Datasheet for ABIN5539967

anti-APOE antibody (AA 109-119)

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Overview

Quantity:	0.1 mg
Target:	APOE
Binding Specificity:	AA 109-119
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This APOE antibody is un-conjugated
Application:	Western Blotting (WB), Immunoprecipitation (IP), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p))

Product Details

Immunogen:	Synthetic peptide corresponding to partial amino acid sequence of human ApoE4 (109-119 aa)
Clone:	1F9
Isotype:	IgG1
Specificity:	This antibody reacts with human ApoE4 (35 kDa). I does not react with human ApoE2 and ApoE3.
Cross-Reactivity (Details):	Does not react with Mouse, Rat, Goat, Rabbit, and Bovine.

Target Details

Target:	APOE

Target Details

Alternative Name:	apolipoprotein e,apo e (APOE Products)
Background:	Apolipoprotein E (ApoE), a 35 kDa plasma protein containing sialic acid, plays a role in triglyceride, cholesterol tr ansport 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 ε 2, ε 3 and ε 4 at a single gene locus respectively. Reportedly ApoE represents an important risk marker for Alzheimer's disease.
UniProt:	P02649
Pathways:	Regulation of Cell Size, Lipid Metabolism

Application Details

Application Notes:	Western blot: 1 µg/mL for chemiluminescence detection system. Immunoprecipitation: 5 µg/2 µL of human serum. Immunohistochemistry on paraffin sections: 10 µg/mL. For details see protocol below.
Protocol:	SDS-PAGE & Western Blotting 1) Boil the samples for 3-5 minutes and centrifuge. Load 0.5 µ L of serum containing ApoE4 and electrophoresis in a 1 mm thick SDS-polyacrylamide gel. 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 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. 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 2 O 2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS-T [0.05 % Tween-20 in PB S] for 5 minutes each. 5)

Application Details

	Remove the slides from PB S, wipe gently around each section and cover tissues with Protein Blocking Agent 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. Incubate for 10 minutes at room temperature. Wash as in step 8). 10) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase. 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 µ L of 30 % H 2 O 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 µ L of serum with 100 µ L 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 µ L 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 µ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µ L/lane for the SDS-PAGE analysis. (See SDS-PAGE & Western blotting.)
Restrictions:	For Research Use only

Handling

Buffer:	Buffer System: PBS (pH 7.2) containing 1 % sucrose. No preservative is contained
Preservative:	Azide free
Storage:	4 °C,-20 °C
Storage Comment:	Prior to reconstitution store at 2-8°C. Following reconstitution store (in aliquots) at -20°C. Avoid repeated freezing and thawing. Shelf life: one year from despatch.
Expiry Date:	12 months