abcam

Product datasheet

Anti-ABCA1 antibody [HJ1] ab66217

敲除验证

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概述

产品名称	Anti-ABCA1抗体[HJ1]
描述	**小鼠单克隆抗体**[HJ1] to ABCA1
宿主	Mouse
经测试应用	**适用于:** WB, Flow Cyt, IHC-P
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Recombinant fragment corresponding to ABCA1 (N terminal). 50 kDa N-terminal extracellular loop of ABCA1 Database link: **O95477**
阳性对照	IHC-P: Liver parenchyma and liver tissue. Flow: HepG2 cells. WB: Hap1 whole cell lysate.
常规说明	This monoclonal antibody to ABCA1 has been knockout validated in Western blot. The expected band for ABCA1 was observed in wild type cells and the band was not seen in knockout cells, although other non-specific bands were also seen. The data are shown on this datasheet. This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

性能

形式	Liquid
存放说明	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
存储溶液	pH: 7.40 Preservative: 0.02% Sodium azide

	Constituents: PBS, 6.97% L-Arginine Some batches contain 6.97% L-Arginine as a stabilizing agent. For lot-specific buffer information, please contact our Scientific Support team.
纯度	Protein G purified
克隆	单克隆
克隆编号	HJ1
同种型	IgG2b
轻链类型	kappa

应用

The Abpromise guarantee Abpromise™承诺保证使用ab66217于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

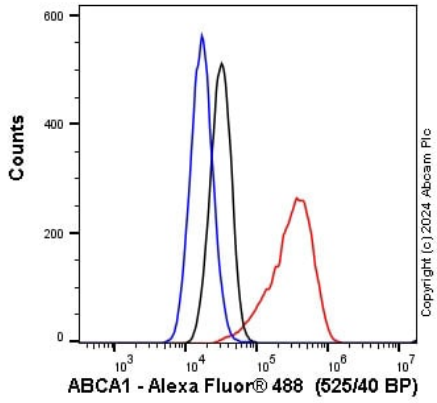
应用	Ab评论	说明
WB	★★☆☆☆ (1)	Use a concentration of 1 µg/ml. Detects a band of approximately 254 kDa (predicted molecular weight: 254 kDa). Abcam recommends using 3% Milk as the blocking agent.
Flow Cyt	★★★☆☆ (1)	Use 2µg for 10^6 cells. (methanol fixed cells) **ab170192** - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.
IHC-P		Use a concentration of 0.2 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

靶标

功能	cAMP-dependent and sulfonylurea-sensitive anion transporter. Key gatekeeper influencing intracellular cholesterol transport.
组织特异性	Widely expressed, but most abundant in macrophages.
疾病相关	Defects in ABCA1 are a cause of high density lipoprotein deficiency type 1 (HDLD1) [MIM:205400]; also known as analphalipoproteinemia or Tangier disease (TGD). HDLD1 is a recessive disorder characterized by absence of high density lipoprotein (HDL) cholesterol from plasma, accumulation of cholesteryl esters, premature coronary artery disease (CAD), hepatosplenomegaly, recurrent peripheral neuropathy and progressive muscle wasting and weakness. Defects in ABCA1 are a cause of high density lipoprotein deficiency type 2 (HDLD2) [MIM:604091]; also known as familial hypoalphalipoproteinemia (FHA). HDLD2 is inherited as autosomal dominant trait. It is characterized by moderately low HDL cholesterol, predilection toward premature coronary artery disease (CAD) and a reduction in cellular cholesterol efflux.
序列相似性	Belongs to the ABC transporter superfamily. ABCA family. Contains 2 ABC transporter domains.

结构域	Multifunctional polypeptide with two homologous halves, each containing an hydrophobic membrane-anchoring domain and an ATP binding cassette (ABC) domain.
翻译后修饰	Phosphorylation on Ser-2054 regulates phospholipid efflux. Palmitoylation by DHHC8 is essential for membrane localization.
细胞定位	Membrane.

图片



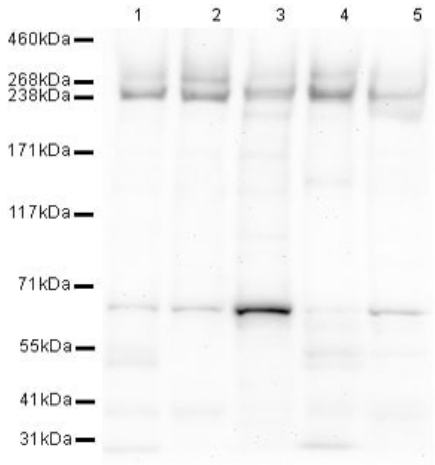
Flow Cytometry (Intracellular) - Anti-ABCA1 antibody [HJ1] (ab66217)

Flow cytometry overlay histogram showing Hep G2 cells stained with ab66217 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were incubated in 1x PBS containing 10%; normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab66217) (1x 10^6 in 100 µl at 5.0 µg/ml (1/0)) for 30 min at 22°C.

The secondary antibody Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Mouse IgG2b, kappa monoclonal [7E10G10] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Western blot - Anti-ABCA1 antibody [HJ1] (ab66217)

All lanes : Anti-ABCA1 antibody [HJ1] (ab66217) at 1 µg/ml

Lane 1 : Brain (Mouse) Tissue Lysate
Lane 2 : Brain (Rat) Tissue Lysate
Lane 3 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
Lane 4 : Liver (Mouse) Tissue Lysate
Lane 5 : Liver (Rat) Tissue Lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat polyclonal Secondary Antibody to Mouse IgG - H&L (HRP), pre-adsorbed at 1/5000 dilution

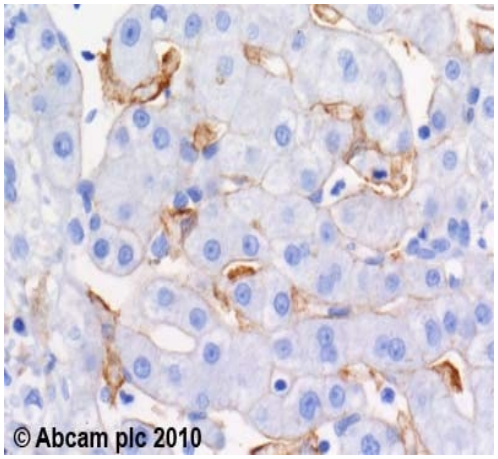
Performed under reducing conditions.

Predicted band size: 254 kDa

Observed band size: 254 kDa
Additional bands at: 70 kDa. We are unsure as to the identity of these extra bands.

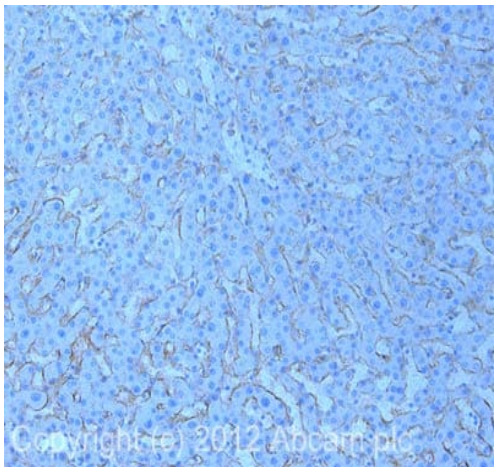
Exposure time: 4 minutes

Abcam recommends using milk as the blocking agent. This blot was produced using a 3-8% Tris Acetate gel under the TA buffer system. The gel was run at 150V for 60 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% Milk before being incubated with ab66217 overnight at 4°C. Antibody binding was detected using an anti-mouse antibody conjugated to HRP, and visualised using ECL development solution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ABCA1 antibody [HJ1] (ab66217)

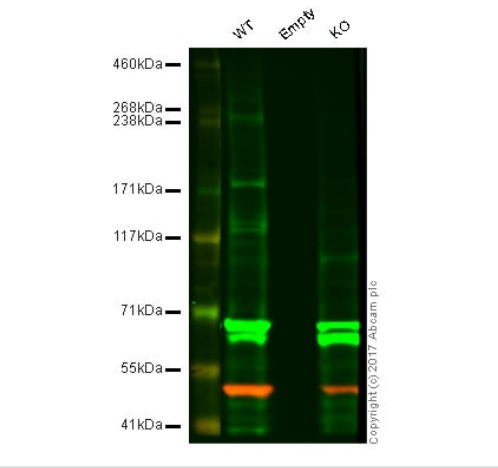
ab66217 (4µg/ml) staining ABCA1 in human liver using an automated system (DAKO Autostainer Plus). Using this protocol there is moderate cell membrane staining throughout the liver parenchyma.
Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer EDTA pH 9.0 in a DAKO PT link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ABCA1 antibody [HJ1] (ab66217)

IHC image of ABCA1 staining in human liver formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab66217, 0.2µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Western blot - Anti-ABCA1 antibody [HJ1] (ab66217)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: Empty
Lane 3: ABCA1 knockout (KO) HAP1 whole cell lysate (20 µg)

Lanes 1 - 3: Merged signal (red and green). Green - ab66217 observed at 240 kDa. Red - loading control, **ab176560**, observed at 50 kDa.

ab66217 detected the expected band for ABCA1 in wild type HAP1 cells and the band was not seen in ABCA1 knockout HAP1 cells, although additional non-specific bands were also seen. Wild-type and ABCA1 knockout samples were subjected to SDS-PAGE. Ab66217 and **ab176560** (Rabbit anti alpha Tubulin loading control) were incubated overnight at 4°C at 1 µg/ml and 1/10000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed **ab216772** and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed **ab216777** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

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