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

MONOCLONAL ANTIBODY

Anti-Apolipoprotein E (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M068-3	3D12	Mouse IgG2a	100 µL	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 ε2, ε3 and ε4 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 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 human apolipoprotein E2, E3 and E4 on Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; 5 µg/2 µL 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:

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SPECIES CROSS REACTIVITY:

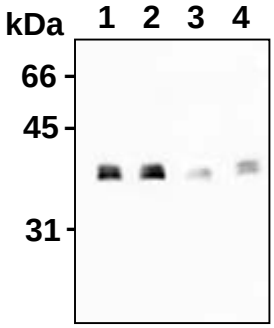
Species	Human	Mouse	Rat	Goat	Rabbit	Bovine
Reactivity on WB	+	-	-	-	-	-

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 µL of human serum suspend with 10 µL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 10 µL 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/cm^2 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)