

Selection of TOP monoclonal antibodies

Datasheets of mAbs against several proteins involved in neurodegeneration



Within this selection we want to highlight some of our best monoclonal antibodies

- TAU & pTAUs:
 - **7E5**: against all TAU isoforms, especially for measurements in CSF and IHC, specific to human
 - **2B8**: selectively binds to continuous exon 4-5 sequences on brain-derived Tau isoforms, specific to human
 - **15E3**: selectively binds the master phosphorylation site at amino acid position 50 (T) of Tau, specific to human
 - **10F8**: specific to human pS202-TAU, does not react with rec. TAU441
 - **2B11**: specific to human pT231-TAU; does not react with rec. TAU441
- beta-Amyloid:
 - **6D11**: specific to N-terminal region of human β -Amyloid, well performing in IHC
 - **NEW mAbs** specific to N-terminal and C-terminal regions of β -Amyloid 1-38, 1-40 and 1-42 are available soon
- alpha-Synuclein:
 - **5G4**: binds to beta-sheets of human alpha-Synuclein, recognizes pathological relevant structures, worldwide unique, patented
 - **10D2**: specific to total human alpha-Synuclein
- TDP43:
 - **2G10**: Highly specific to TDP43, tested with patient samples
 - **NEW phospho-TDP43 mAbs** to Phospho 409 and Phospho 410
- Prion protein:
 - **14D11**: Reactive to prion protein from human, sheep and cattle
- Calpain:
 - **1E8**: reactive to both subunits of Calpain-2 from human
- Cyclooxygenase:
 - **5F6**: very specific only to Cyclooxygenase 1 from human, mouse, rat
 - **5E10**: reactive to Cyclooxygenase 2 from human, mouse, rat



Anti-human TAU total mab 7E5

Catalogue number	Package size
847-0102006301	100 µg
847-0102006303	1 mg

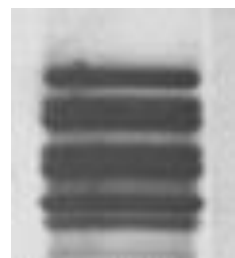
For research use only.

Product:	monoclonal antibody
Clone:	7E5
Host:	Mouse
Isotype:	IgG3 _k
Immunogen:	human TAU 441
Specificity:	Epitope: RGAAPPGQKQQA, human TAU amino acids 156-165
Purification:	purified by affinity chromatography
Purity:	> 95% by SDS-PAGE
Solution:	Carbonate buffer pH 9.6 without additives
Storage:	at -20 °C (repeated thawing and freezing should be avoided)
Applications:	ELISA, Western Blot, Immunohistochemistry
Dilution:	1 µg/ml in Western Blot
Reactivity:	TAU 441 all isoforms, TAU total in CSF

Detection of all 6 TAU isoforms blotted on nitrocellulose by means of mab 7E5.

10 µl of standard TAU ladder (rPeptide) were separated by SDS-PAGE using 8-14% gradient gel and blotted on nitrocellulose 0.45 µm.

After blocking with 5% skim milk powder in TRIS pH 10 mab 7E5 was incubated in blocking solution (1 µg mab/ml) at RT overnight. Bound antibody was detected using Anti-Mouse IgG HRP conjugate and Western blot staining.



2N4R
 2N3R
 1N4R
 1N3R
 0N4R
 0N4R

Anti-human Brain derived TAU mab 2B8

Catalogue number

847-0102007601

847-0102007603

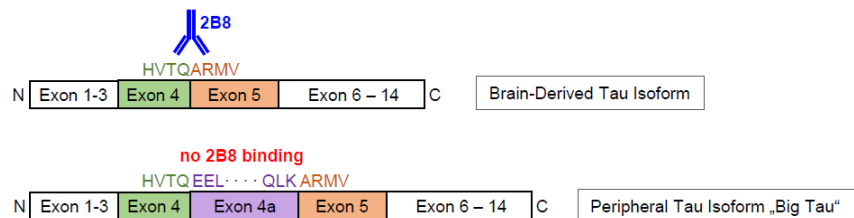
Package size

100 µg

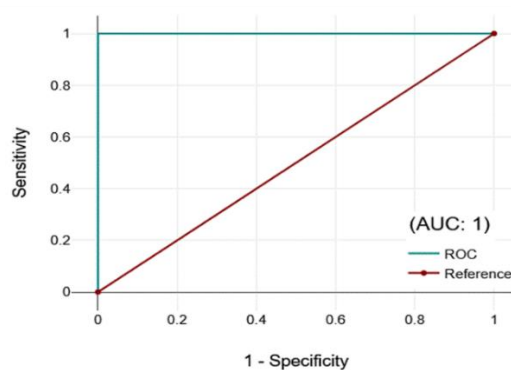
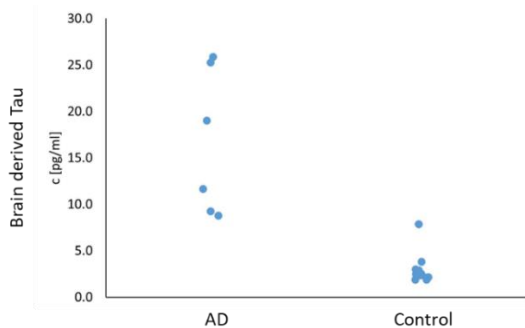
1 mg

For research use only.

Product:	monoclonal antibody
Clone:	2B8
Host:	mouse
Isotype:	IgG1 _k
Immunogen:	human TAU441 (2N4R)
Specificity:	Epitope: HVTQARMV, human TAU441 (2N4R) amino acids 121-128



Purification:	purified by affinity chromatography
Purity:	> 95% by SDS-PAGE
Solution:	PBS ph 7.4 w/o additives
Storage:	at -20 °C (repeated thawing and freezing should be avoided)
Applications:	ELISA, Western Blot, Immunohistochemistry, Immunoprecipitation, SIMOA, Luminex
Reactivity:	TAU441 (2N4R) all isoforms, TAU total in CSF, brain derived TAU



Pre-analytical enrichment of Tau from Plasma samples with the Neuro IP-Kit (847-0108000108) and subsequent measurement of brain-derived Tau using in-House SIMOA Assays shows excellent differentiation between Alzheimer (AD-Pools, n=7) and Controls (Control-Pools, n=12).



Anti-human phospho-50 TAU mab 15E3

Catalogue number

847-0102008001
 847-0102008003

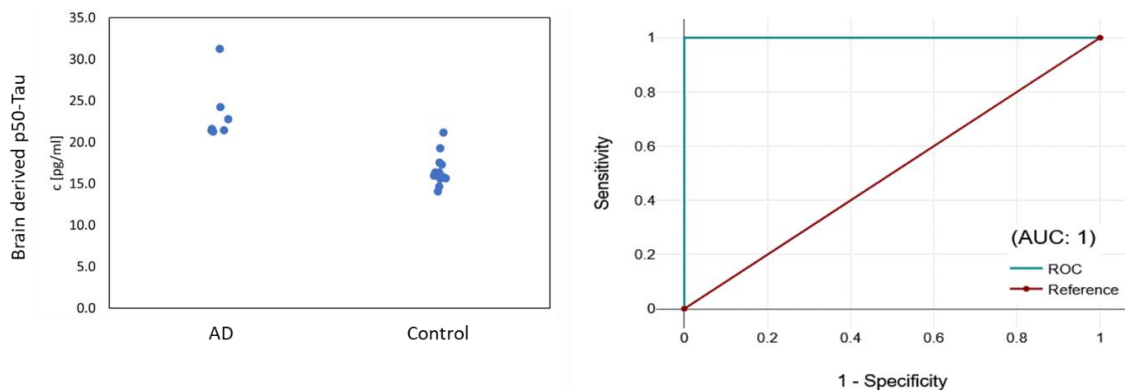
Package size

100 µg
 1 mg

For research use only.

Product:	monoclonal antibody
Clone:	15E3
Host:	Mouse
Isotype:	IgG1 _k
Immunogen:	Synthetic peptide LKESPLQT(Pi)TEDGS
Specificity:	human TAU phosphorylated at amino acid position 50 (T)
Purification:	purified by affinity chromatography
Purity:	> 95% by SDS-PAGE
Solution:	PBS ph 7.4 w/o additives
Storage:	at -20 °C (repeated thawing and freezing should be avoided)
Applications:	ELISA, Western Blot, Immunohistochemistry
Dilution:	1 µg/ml in Western Blot
Reactivity:	CSF, phosphorylated TAU at amino acid position 50 (T)

P50-Tau has the potential to distinguish AD from control patients.



Pre-analytical enrichment of Tau from Plasma samples with the Neuro IP-Kit (847-0108000108) and subsequent measurement of threonine-50 phosphorylated, brain derived Tau (p50-BD-Tau) in an in-House SIMOA Assay shows excellent differentiation between Alzheimer (AD-Pools, n=7) and Controls (Control-Pools, n=12).

Anti-human phospho-202 TAU mab 10F8

Catalogue number	Package size
847-0102004501	100 µg
847-0102004503	1 mg

For research use only.

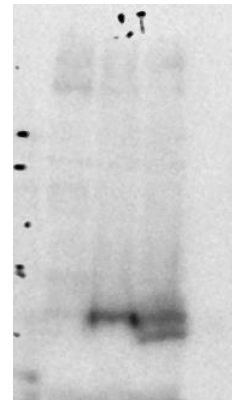
Product:	monoclonal antibody
Clone:	10F8
Host:	Mouse
Isotype:	IgG1 _k
Immunogen:	Synthetic peptide SGYSSPGS(Pi)PGTPG <i>Design and synthesis were kindly performed by group of Prof. Dr. Ralf Hoffmann (BBZ, University of Leipzig, Germany).</i>
Specificity:	human TAU sequence SGYSSPGSPGTPG phosphorylated at amino acid position 202 (S)
Purification:	purified by affinity chromatography
Purity:	> 95% by SDS-PAGE
Solution:	PBS pH 7.4 without additives
Storage:	at -20 °C (repeated thawing and freezing should be avoided)
Applications:	ELISA, Western Blot, Immunohistochemistry
Dilution:	1 µg/ml in Western Blot
Reactivity:	PHF TAU, phosphorylated TAU at amino acid position 202, TAU

Brain homogenates of mice were separated by SDS-PAGE and blotted on nitrocellulose by Western blotting.
Lane1: homogenate of Balb/c wild type
Lane2: homogenate human TAU transgen P301L mutation, female
Lane3: like lane (2), male
Lane4: human recombinant Tau 441
Bound antibody was detected using horseradish peroxidase conjugated goat anti-mouse IgG and ECL substrate solution.
Mab 10F8 reacted specifically with the phosphorylated human TAU but not with recombinant TAU441.

Analyses were kindly performed by Dr. Max Holzer (University of Leipzig, Paul-Flechsig-Institute, Germany).

MW (kDa) 1 2 3 4

170
130
95
72
55



Anti-human phospho-231 TAU mab 2B11

Catalogue number	Package size
0102003501	100 µg
0102003503	1 mg

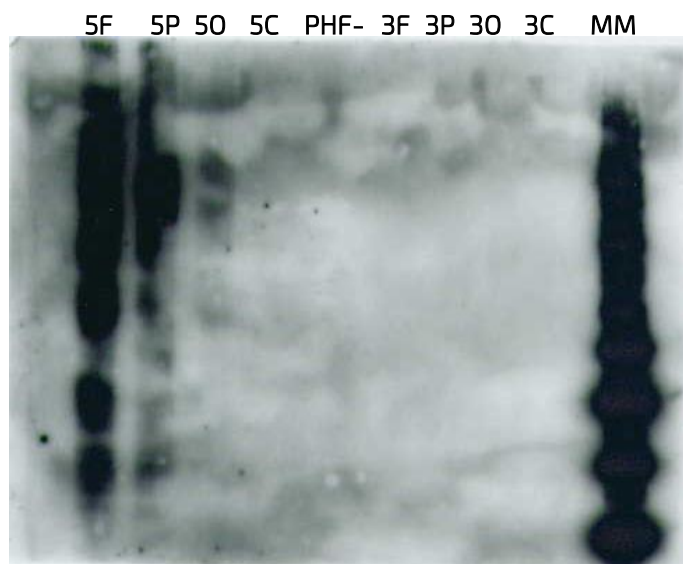
For research use only.

Product:	monoclonal antibody
Clone:	2B11
Host:	Mouse
Isotype:	IgG _{1k}
Immunogen:	Synthetic peptide KKVAVVRT(Pi)PPKSPSS
Specificity:	human TAU phosphorylated at amino acid position 231 (T)
Purification:	purified by affinity chromatography
Purity:	> 95% by SDS-PAGE
Solution:	PBS pH 7.4 without additives
Storage:	at -20 °C (repeated thawing and freezing should be avoided)
Applications:	ELISA, Western Blot, Immunohistochemistry
Dilution:	1 µg/ml in Western Blot
Reactivity:	PHF TAU, phosphorylated TAU at amino acid position 231 (T)

Brain homogenate from patients of braak stage of 5 and 3 for AD pathology, respectively (4 brain areas for each patients (F: frontal; P: parietal; O: occipital; C: cerebellum) and PHF preparation from healthy brain (PHF) were separated by SDS-PAGE and blotted on nitrocellulose membrane. NC membrane was incubated by 5 µg/ml of mab 2B11.

Bound antibody was detected using horseradish peroxidase conjugated goat anti-mouse IgG antibody and ECL staining. Mab 2B11 reacted specifically with the phosphorylated TAU and fragments of lower molecular weight in frontal, parietal occipital areas of braak stage 5 AD patient.

Analyses were kindly performed by Seguin Jérémie (CBPE, Hôpitaux de Lyon, France).



Anti-human β -amyloid mab 6D11

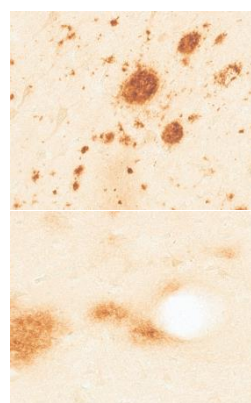
Catalogue number	Package size
847-0102006501	100 μ g
847-0102006503	1 mg

For research use only.

Product:	monoclonal antibody
Clone:	6D11
Host:	Mouse
Isotype:	IgG1 _k
Immunogen:	beta-amyloid 1-42
Specificity:	β -amyloid pathological related deposits, reacted with peptide fragment 1-11 of beta-amyloid
Purification:	purified by affinity chromatography
Purity:	> 95% by SDS-PAGE
Solution:	PBS pH 7.4 without additives
Storage:	at -20°C (repeated thawing and freezing should be avoided)
Applications:	ELISA, Western Blot, Immunohistochemistry (see figure below)
Dilution:	2 ng/ml in ELISA, 1 μ g/ml in Western Blot, 1-5 μ g/ml in IHC
Reactivity:	β -amyloid pathological related deposits

Detection of β -amyloid pathological related deposits in *Cortex* tissue of an Alzheimer disease patient using mab 6D11, dilution 1:100.

Immunohistochemical analysis were kindly performed by group of Prof. Dr. Steffen Rossner, Paul-Flechsig-Institute Leipzig.



Anti-human α -Synuclein mab 5G4

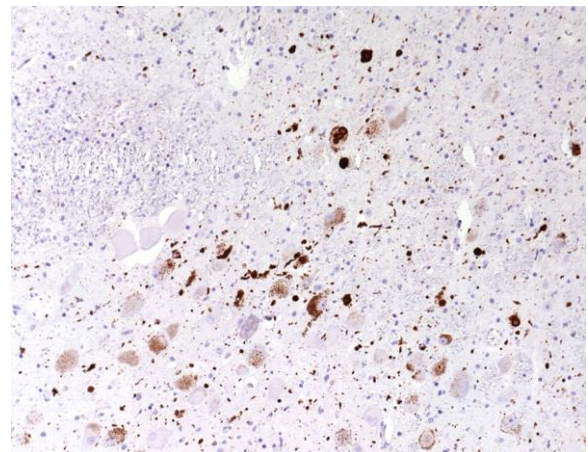
Catalogue number	Package size
847-0102004001	100 μ g
847-0102004003	1 mg

For research use only.

Product:	monoclonal antibody
Clone:	5G4
Host:	Mouse
Isotype:	IgG1 _k
Immunogen:	amino acid sequence 44-57 TKEGVVHGVATVAE
Specificity:	human α -Synuclein oligomers only, amino acids 47-52 (EGVHGVVA) in β -sheet
Purification:	purified by affinity chromatography
Purity:	> 95% by SDS-PAGE
Solution:	PBS pH 7.4 without additives
Storage:	at -20°C (repeated thawing and freezing should be avoided)
Applications:	ELISA, Western Blot, Immunohistochemistry (see figure below)
Dilution:	2 ng/ml in ELISA, 1 μ g/ml in Western Blot, 1-5 μ g/ml in IHC
Reactivity:	human α -Synuclein pathological related forms only

Detection of α -Synuclein aggregates using monoclonal antibody 5G4 in Loc. Coeruleus of Parkinson disease brain tissue.

Analyses were kindly performed by Dr. Gabor Kovacs, Medical University Vienna, Institute of Neurology, Austria.



Anti-human α -Synuclein mab 10D2

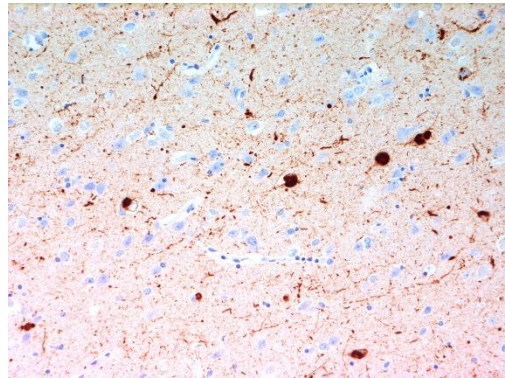
Catalogue number	Package size
847-0102004701	100 μ g
847-0102004703	1 mg

For research use only.

Product:	monoclonal antibody
Clone:	10D2
Host:	mouse
Isotype:	IgG1 _k
Immunogen:	recombinant human α -Synuclein
Specificity:	human α -Synuclein, amino acids 119-126 (DPDNEAYE)
Purification:	purified by affinity chromatography
Purity:	> 95% by SDS-PAGE
Solution:	PBS pH 7.4 without additives
Storage:	at -20°C (repeated thawing and freezing should be avoided)
Applications:	ELISA, Western Blot, Immunohistochemistry
Dilution:	2 ng/ml in ELISA, 1-2 μ g/ml in Western Blot, 1-5 μ g/ml in IHC
Reactivity:	human α -Synuclein

Immunostaining for α -Synuclein in the Amygdala of an individual patient with Lewy body disease.

Kindly provided by G. Kovacs, Vienna



Anti-human TDP43 mab 2G10

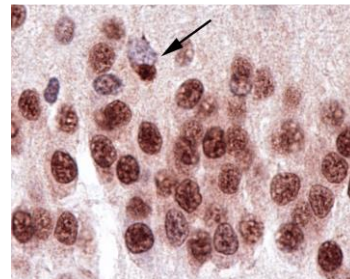
Catalogue number	Package size
847-0102007401	100 µg
847-0102007403	1 mg

For research use only

Product:	monoclonal antibody
Clone:	2G10
Host:	mouse
Isotype:	IgG1 _k
Immunogen:	recombinant human TDP43
Specificity:	Amino acids MTEDELREFFSQYGDVM of TDP43 protein
Purification:	purified by affinity chromatography
Purity:	> 95% by SDS-PAGE
Solution:	PBS pH 7.4 without additives
Storage:	at -20 °C (repeated thawing and freezing should be avoided)
Applications:	ELISA, Western Blot
Dilution:	0.1 - 1 µg/ml in Western Blot and IHC
Reactivity:	TDP43

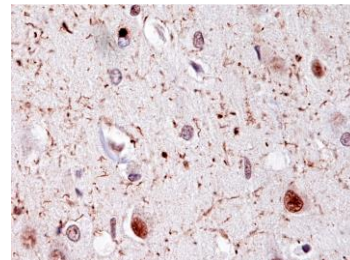
Prominent nuclear physiological nuclear immunostaining of TDP-43 and a neuronal cytoplasmic inclusion body in a neuron lacking the physiological nuclear staining (arrow) using **2G10** antibody in a case with FTLD-TDP.

Results are kindly provided by Prof. Dr. Gabor G. Kovacs MUW (Vienna, Austria).



Fine neuropil threads in the hippocampus and neuronal cytoplasmic inclusions detected by **2G10** antibody in an Alzheimer diseased case with concomitant limbic TDP-43 proteinopathy.

Results are kindly provided by Prof. Dr. Gabor G. Kovacs MUW (Vienna, Austria).



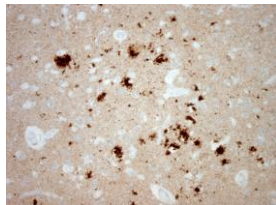
Anti-human prion protein mab 14D11

Catalogue number
847-0102001704

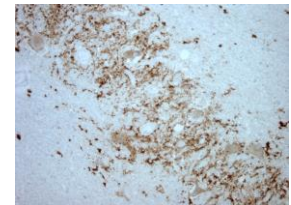
Package size
50 µg

For research use only.

Product:	monoclonal antibody
Clone:	14D11
Host:	mouse
Isotype:	IgG1 _k
Immunogen:	recombinant human prion protein
Specificity:	bovine prion protein aa GSDYEDRYREN ... MHRYPNQVYYRP
Purification:	purified by affinity chromatography
Purity:	> 95% by SDS-PAGE
Solution:	PBS pH 7.4 without additives
Storage:	at -20 °C (repeated thawing and freezing should be avoided)
Applications:	ELISA, Western Blot, Immunohistochemistry (see figure below)
Dilution:	0.2 – 1 µg/ml in Western Blot
Reactivity:	prion protein from human, sheep and cattle



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).



Anti-Calpain 2 mab 1E8

Catalogue number	Package size
0102000601	100 µg
0102000603	1 mg

For research use only.

Product:	monoclonal antibody
Clone:	1E8
Host:	mouse
Isotype:	IgG1 _k
Immunogen:	bovine m-calpain purified from heart muscle cells
Specificity:	80 kDa subunit of m-calpain
Purification:	purified by affinity chromatography
Purity:	> 95% by SDS-PAGE
Solution:	PBS pH 7.4 without additives
Storage:	at -20 °C (repeated thawing and freezing should be avoided)
Applications:	ELISA, Western Blot, Immunohistochemistry (see figure below)
Dilution:	1-10 ng/ml in ELISA, 1-2 µg/ml in Western Blot
Reactivity:	Calpain-2 from cattle and human

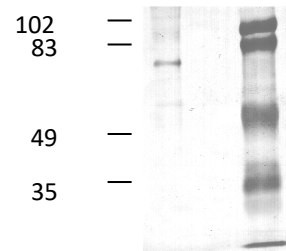
Purified bovine Calpain-2 (1) was separated by SDS-PAGE under reducing conditions and blotted on nitrocellulose membrane.

NC membrane was incubated by 1 µg/ml of mab 1E8.

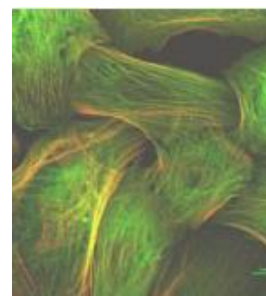
Bound antibody was detected using horseradish peroxidase conjugated goat anti-mouse IgG/M antibody (ZYMED) and H₂O₂/TMB substrate solution.

Mab 1E8 reacted specifically with Calpain-2.

MW (kDa) 1 2



Immunofluorescence of intracellular calpain in bovine lens epithelial cells using mab 1E8 (green).



Anti-Cyclooxygenase 1 mab 5F6

Catalogue number

Package size

847-0102000101

100 µg

847-0102000103

1 mg

For research use only.

Product:	monoclonal antibody
Clone:	5F6/F4
Host:	mouse
Isotype:	IgG1 _k
Immunogen:	COX-1 purified from HL-60 cells
Specificity:	constitutively isoform of the human intracellular Cyclooxygenase 1
Purification:	purified by affinity chromatography
Purity:	> 95% by SDS-PAGE
Solution:	PBS pH 7.4 without additives
Storage:	at -20 °C (repeated thawing and freezing should be avoided)
Applications:	ELISA, Western Blot (see figure below)
Dilution:	1-10 ng/ml in ELISA, 1-2 µg/ml in Western Blot
Reactivity:	Cyclooxygenase 1 from human, mouse, rat

Recombinant Cox-1 standard (2) was separated by SDS-PAGE under reducing conditions and blotted on nitrocellulose membrane.

NC membrane was incubated by 1 µg/ml of mab 5F6.

Bound antibody was detected using horseradish peroxidase conjugated goat anti-mouse IgG/M antibody (ZYMED) and H₂O₂/TMB substrate solution.

Mab 5F6 reacted specifically with Cox-1.

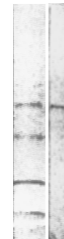
MW (kDa) 1 2

69 —

45 —

29 —

21 —



Anti-Cyclooxygenase 2 mab 5E10

Catalogue number	Package size
847-0102000201	100 µg
847-0102000203	1 mg

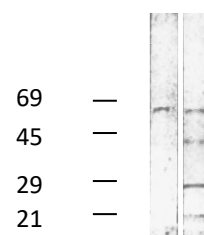
For research use only.

Product:	monoclonal antibody
Clone:	5E10/D10
Host:	mouse
Isotype:	IgG _{1k}
Immunogen:	COX-2 purified from HL-60 cells
Specificity:	target of NSAID Cyclooxygenase-2
Purification:	purified by affinity chromatography
Purity:	> 95% by SDS-PAGE
Solution:	PBS pH 7.4 without additives
Storage:	at -20 °C (repeated thawing and freezing should be avoided)
Applications:	ELISA, Western Blot (see figure below)
Dilution:	1-10 ng/ml in ELISA, 1-2 µg/ml in Western Blot
Reactivity:	Cyclooxygenase 2 from human, mouse, rat

Recombinant Cox-2 standard (1) was separated by SDS-PAGE under reducing conditions and blotted on nitrocellulose membrane.

NC membrane was incubated by 1 µg/ml of mab 5E10. Bound antibody was detected using horseradish peroxidase conjugated goat anti-mouse IgG/M antibody (ZYMED) and H₂O₂/TMB substrate solution. Mab 5E10 reacted specifically with Cox-2.

MW (kDa) 1 2



Order Information

Antibodies for TDP43

Order number [x] = [1]: 100 µg, [3]: 1 mg	Clon	Reactivity
847-010200740[x]	2G10	TDP43 (200-220)
847-010200770[x]	21B2	TDP43 (80-90)
847-010200780[x]	19C9	TDP43 (80-90)
847-010200790[x]	35G7	TDP43 (260-270)
847-010200810[x]	1E6	Phospho-TDP43 (Ser409/410)
847-010200820[x]	2F11	Phospho-TDP43 (Ser409/410)
847-010200820[x]	11C10	Phospho-TDP43 (Ser409/410)

Antibodies for alpha-Synuclein

Order number [x] = [1]: 100 µg, [3]: 1 mg	Clon	Reactivity
847-010200180[x]	10C3	Human α -synuclein
847-010200400[x]	5G4	β -sheet oligomers of human α -synuclein
847-010200470[x]	10D2	Human α -synuclein
847-010300090[x]	polyclonal	Human α -synuclein

Antibodies for Tau protein & beta-Amyloid

Order number [x] = [1]: 100 µg, [3]: 1 mg	Clon	Reactivity
847-010200380[x]	1E7	Phospho-Tau (Thr181)
847-010200390[x]	8B11	Phospho-Tau (Thr181)
847-010200620[x]	8D2	Phospho-Tau (Thr181)
847-010200610[x]	10D3	Phospho-Tau (Thr181)
847-010200860[x]	14B6	Phospho-Tau (Thr181)
847-010200320[x]	1F3	Phospho-Tau (Ser199)
847-010200460[x]	9C8	Phospho-Tau (Ser199+Ser202)
847-010200450[x]	10F8	Phospho-Tau (Ser202)
847-010200880[x]	9G8	Phospho-Tau (Thr217)
847-010200350[x]	2B11	Phospho-Tau (Thr231)
847-010200360[x]	5G7	Phospho-Tau (Thr231)
847-010200370[x]	9D8	Phospho-Tau (Thr231)
847-010200310[x]	4C10	Phospho-Tau (Thr231)
847-010200870[x]	8G5	Phospho-Tau (Thr231)
847-010200440[x]	3G3	Phospho-Tau (Thr231+Ser235)
847-010200800[x]	15E3	Phospho-Tau (Thr50)
847-010200480[x]	4B5	Tau-441 (2N4R)
847-010200510[x]	8F10	Tau-441 (2N4R)
847-010200520[x]	12C2	Tau-441 (2N4R)
847-010200530[x]	18B5	Tau-441 (2N4R)
847-010200630[x]	7E5	Tau all isoforms
847-010200640[x]	2B6	Exon 3 human Tau
847-010200660[x]	9E11	Exon 2 and 3 human Tau
847-010200760[x]	2B8	Brain-derived Tau
847-010300100[x]	polyclonal	Tau-441 (2N4R)
847-010200650[x]	6D11	Beta-Amyloid
847-010200840[x]	2C7	Beta-Amyloid 1-12
847-010200850[x]	3C4	Beta-Amyloid 1-12



Antibodies for Prion Protein

Order number [x] = [1]: 100 µg, [3]: 1 mg	Clon	Reactivity
847-010200120[x]	5C4	Human, cattle, sheep, and deer prion protein
847-010200130[x]	1E2	Human and cattle prion protein
847-010200150[x]	6G3	Human, cattle, sheep, and deer prion protein
847-010200160[x]	5B9	Human and cattle prion protein
847-010200410[x]	6E2	Human and cattle prion protein
847-010200420[x]	7D5	Human and cattle prion protein
847-010200430[x]	5G11	Human and cattle prion protein
847-0102001704*	14D11	Human, sheep, and cattle prion protein, PrP ^{res}
847-010200070[x]	4F7	Bovine and human prion protein, PrP ^{res}
847-010200080[x]	1E5	Bovine and human prion protein, PrP ^{res}
847-010200090[x]	3E7	Bovine, human, and ovine prion protein, PrP ^{res}
847-010200100[x]	3B8	Bovine and ovine prion protein, PrP ^{res}
847-010200110[x]	7B6	Bovine, human, sheep, and deer prion protein
847-010300010[x]	pAB R10 polyclonal	(sheep, human, cattle, deer, and mouse) prion

* = 50 µg

Antibody for Calpain

Order number [x] = [1]: 100 µg, [3]: 1 mg	Clon	Reactivity
847-010200060[x]	1E8	Calpain-2 from cattle and human

Antibodies for Cyclooxygenase

Order number [x] = [1]: 100 µg, [3]: 1 mg	Clon	Reactivity
847-010200010[x]	5F6	Cyclooxygenase 1 from human, mouse, rat
847-010200020[x]	5E10	Cyclooxygenase 2 from human, mouse, rat

Recommendations for Use:

Fast and Easy Implementation of Antibodies

The use of our 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 ROBOSCREEN'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 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 or 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.
- Stain tissue slides with DAB according to manufacturer's IFU.

Western blot

- Block transferred antigen onto nitrocellulose with 5% skim milk powder in TRIS buffer pH10 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 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 pH7.4 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.
- 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.
- 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.



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