abcam

Product datasheet

Anti-Apolipoprotein E antibody [EP1374Y] ab52607

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Apolipoprotein E antibody [EP1374Y]
Description	Rabbit monoclonal [EP1374Y] to Apolipoprotein E
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK293, HAP1, and HepG2 cell lysate, Human liver, cerebellum and serum lysate; Flow Cyt (intra): HepG2 cells; IP: HepG2 and HAP1 lysates, and Human serum; ICC/IF: HepG2 cell lysate; IHC: Astrocytoma tissue.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 59% PBS, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EP1374Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab52607 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/20.
WB	★★★★☆ (1)	1/1000 - 1/10000. Detects a band of approximately 36 kDa (predicted molecular weight: 36 kDa).
IP		1/20.
IHC-P		1/800. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF	★★★★☆ (1)	1/50.

Target

Function Mediates the binding, internalization, and catabolism of lipoprotein particles. It can serve as a ligand for the LDL (apo B/E) receptor and for the specific apo-E receptor (chylomicron remnant) of hepatic tissues.

Tissue specificity Occurs in all lipoprotein fractions in plasma. It constitutes 10-20% of very low density lipoproteins (VLDL) and 1-2% of high density lipoproteins (HDL). APOE is produced in most organs. Significant quantities are produced in liver, brain, spleen, lung, adrenal, ovary, kidney and muscle.

Involvement in disease Defects in APOE are a cause of hyperlipoproteinemia type 3 (HLPP3) [MIM:107741]; also known as familial dysbetalipoproteinemia. Individuals with HLPP3 are clinically characterized by xanthomas, yellowish lipid deposits in the palmar crease, or less specific on tendons and on elbows. The disorder rarely manifests before the third decade in men. In women, it is usually expressed only after the menopause. The vast majority of the patients are homozygous for APOE*2 alleles. More severe cases of HLPP3 have also been observed in individuals heterozygous for rare APOE variants. The influence of APOE on lipid levels is often suggested to have major implications for the risk of coronary artery disease (CAD). Individuals carrying the common APOE*4 variant are at higher risk of CAD.
Genetic variations in APOE are associated with Alzheimer disease type 2 (AD2) [MIM:104310]. It is a late-onset neurodegenerative disorder characterized by progressive dementia, loss of cognitive abilities, and deposition of fibrillar amyloid proteins as intraneuronal neurofibrillary tangles, extracellular amyloid plaques and vascular amyloid deposits. The major constituent of these plaques is the neurotoxic amyloid-beta-APP 40-42 peptide (s), derived proteolytically from the transmembrane precursor protein APP by sequential secretase processing. The cytotoxic C-terminal fragments (CTFs) and the caspase-cleaved products such as C31 derived from APP, are also implicated in neuronal death. Note=The APOE*4 allele is genetically associated with the common late onset familial and sporadic forms of Alzheimer disease. Risk for AD increased from 20% to 90% and mean age at onset decreased from 84 to 68 years with increasing number of

APOE*4 alleles in 42 families with late onset AD. Thus APOE*4 gene dose is a major risk factor for late onset AD and, in these families, homozygosity for APOE*4 was virtually sufficient to cause AD by age 80. The mechanism by which APOE*4 participates in pathogenesis is not known.
Defects in APOE are a cause of sea-blue histiocyte disease (SBHD) [MIM:269600]; also known as sea-blue histiocytosis. This disorder is characterized by splenomegaly, mild thrombocytopenia and, in the bone marrow, numerous histiocytes containing cytoplasmic granules which stain bright blue with the usual hematologic stains. The syndrome is the consequence of an inherited metabolic defect analogous to Gaucher disease and other sphingolipidoses.
Defects in APOE are a cause of lipoprotein glomerulopathy (LPG) [MIM:611771]. LPG is an uncommon kidney disease characterized by proteinuria, progressive kidney failure, and distinctive lipoprotein thrombi in glomerular capillaries. It mainly affects people of Japanese and Chinese origin. The disorder has rarely been described in Caucasians.

Sequence similarities

Belongs to the apolipoprotein A1/A4/E family.

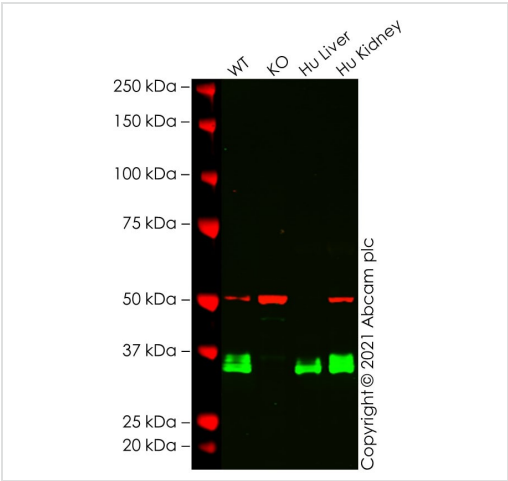
Post-translational modifications

Synthesized with the sialic acid attached by O-glycosidic linkage and is subsequently desialylated in plasma. O-glycosylated with core 1 or possibly core 8 glycans. Thr-307 is a minor glycosylation site compared to Ser-308.
Glycated in plasma VLDL of normal subjects, and of hyperglycemic diabetic patients at a higher level (2-3 fold).
Phosphorylation sites are present in the extracelllular medium.

Cellular localization

Secreted.

Images



Western blot - Anti-Apolipoprotein E antibody [EP1374Y] (ab52607)

All lanes : Anti-Apolipoprotein E antibody [EP1374Y] (ab52607) at 1/1000 dilution

Lane 1 : Wild-type HepG2 cell lysate
Lane 2 : APOE knockout HepG2 cell lysate
Lane 3 : Human Liver cell lysate
Lane 4 : Human Kidney cell lysate

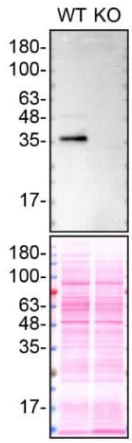
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 36 kDa
Observed band size: 34 kDa

False colour image of Western blot: Anti-Apolipoprotein E antibody [EP1374Y] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab52607 was shown to bind specifically to Apolipoprotein E. A band was observed at 34-37

kDa in wild-type HepG2 cell lysates with no signal observed at this size in APOE knockout cell line. To generate this image, wild-type and APOE knockout HepG2 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-Apolipoprotein E antibody [EP1374Y] (ab52607)

All lanes : Anti-Apolipoprotein E antibody [EP1374Y] (ab52607) at 1/1000 dilution

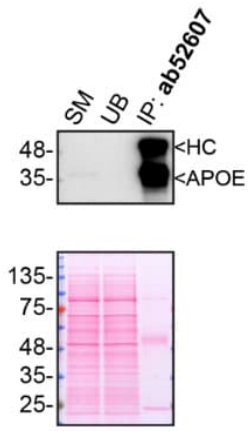
Lane 1 : Wild-type HAP1 lysate
Lane 2 : APOE Knockout HAP1 lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 36 kDa
Observed band size: 36 kDa

ab52607 was shown to react with APOE in wild-type HAP1 cells in Western blot with loss of signal observed in a APOE knockout cell line. Wild-type HAP1 and APOE knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab52607 overnight at 4 °C at a 1/1000 dilution. Blots were incubated with secondary antibodies at 0.2ug/mL before imaging.

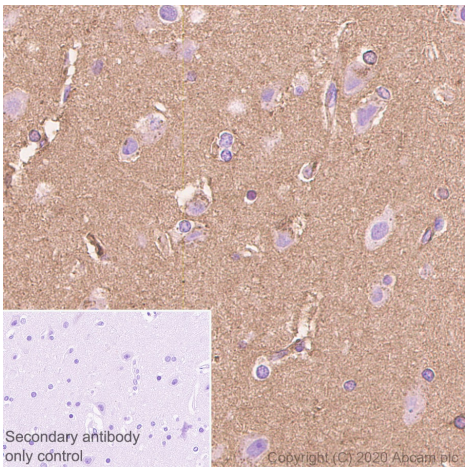
These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.



Immunoprecipitation - Anti-Apolipoprotein E antibody [EP1374Y] (ab52607)

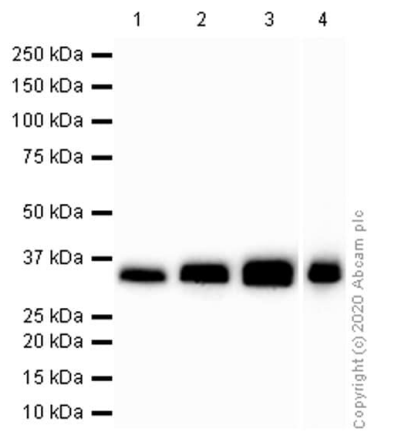
Immunoprecipitation of APOE in HAP1 cells. Lysates were prepared and immunoprecipitation was performed using 2.0 µg of ab52607 pre-coupled to Protein A beads. Samples were then washed and processed for western blot.

These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Apolipoprotein E antibody [EP1374Y] (ab52607)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human astrocytoma tissue sections labeling Apolipoprotein E with purified ab52607 at 1/800 dilution (0.13 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



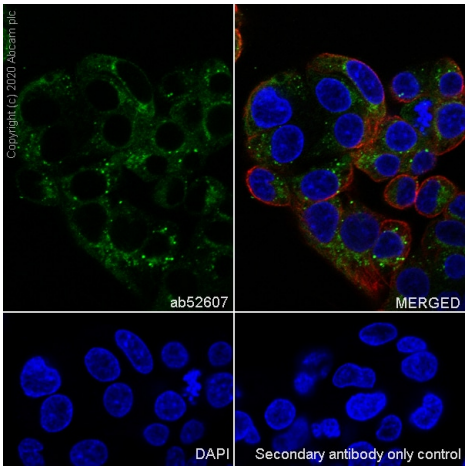
Western blot - Anti-Apolipoprotein E antibody [EP1374Y] (ab52607)

All lanes : Anti-Apolipoprotein E antibody [EP1374Y] (ab52607) at 1/10000 dilution (Purified)

Lane 1 : Human liver lysate at 20 µg
Lane 2 : Human cerebellum lysate at 20 µg
Lane 3 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate at 20 µg
Lane 4 : Human serum lysate at 15 µg

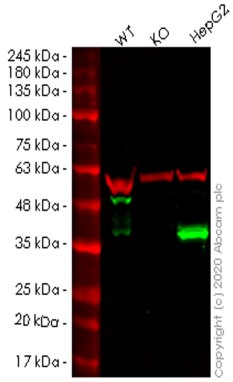
Secondary
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 36 kDa



Immunocytochemistry/ Immunofluorescence - Anti-Apolipoprotein E antibody [EP1374Y] (ab52607)

Immunocytochemistry analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling Apolipoprotein E with Purified ab52607 at 1:50 dilution (2.0 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488,**ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-Apolipoprotein E antibody [EP1374Y] (ab52607)

All lanes : Anti-Apolipoprotein E antibody [EP1374Y] (ab52607) at 1/1000 dilution (Unpurified)

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : APOE knockout HEK293T cell lysate
Lane 3 : HepG2 cell lysate

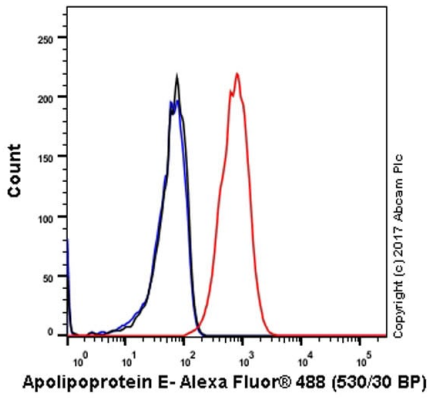
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 36 kDa
Observed band size: 36 kDa

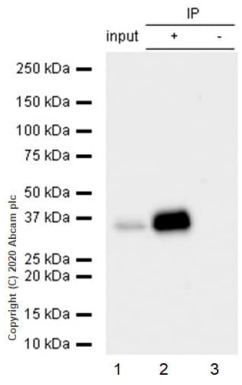
Lanes 1-3: Merged signal (red and green). Green - ab52607 observed at 36 kDa. Red - loading control **ab7291** observed at 50 kDa.

ab52607 Anti-Apolipoprotein E antibody [EP1374Y] was shown to specifically react with Apolipoprotein E in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab266546** (knockout cell lysate **ab256838**) was used. Wild-type and Apolipoprotein E knockout samples were subjected to SDS-PAGE. ab52607 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated at room temperature for 2. 5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



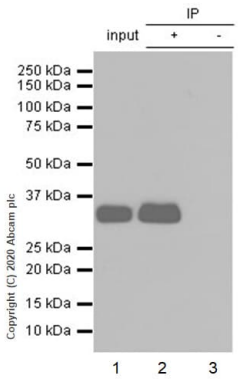
Flow Cytometry (Intracellular) - Anti-Apolipoprotein E antibody [EP1374Y] (ab52607)

Intracellular Flow Cytometry analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling Apolipoprotein E (red) with purified ab52607 (Unpurified) at a 1/250 dilution (10ug/mL). Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti rabbit IgG (Alexa Fluor®488) (**ab150077**) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (Black) (**ab172730**). Blue (unlabeled control) - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunoprecipitation - Anti-Apolipoprotein E antibody [EP1374Y] (ab52607)

Purified ab52607 at 1/20 dilution (0.5µg) immunoprecipitating Apolipoprotein E in HepG2 whole cell lysate.
Lane 1 (input): HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab52607 + HepG2 whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab52607 in HepG2 whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/5000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 36 kDa



Immunoprecipitation - Anti-Apolipoprotein E antibody [EP1374Y] (ab52607)

Purified ab52607 at 1/20 dilution (0.5µg) immunoprecipitating Apolipoprotein E in Human serum.

Lane 1 (input): Human serum 10µg

Lane 2 (+): ab52607 + Human serum.

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab52607 in Human serum.

VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/1000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 36 kDa

Anti-Apolipoprotein E antibody [EP1374Y] (ab52607)

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