

MONOCLONAL ANTIBODY

Anti-Apolipoprotein E (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M068-3	3D12	Mouse IgG2a	100 μ L	1 mg/mL

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 Apo E2, E3 and E4 coded for by three alleles of ϵ 2, ϵ 3 and ϵ 4 at a single gene locus respectively.

SOURCE: This antibody was purified from hybridoma (clone 3D12) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with Balb/c mouse splenocyte immunized with recombinant human apolipoprotein E3.

FORMULATION: 100 μ g IgG in 100 μ L 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 human apolipoprotein E2, E3 and E4 on Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting; 1 μ g/mL for chemiluminescence detection system

Immunoprecipitation; 5 μ g/2 μ L of human serum

Immunohistochemistry; Not tested*

*It is reported that clone 3D12 can be used in this application in the web site: Human Protein Atlas (<http://www.proteinatlas.org/index.php>)

Immunocytochemistry; Not tested

Flow cytometry; Not tested

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

INTENDED USE:

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

SPECIES CROSS REACTIVITY:

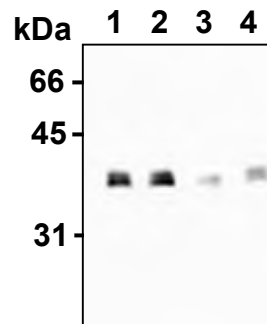
Species	Human	Mouse	Rat	Goat	Rabbit	Bovine
Reactivity on WB	+	-	-	-	-	-

REFERENCES:

- 1) Levy, O., *et al.*, *EMBO Mol. Med.* **7**, 211-226 (2015) [IHC]
- 2) Zhang, G., *et al.*, *Diagn. Pathol.* **9**, 200 (2014) [IHC]
- 3) Lindén, M., *et al.*, *BJU Int.* **112**, 407-415 (2013) [IHC]
- 4) Lindén, M., *et al.*, *Proteomics* **12**, 135-144 (2012) [WB, IHC]
- 5) He, X., *et al.*, *J. Neurosci.* **27**, 4052-4060 (2007) [WB]
- 6) Yamauchi, K., *et al.*, *Clin. Chem.* **45**, 497-504 (1999)

RELATED PRODUCTS:

- M067-3 Anti-Apolipoprotein E4 (Human) mAb (1F9)
- D273-3 Anti-ApoER2 (LA8) (Mouse) mAb (25G5)
- M076-3 Mouse IgG2a (isotype control) (6H3)
- 7635 ApoE4/Pan-ApoE ELISA Kit



Western blot analysis of Apolipoprotein E expression on human serum (1-4) using M068-3.

PROTOCOLS:

SDS-PAGE & Western Blotting

- 1) 1 μ L of human serum suspend with 10 μ L of Laemmli's sample buffer.
- 2) Boil the samples for 2 minutes and centrifuge. Load 10 μ L of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 3) 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 manufacturer's manual for precise transfer procedure.

- 4) 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°C.
- 5) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 7) Incubate the membrane with 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.
- 8) Wash the membrane with PBS-T (5 minutes x 3 times).
- 9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
- 10) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 11) Expose to an X-ray film in a dark room for 5 minutes.
- 12) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Human serum)

Immunoprecipitation

- 1) Add 5 µg of antibody into 2 µL of serum with 100 µL of cold Lysis buffer [50 mM HEPES-KOH (pH 7.5), 250 mM NaCl, 0.1% NP-40, 5 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Mix well and incubate it at 4°C with rotating for 60 minutes.
- 2) Add 20 µL of 50% protein A agarose beads into the tube. Mix well and incubate with gentle agitation for 30-60 minutes at 4°C.
- 3) Wash the beads 3-5 times with ice-cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 4) Resuspend the beads in 30 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 15 µL/lane for the SDS-PAGE analysis.

(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; Human serum)