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

MONOCLONAL ANTIBODY

Anti-Apolipoprotein E4

Code No.	Clone	Subclass	Quantity	Form
M067-3	1F9	Mouse IgG1	100 µg	Lyophilized

BACKGROUND: Apolipoprotein E (ApoE), a 35 kDa plasma protein containing sialic acid, plays a role in triglyceride, cholesterol transport and metabolism, and known to be synthesized in liver, brain and other organs. ApoE is a polymorphic apolipoprotein exhibiting three isoforms such as ApoE2, E3 and E4 coded for by three alleles of ε2, ε3 and ε4 at a single gene locus respectively. Reportedly ApoE represents an important risk marker for Alzheimer's disease.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone 1F9) was established by fusion of mouse myeloma cell SP2/0 with Balb/c mouse splenocyte immunized with the synthetic peptide corresponding to partial amino acid sequence of human ApoE4 (109-119 aa).

FORMULATION: This antibody is lyophilized form. Prepare a stock solution by dissolving the lyophilized antibody in 100 µL of distilled water. After reconstitution, the IgG concentration will be 1 mg/mL in PBS (pH 7.2)/ 1% sucrose. No preservative is contained.

STORAGE: This antibody is stable for one year from the date of shipment when stored at 4°C. After reconstitution, avoid repeated freezing and thawing. For storage, prepare appropriate aliquots and freeze them at -20°C.

REACTIVITY: This antibody reacts with human ApoE4 (35 kDa) on Western blotting, Immunoprecipitation and Immunohistochemistry. This antibody does not react with human ApoE2 and ApoE3.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; 5 µg/2 µL of human serum
Immunohistochemistry; 10 µg/mL
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Goat	Rabbit	Bovine
Reactivity	+	-	-	-	-	-

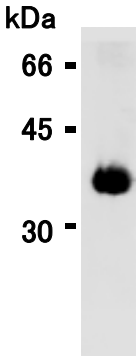
REFERENCES:

1) He, X., *et al., J. Neurosci.* **27**, 4052-4060 (2007)
2) Mann, K. M., *et al., Hum. Mol. Genet.* **13**, 1959-1968 (2004)
3) Yamauchi, K., *et al., Clin. Chem.* **45**, 497-504 (1999)
4) Coeder, E., *et al., Science* **261**, 921-923 (1993)
5) Saunders, A., *et al., Neurology* **43**, 1467-1472 (1993)

This antibody is used in reference number 1).

RELATED PRODUCTS:

M068-3	anti-Apolipoprotein E (3D12)
M046-3	anti-Amyloid β N-terminal specific (2C8)
M066-3	anti-Amyloid β/Amyloid precursor Protein (4E12)
M009-3	anti-Amyloid precursor Protein (3E9)
7635	ApoE4/Pan-ApoE ELISA Kit



Western blot analysis of Apolipoprotein E4 expression in human serum using M067-3.

PROTOCOLS:

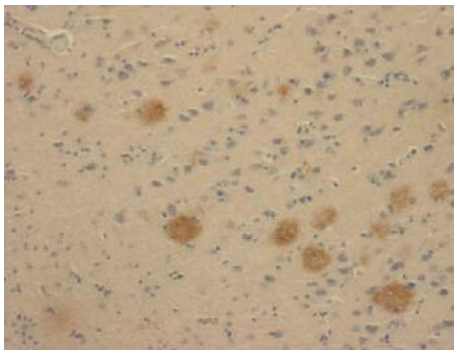
SDS-PAGE & Western Blotting

1) Boil the samples for 3-5 minutes and centrifuge. Load 0.5 µL of serum containing ApoE4 and electrophoresis in a 1 mm thick SDS-polyacrylamide gel.

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL https://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

2) 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.
3) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4^{o}C.
4) 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.)
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
6) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
7) Wash the membrane with PBS-T (5 minutes x 3 times).
8) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
9) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
10) Expose to an X-ray film in a dark room for 3 minutes.
11) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; human serum)



Immunohistochemical detection of Apolipoprotein E4 on cerebrum of Alzheimer's disease paraffin embedded section with M067-3.

Immunohistochemical staining for paraffin-embedded sections: SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Remove the slides from PBS and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS-T [0.05% Tween-20 in PBS] for 5 minutes each.
5) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; IMMUNOTECH, code no. IM-2391) for 5 minutes to block non-specific staining. Do not wash.
6) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the **APPLICATIONS**.
7) Incubate the sections for 1 hour at room temperature or over night at 4 ^{o}C.
8) Wash the slides 3 times in PBS-T for 10 minutes each.
9) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8).
10) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8).
11) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
14) Now ready for mounting.

(Positive control for Immunohistochemistry; cerebrum of Alzheimer's disease)

Immunoprecipitation

1) Add primary antibody as suggest in the **APPLICATIONS** into 2 µL of serum with 100 µL of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol).
2) Mix well and incubate with gentle agitation for 30-120 minutes at 4^{o}C.
3) Add 20 µL of 50% protein G agarose resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4^{o}C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)