

## Mouse monoclonal antibody to Amyloid beta peptide (A-beta 1-40/42), [MOAB-2] - Biotinylated

**Catalogue No.:** M-1742-50-B

**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 (Abeta) associated with the pathology characteristic of Alzheimer's disease (AD) remains unclear. In particular, the neurotoxicity of intraneuronal Abeta accumulation is an area of considerable research and controversy principally because antibodies thought to be specific for Abeta have been shown to actually detect intraneuronal APP and not Abeta exclusively.

MOAB-2 (mouse IgG2b) is a pan-specific, high-titer antibody to Abeta 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 Abeta40 and Abeta42. 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 Abeta, distinct from Abeta associated with the cell membrane. MOAB-2 demonstrated strong intraneuronal and extra-cellular immunoreactivity in 5xFAD and 3xTg mouse brain tissues.

Biosensis now offers biotinylated MOAB-2 antibody allowing more flexibility in experimental design by using the biotin-avidin/streptavidin detection method. Biotinylated MOAB-2 antibody may also help to reduce background staining in difficult-to-stain tissues and increase detection sensitivity. The ability of biotinylated MOAB-2 antibody to detect amyloid beta has been validated by IHC.

Purified, non-biotinylated MOAB-2 antibody is available here.

**Related products:** Purified, non-biotinylated MOAB-2 antibody, cat# M-1586-100 Oligomeric Abeta ELISA Kit, cat# BEK-2215-1P/2P

**Batch No.:** See product label

**Unit size:** 50 ug

**Antigen:** Recombinant human amyloid beta protein 42 (A $\beta$ 42):  
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

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

**Antigen Length:**

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	42 amino acids
<b>Antibody Type:</b>	Mouse monoclonal
<b>Isotype:</b>	IgG2b, lambda
<b>Clone:</b>	MOAB-2
<b>Other Names:</b>	Beta-APP42; Beta-APP40; Beta-amyloid protein 42; Beta-amyloid protein 40; ABPP; APPI; Amyloid beta A4 protein; MOAB2; MOAB-2; Alzheimer's antibody; AB40; AB42; abeta
<b>Accession:</b>	P05067 A4_HUMAN
<b>Produced in:</b>	Mouse
<b>Molecular Weight:</b>	With Formic acid extractions and standard reducing western blotting procedures, beta-amyloid peptide migrates between 3-6 kDa (see Figure 1).
<b>Purity:</b>	Antibody was purified from cell culture supernatant by Protein G chromatography, biotinylated and buffer-exchanged into PBS, pH 7.4 buffer
<b>Applications:</b>	The biotinylated MOAB-2 antibody has been tested by IHC (1:500 - 1:2,000 dilution) and is also expected to work in applications validated for the unlabelled antibody (M-1586-100) at same or higher dilutions: Western Blotting (WB), Immunohistochemistry (IHC), Immunohistochemistry/paraffin embedded IHC(P), Immunoprecipitation (IP), Immunofluorescence (IF), ELISA. Western Blotting: MOAB-2 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. Suggested dilution of 1:2000-1:5,000 for WB, standard ECL detection systems. Tissue samples for the detection of beta-amyloid should be prepared as detailed in Youmans KL et al., 2011 (Journal of Neuroscience Methods 196: 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. Immunohistochemistry: Suggested dilution for biotinylated MOAB-2 in IHC is 1:500-1:2,000. Fresh frozen, 4% paraformaldehyde fixed frozen, or formalin fixed paraffin embedded tissues are all suitable. 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). The antibody also reacts with archival formalin-fixed, paraffin-embedded tissue samples with antigen Heat Induced Epitope Retrieval (HIER). Recommended buffer for HIER is citrate, pH 6.0. Signal was weak without antigen retrieval. Immunoreactivity was observed in intraneuronal-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. In addition, MOAB-2 demonstrated no significant differences in A-beta 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 A-beta compared to without FA (incubated in 88% FA 8 min, Youmans KL et al., 2012). Free floating

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tissue sections were permeabilized in TBS containing 0.25% Triton X-100 (TBSX; 3 x 10 min), blocked with 3% horse serum in TBSX (3 x 10 min) followed by 1% horse serum in TBSX (2 x 10 min) and incubated with appropriate primary antibodies diluted in TBSX containing 1% horse serum overnight. See Youmans KL et al., 2012, for full IHC(P) protocol and method details. Immunofluorescence: 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 x 10 min), then incubated in 88% FA (8 min), and then permeabilized in TBSX (3 x 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). Immunoprecipitation: For IP, the suggested dilution is 1:200 to 1:1,000 for labelled beta-amyloid using SA-coated beads as the capture vehicle, similar to the protocols employed by Youmans KL et al., 2012. ELISA: In an ELISA, a dilution of 1:50-1:1,000 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.

<b>Specificity:</b>	MOAB-2 detects preparations enriched in U-, O-, F-A $\beta$ 42, and U-A $\beta$ 40 by dot-blot, and is thus a pan-specific A $\beta$ antibody. However, MOAB-2 is selective for the more neurotoxic A $\beta$ 42 compared to A $\beta$ 40. Indeed, MOAB-2 demonstrated a titration against antigen concentration, and detects A $\beta$ 40 at 2.5 pmol, but U-, O- and F-A $\beta$ 42 at antigen concentrations as low as ~ 0.1 pmol (Youmans. KL et al., 2012; PMID: 22423893). MOAB-2 does not detect APP (Amyloid Precursor Protein).
<b>Species Against:</b>	Human
<b>Antibody Against:</b>	Human
<b>Cross-reactivity:</b>	Human, rat, other species not yet tested. By Dot Blot, MOAB-2 detected rat A Beta 40 and human A Beta 40, albeit with less affinity than for A Beta 42 (Youmans KL et al., 2012).
<b>Conjugate:</b>	Biotin
<b>Form:</b>	Lyophilized from PBS buffer, pH 7.4; contains no preservative.
<b>Appearance:</b>	Dry powder.
<b>Reconstitution:</b>	Spin vial briefly before opening. Reconstitute in 50 uL of sterile water to give a concentration of 1 mg/mL. Centrifuge to remove any insoluble material. Final buffer is PBS, pH 7.4 without preservative.
<b>Storage:</b>	After reconstitution keep aliquots at -20C to -70C for a higher stability. At 2-8C keep up to one week; 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.
<b>Expiry Date:</b>	12 months after purchase.

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**Specific References:** Ruan, CS. et al. (2017) Sortilin inhibits amyloid pathology by regulating non-specific degradation of APP. *Exp Neurol*. [Epub ahead of print] Application: IHC

References for non-biotinylated MOAB-2 antibody (M-1586-100):

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

Huang, TY. et al. (2017) SORLA attenuates EphA4 signaling and amyloid  $\beta$ -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- $\beta$  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- $\beta$  induced angiogenesis deficits in vitro. *J Cereb Blood Flow Metab*. [Epub ahead of print] Application: IF

Löffler, T. et al. (2016) Decreased Plasma A $\beta$  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.

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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 $\beta$ 42 hotspots around plaques. Nat Commun. 6:6176. Application: IF

Iulita 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]

Tai LM et al (2013) Levels of soluble apolipoprotein E/amyloid- $\beta$  (A $\beta$ ) complex are reduced and oligomeric A $\beta$  increased with APOE4 and Alzheimer disease in a transgenic mouse model and human samples. J Biol Chem. 2013 Feb 22;288(8):5914-26.

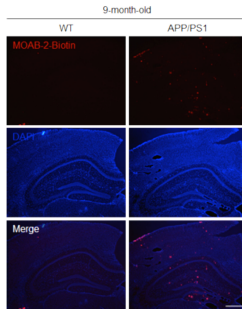
K.L. Youmans et al (2012) Intraneuronal Abeta detection in 5xFAD mice by a new Abeta-specific antibody Mol Neurodegener. 2012 Mar 16;7(1):8.

K.L. Youmans et al (2011) Amyloid- $\beta$ 42 alters apolipoprotein E solubility in brains of mice with five familial AD mutations J Neurosci Methods. 2011 Mar 15;196(1):51-9.

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## Mouse monoclonal antibody to Amyloid beta peptide (A-beta 1-40/42), [MOAB-2] - Biotinylated



Immunohistochemical detection of amyloid plaques in the brain of 9 months old WT and APP/PS1 mice. Brain tissues were fixed with 4% paraformaldehyde and dehydrated in 30% sucrose solution. 30  $\mu$ m frozen sections were prepared and blocked in 5% Normal horse serum at room temperature for 1h. Then, sections were incubated with biotinylated MOAB-2 antibody (1:1000, red), overnight at 4°C. MOAB-2 binding was visualized with a streptavidin-Cy3 conjugate (1:1000, 1h). Cell nuclei were counterstained with DAPI dye. Figure courtesy of Dr C.S. Ruan, University of South Australia.

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