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Product datasheet for AM26575PU-N

Amyloid Precursor Protein (APP) (APP695) (18-38) Mouse Monoclonal Antibody [Clone ID: 3E9]

Product data:

Product Type:	Primary Antibodies
Clone Name:	3E9
Applications:	WB
Recommended Dilution:	Western blot: 5-10 µg/ml for chemiluminescence detection system. For details see protocol below.
Reactivity:	Human, Mouse
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Synthetic peptide corresponding to APP695 (18-38 aa)
Specificity:	This antibody reacts with Amyloid Precursor Protein.
Formulation:	PBS (pH 7.2) State: Azide Free State: Lyophilized Ig fraction Stabilizer: 1% sucrose Preservative: 0.09% NaN3
Reconstitution Method:	Restore with 100 µL of distilled water.
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Prior to reconstitution store at 2-8°C. Following reconstitution store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	amyloid beta precursor protein
Database Link:	Entrez Gene 351 Human P05067

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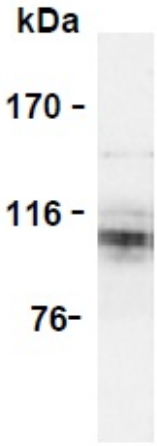
Background: Alzheimer's disease (AD) is the most common form of dementia in the elderly. The neuropathological hallmarks are neurofibrillary tangles and senile plaques. The major protein component of the plaques consists of 39-42 amino acids peptide (ß-amyloid/Aß). Aß occurs in two predominant forms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

Synonyms: Alzheimer disease amyloid protein, Amyloid Precursor Protein, ABPP, APPI, PreA4, Cerebral vascular amyloid peptide, CVAP

Note: This product was originally produced by MBL International.

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 4oC 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 4oC 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/cm2 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 4oC.
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 HRP-conjugated anti-mouse IgG 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)

Product images:



Western blot analysis of Amyloid Precursor Protein expression in mouse brain using AM26575PU-N.