



MOAB-2 Mouse Monoclonal antibody to Amyloid beta peptide (A beta 40/42), purified

Catalogue No.: M-1586-100

Description: The amyloid beta peptide is derived from the cleavage of the Amyloid precursor protein (APP) and varies in length from 39 to 43 amino acids. However, the form(s) of amyloid-beta peptide (A β) associated with the pathology characteristic of Alzheimer's disease (AD) remains unclear. In particular, the neurotoxicity of intraneuronal A β accumulation is an area of considerable research and controversy principally because antibodies thought to be specific for A β have been shown to actually detect intraneuronal APP and not A β exclusively.

MOAB-2 (mouse IgG2b) is a pan-specific, high-titer antibody to A β residues 1-4 as demonstrated by biochemical and immunohistochemical analyses (IHC), and is highly specific just to amyloid beta peptide.

MOAB-2 did not detect APP or APP-CTFs in cell culture media/lysates (HEK-APPSwe or HEK APPSwe/BACE1) or in brain homogenates from transgenic mice expressing 5 familial AD (FAD) mutation (5xFAD mice).

Using IHC on 5xFAD brain tissue, MOAB-2 immunoreactivity co-localized with C-terminal antibodies specific for A β 40 and A β 42. MOAB-2 did not co-localize with either N- or C-terminal antibodies to APP. In addition, no MOAB-2-immunoreactivity was observed in the brains of 5xFAD/BACE-/- mice, although significant amounts of APP were detected by N- and C-terminal antibodies to APP, as well as by 6E10.

In both 5xFAD and 3xTg mouse brain tissue, MOAB-2 co-localized with cathepsin-D, a marker for acidic organelles, further evidence for intraneuronal A β , distinct from A β associated with the cell membrane. MOAB-2 demonstrated strong intraneuronal and extra-cellular immunoreactivity in 5xFAD and 3xTg mouse brain tissues.

Related products: Biotinylated MOAB-2 antibody, cat# M-1742-50-B
Oligomeric A β ELISA Kit, cat# BEK-2215-1P/2P

Batch No.: See product label

Unit size: 100 μ g

Antigen: Recombinant human amyloid beta protein 42 (A β 42):
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

Antigen Location: Epitope maps to residues 1-4 of human amyloid beta peptide 40/42

Antigen Length: 42 amino acids

Antibody Type: Monoclonal

Isotype: IgG2b, lambda

Clone: MOAB-2

Other Names: Beta-APP42; Beta-APP40; Beta-amyloid protein 42; Beta-amyloid protein 40; ABPP; APPI;

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	Amyloid beta A4 protein;MOAB2;MOAB-2; Alzheimer's antibody;AB40;AB42;abeta
Accession:	P05067 A4_HUMAN;
Produced in:	Mouse
Molecular Weight:	With Formic acid extractions and standard reduced western procedures beta-amyloid peptide migrates between 3-6 kDa (see figure 1).
Purity:	This product is a Protein A purified mouse IgG _b in 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, 0.01% sodium azide, pH 7.2.
Applications:	<p>Western Blotting (WB), Immunohistochemistry (IH), Immunohistochemistry/paraffin embedded IH(P), Immunoprecipitation (IP), Immunofluorescence (IF), ELISA.</p> <p>Antibody has been tested in WB using purified synthetic beta-amyloid preparations and from transgenic mouse brain formic acid extracts (see figure 1). Formic acid extraction/concentration is required for western blot detection from extracts. MOAB-2 antibody is specific for beta-amyloid and does not detect APP. Suggested dilution of 1:2000-1:5,000 for WB, standard ECL detection systems.</p> <p>Tissue samples for the detection of beta-amyloid should be prepared as detailed in K.L. Youmans et al. {Journal of Neuroscience Methods 196 (2011) 51–59} for best results. Detection of beta-amyloid 40/42 in direct westerns can be difficult; Dot-blot of prepared samples are recommended as detailed in Youmans. KL et al 2012.</p> <p>IR or fluorescent detection systems not yet tested, they but are expected to work well with higher primary antibody dilutions because of the increased sensitivity of the detection methods. Suggested dilutions for IHC are 1:50-1:1,000. Fresh frozen, 4% paraformaldehyde fixed frozen, or formalin fixed paraffin embedded tissues are all suitable. Optimal dilutions must be determined by the end user. Antigen retrieval is required in fixed tissues for optimal staining. Antibody was tested on 4% paraformaldehyde/0.1% glutaraldehyde fixed frozen tissue from 3xTg and 5xFAD mice. MOAB-2 antibody detects intraneuronal and extracellular beta-amyloid in IHC and does not detect APP {Youmans KL et al 2012}.</p> <p>The antibody also reacts with archival formalin-fixed, paraffin-embedded tissue samples with antigen Heat Induced Epitope Retrieval (HIER): Recommended Citrate, pH 6.0 buffer for HIER. Signal was weak without antigen retrieval. Immunoreactivity was expressed in intraneural-amyloid deposition (plaque) in Alzheimer's brain. MoAB-2 was found to be extremely clean and with an excellent signal to noise ratio with no neuro-cellular diffusive staining.</p> <p>In addition MOAB-2 demonstrated no significant differences in Aβ detection using paraffin fixed, free-floating sections {Youmans KL et al 2012}. Formic acid (FA) treatment resulted in optimal detection of both intraneuronal and extracellular Aβ compared to without FA (incubated in 88% FA 8 min, Youmans KL et al 2012). Free floating tissue sections were permeabilized in TBS containing 0.25% Triton X-100 (TBSX; 3 \times 10 min), blocked with 3% horse serum in TBSX (3 \times 10 min) followed by 1% horse serum in TBSX (2 \times 10 min) and incubated with appropriate primary antibodies diluted in TBSX containing 1% horse serum overnight. See Youmans KL et</p>

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al 2012 for full IH(P) protocol and method details.

For IF, suggested dilution is 1:100-1:500. The antibody was tested on 4% PFA fixed frozen tissue. Fixed tissues were washed in TBS (3 × 10 min), then incubated in 88% FA (8 min), and then permeabilized in TBSX (3 × 10 min), and blocked in TBSX containing 5% bovine serum albumin (BSA; 1 hr). Sections were subsequently incubated with appropriate primary antibodies diluted in TBSX containing 2% BSA overnight on an oscillatory rotator. Detection was via fluorescently labelled absorbed secondary antibodies {Youmans KL et al 2012}.

For IP, the suggested dilution is 1:200 to 1:1,000 for labeled beta-amyloid using Protein A/G conjugated beads as the capture vehicle {Youmans KL et al 2012}.

In an ELISA, a dilution of 1:50-1:1000 is suggested. The antibody has been tested in ELISAs on synthetic beta-amyloid and tissue homogenates from beta-amyloid-Tg mice. Biosensis recommends optimal dilutions/concentrations should be determined by the end user for all applications. Dilutions provided are only meant to serve as a basic guide.

Specificity: MOAB-2 detects preparations enriched in U-, O-, F-A β 42, and U-A β 40 by dot-blot, and is thus a pan-specific A β antibody. However, MOAB-2 is selective for the more neurotoxic A β 42 compared to A β 40. Indeed, MOAB-2 demonstrated a titration against antigen concentration, and detects A β 40 at 2.5 pmol but U-, O- and F-A β 42 at antigen concentrations as low as ~ 0.1 pmol {Youmans. KL et al 2012}. MOAB-2 does not detect APP (Amyloid precursor protein).

Species Against: Human

Antibody Against: Human

Cross-reactivity: Human, Rat, other species not yet tested.

By Dot blot, MOAB-2 detected rat A β 40 and human A β 40, albeit with less affinity than for A β 42. {Youmans. KL et al 2012}

Form: Lyophilized, from a Protein A purified preparation in 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, 0.01% sodium azide, pH 7.2; contains 0.01% sodium azide as a preservative.

Appearance: dry powder

Reconstitution: Reconstitute in 100 μ l of sterile water. Centrifuge to remove any insoluble material. Final buffer is 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, 0.01% sodium azide, pH 7.2.

Storage: After reconstitution keep aliquots at -20° to -70C for a higher stability. At 4°C keep up to one week, insulated, protected from light; use sterile methods and pipettes. Highly purified glycerol (1:1) may be added for an additional stability. Avoid repetitive freeze/thaw cycles. Keep tightly closed when not in use and protected from light.

Expiry Date: 12 months after purchase

Specific References: Zhu, B. et al. (2017) ER-associated degradation regulates Alzheimer's amyloid pathology and memory function by modulating γ -secretase activity. Nat Commun. 8(1):1472.
Application: IHC

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Huang, TY. et al. (2017) SORLA attenuates EphA4 signaling and amyloid β -induced neurodegeneration. *J Exp Med.* pii: jem.20171413. [Epub ahead of print]. Application: IHC

Felecia, M. et al. (2017) Peripheral Inflammation, Apolipoprotein E4, and Amyloid- β Interact to Induce Cognitive and Cerebrovascular Dysfunction. *ASN Neuro.* 9(4):1759091417719201. Application: IHC/IF

Thomas, R. et al. (2016) Epidermal growth factor prevents APOE4 and amyloid-beta-induced cognitive and cerebrovascular deficits in female mice. *Acta Neuropathol Commun.* 4(1):111 Application: IHC

Koster, KP. et al. (2016) Epidermal growth factor prevents oligomeric amyloid- β induced angiogenesis deficits in vitro. *J Cereb Blood Flow Metab.* [Epub ahead of print] Application: IF

Löffler, T. et al. (2016) Decreased Plasma A β in Hyperlipidemic APPSL Transgenic Mice Is Associated with BBB Dysfunction. *Front. Neurosci.* Application: IF

Kobro-Flatmoen, A. et al. (2016) Reelin-immunoreactive neurons in entorhinal cortex layer II selectively express intracellular amyloid in early Alzheimer's disease. *Neurobiology of Disease.* 93:172-183. Application: IHC

Tai, LM. et al. (2016) The role of APOE in cerebrovascular dysfunction. *Acta Neuropathol.* 131(5):709-23. Application: IF

Kim, YH. et al. (2015) A 3D human neural cell culture system for modeling Alzheimer's disease. *Nat Prot.* 10(7):985-1006. Application: WB

Condello, C. et al. (2015) Microglia constitute a barrier that prevents neurotoxic protofibrillar A β 42 hotspots around plaques. *Nat Commun.* 6:6176. Application: IF

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lulita MF et al (2014) Intracellular Abeta pathology and early cognitive impairments in a transgenic rat model overexpressing human amyloid precursor protein: a multidimensional study.

Acta Neuropathol Commun. 6:61. Application: IF, IH

Smith BR et al (2014) Neuronal inclusions of alpha-synuclein contribute to the pathogenesis of Krabbe disease.

J Pathol. Apr;235(5):509-21. Application: IF

General References:

Tai LM et al (2016) "The role of APOE in cerebrovascular dysfunction."Acta Neuropathol. 2016 Feb 16. [Epub ahead of print]

K.L. Youmans et al (2012) Intraneuronal Abeta detection in 5xFAD mice by a new Abeta-specific antibody

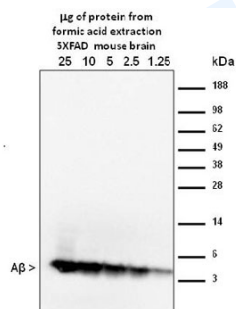
Mol Neurodegener. 2012 Mar 16;7(1):8.

Tai LM et al (2013) Levels of soluble apolipoprotein E/amyloid- β (A β) complex are reduced and oligomeric A β increased with APOE4 and Alzheimer disease in a transgenic mouse model and human samples.

J Biol Chem. 2013 Feb 22;288(8):5914-26.

K.L. Youmans et al (2011) Amyloid- β 42 alters apolipoprotein E solubility in brains of mice with five familial AD mutations

J Neurosci Methods. 2011 Mar 15;196(1):51-9.



Sample combined cortex and hippocampus from 12 month old 5xFAD mice. 1.25-25 mg of FA extraction were analyzed using the standard Western blot procedure as described in Youmans, Molecular Neurodegeneration, 2012. MOAB-2 was used at a concentration of 1 μ g/ml.

Extraction protocol: Modified 3-step sequential extraction as described in Youmans et al, J Neurosci Methods, 2011. The modification involved using less volume of formic acid (FA) in step 3 to give a more concentrated extraction. Therefore, 70% FA was added at a concentration of 600mg/ml rather than 150mg/ml as described in Youmans et al. The final protein concentration of the neutralized formic acid extraction was 0.9 mg/ml

Youmans, KL et al (2011) J. Neuroscience Methods 196(1):51-59.
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3049315>.

Youmans, KL et al (2012) Mol. Neurodegener. 7:8.
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3355009>.

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