deer prion protein
847-0102001203		1.0 mg	
847-0102001301	Anti-human prion protein 1E2, monoclonal	0.1 mg	Human and cattle prion protein
847-0102001303		1.0 mg	
847-0102001501	Anti-human prion protein 6G3, monoclonal	0.1 mg	Human, cattle, sheep and deer prion protein
847-0102001503		1.0 mg	
847-0102001601	Anti-human prion protein 5B9, monoclonal	0.1 mg	Human and cattle prion protein
847-0102001603		1.0 mg	
847-0102004101	Anti-human prion protein 6E2, monoclonal	0.1 mg	Human and cattle prion protein
847-0102004103		1.0 mg	
847-0102004201	Anti-human prion protein 7D5, monoclonal	0.1 mg	Human and cattle prion protein
847-0102004203		1.0 mg	
847-0102004301	Anti-human prion protein 5G11, monoclonal	0.1 mg	Human and cattle prion protein
847-0102004303		1.0 mg	
847-0102001704	Anti-human prion protein 14D11, monoclonal	0.05 mg	Human, sheep and cattle prion protein
847-0102002701	Anti-mouse prion protein T188, monoclonal	0.1 mg	Murine and ovine prion protein
847-0102002703		1.0 mg	
847-0102002801	Anti-mouse prion protein T325, monoclonal	0.1 mg	Murine, cervid, bovine and ovine prion protein
847-0102002803		1.0 mg	
847-0102000701	Anti-bovine prion protein 4F7, monoclonal	0.1 mg	Bovine and human prion protein, PrP <sup>res</sup>
847-0102000703		1.0 mg	
847-0102000801	Anti-bovine prion protein 1E5, monoclonal	0.1 mg	Bovine and human prion protein, PrP <sup>res</sup>
847-0102000803		1.0 mg	
847-0102000901	Anti-bovine prion protein 3E7, monoclonal	0.1 mg	Bovine, human and ovine prion protein, PrP <sup>res</sup>
847-0102000903		1.0 mg	
847-0102001001	Anti-bovine prion protein 3B8, monoclonal	0.1 mg	Bovine and ovine prion protein, PrP <sup>res</sup>
847-0102001003		1.0 mg	
847-0102001101	Anti-bovine prion protein 7B6, monoclonal	0.1 mg	Bovine, human, sheep and deer prion protein
847-0102001103		1.0 mg	
847-0102002901	Anti-ovine prion protein 683, monoclonal	0.1 mg	Prion protein of murine and ovine sequences other than the APR genotype
847-0102002903		1.0 mg	
847-0102003001	Anti-ovine prion protein A516, monoclonal	0.1 mg	Ovine and murine prion protein
847-0102003003		1.0 mg	
847-0103000101	Anti-bovine prion protein pAB R10, polyclonal	0.1 mg	Sheep, human, cattle, deer and mouse prion protein
847-0103000103		1.0 mg	

## Related products

Order number	Product	Quantity
847-0101000101	Recombinant bovine prion protein	0.1 mg
847-0101000102		0.5 mg
847-0101000103		1.0 mg
847-0101000301	Recombinant human prion protein	0.1 mg
847-0101000302		0.5 mg
847-0101000303		1.0 mg
847-0101000601	Recombinant sheep prion protein	0.1 mg
847-0101000602		0.5 mg
847-0101000603		1.0 mg
847-0101000701	Recombinant deer prion protein	0.1 mg
847-0101000702		0.5 mg
847-0101000703		1.0 mg
847-0101007101	Recombinant mouse prion protein	0.1 mg
847-0101007102		0.5 mg
847-0101007103		1.0 mg
847-0101007201	Recombinant sheep prion protein, genotype ARR	0.1 mg
847-0101007202		0.5 mg
847-0101007203		1.0 mg
847-0101007301	Recombinant sheep prion protein, genotype AF141RQ	0.1 mg
847-0101007302		0.5 mg
847-0101007303		1.0 mg
847-0101007501	Recombinant sheep prion protein, genotype AHQ	0.1 mg
847-0101007502		0.5 mg
847-0101007503		1.0 mg
847-0101007601	Recombinant sheep prion protein, genotype VRQ	0.1 mg
847-0101007602		0.5 mg
847-0101007603		1.0 mg



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AJ Roboscreen GmbH  
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as well as further technical development

