**For Research Use Only.
Not for use in diagnostic procedures.**

MONOCLONAL ANTIBODY

Anti-Amyloid Precursor Protein mAb

Code No.	Clone	Subclass	Quantity	Concentration
M009-3	3E9	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Alzheimer's disease (AD) is the most common form of dementia in the elderly. The neuropathological hallmarks are neurofibrillary tangles and senile plaques. The major protein component of the plaques consists of 39-42 amino acids peptide (β-amyloid/Aβ). Aβ occurs in two predominant forms with different COOH-termini, Aβ 40 and Aβ 42, and overproduction of Aβ 42 has been suggested to be the cause of familial earlyonset AD. Aβ generation depends on proteolytic cleavage of the amyloid precursor protein (APP) by two proteases: β-secretase and γ-secretase. Recent study suggested that a transmembrane aspartic protease, termed β-site APP-cleaving enzyme (BACE), functionally acts as the β-secretase.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone 3E9) was established by fusion of mouse myeloma cell SP2/0-Ag14 with Balb/c mouse splenocyte immunized with the synthetic peptide, LEVPTDGNAGLLAEPQIAMFC, which corresponding to APP695 (18-38 aa).

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with Amyloid Precursor Protein on Western blotting.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Tissue and cell	transfectant	brain	Not Tested
Reactivity on WB	+	+	

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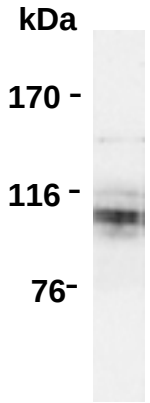
APPLICATIONS:
Western blotting; 5-10 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Reference 1) and 3)

Detailed procedure is provided in the following **PROTOCOL**.

REFERENCES:
1) Sokolow, S., *et al., Neurobiol Aging.* **33**, 1545-1555 (2012) [FCM]
2) Sakai, T. and Hohjoh, H. *Cell Biol Int.* **30**, 952-956 (2006) [WB]
3) Gylys, K. H., *et al., Am J Pathol.* **165**, 1809-1817 (2004) [FCM]
4) Golde, T., *et al., Science* **255**, 728-730 (1992)
5) Selkoe, D. J., *et al., Cell* **58**, 611-612 (1989)
6) Kang, H., *et al., Nature* **325**, 733-736 (1987)

RELATED PRODUCTS:
M046-3 Anti-Amyloid β Protein, N-terminal Specific (Human) mAb (2C8)
M066-3 Anti-Amyloid β/Amyloid Precursor Protein (Human) mAb (4E12)



Western blot analysis of Amyloid Precursor Protein expression in mouse brain using M009-3.

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PROTOCOL:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4^{o}C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4^{o}C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^{2} for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4^{o}C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; mouse brain)