

Product datasheet

Anti-ABCA1 antibody [HJ1] ab66217

KO VALIDATED

★★★★☆ [3 Abreviews](#) [15 References](#) [5 Images](#)

Overview

Product name	Anti-ABCA1 antibody [HJ1]
Description	Mouse monoclonal [HJ1] to ABCA1
Host species	Mouse
Tested applications	Suitable for: WB, Flow Cyt, IHC-P
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Recombinant fragment corresponding to ABCA1 (N terminal). 50 kDa N-terminal extracellular loop of ABCA1 Database link: O95477
Positive control	IHC-P: Liver parenchyma and liver tissue. Flow: HepG2 cells. WB: Hap1 whole cell lysate.
General notes	<p>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.</p> <p>This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com.</p> <p>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.</p> <p>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</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer	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.

Purity	Protein G purified
Clonality	Monoclonal
Clone number	HJ1
Isotype	IgG2b
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab66217 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
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.

Target

Function cAMP-dependent and sulfonyleurea-sensitive anion transporter. Key gatekeeper influencing intracellular cholesterol transport.

Tissue specificity Widely expressed, but most abundant in macrophages.

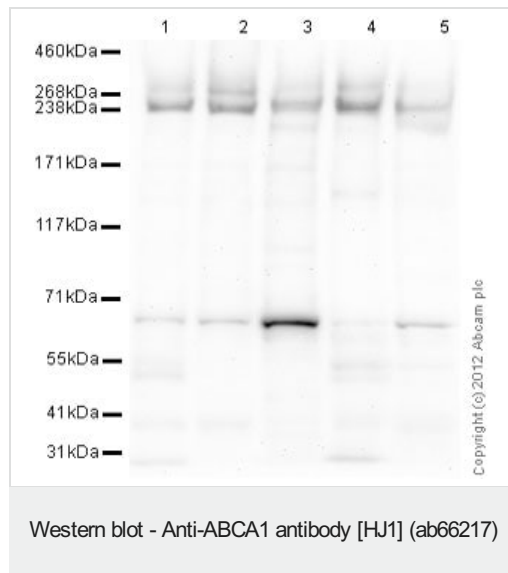
Involvement in disease Defects in ABCA1 are a cause of high density lipoprotein deficiency type 1 (HDL1) [MIM:205400]; also known as analphalipoproteinemia or Tangier disease (TGD). HDL1 is a recessive disorder characterized by absence of high density lipoprotein (HDL) cholesterol from plasma, accumulation 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 (HDL2) [MIM:604091]; also known as familial hypoalphalipoproteinemia (FHA). HDL2 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.

Sequence similarities Belongs to the ABC transporter superfamily. ABCA family.
Contains 2 ABC transporter domains.

Domain	Multifunctional polypeptide with two homologous halves, each containing an hydrophobic membrane-anchoring domain and an ATP binding cassette (ABC) domain.
Post-translational modifications	Phosphorylation on Ser-2054 regulates phospholipid efflux. Palmitoylation by DHHC8 is essential for membrane localization.
Cellular localization	Membrane.

Images



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.

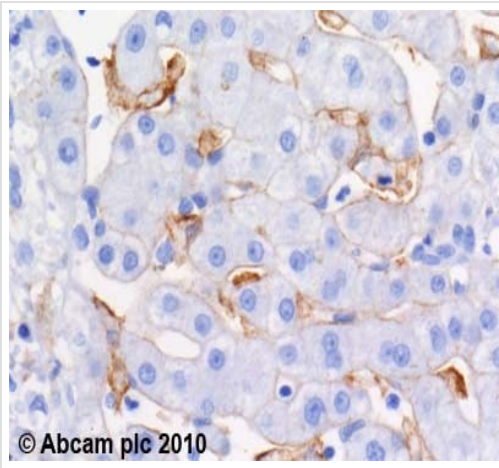
Predicted band size: 254 kDa

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

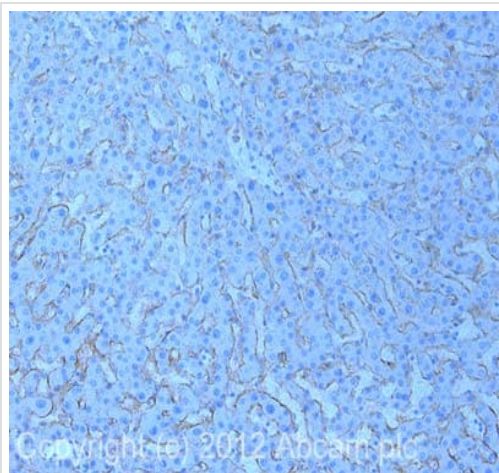
Abcam recommends using milk as the blocking agent. This blot was produced using a 3-8% Tris Acetate gel under the TA buffer system. The gel was run at 150V for 60 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% 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.

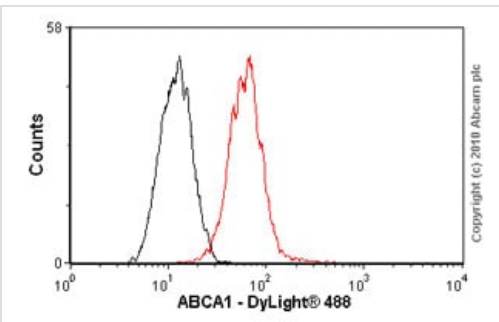
Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer EDTA pH 9.0 in a DAKO PT link. Slides were peroxidase blocked in 3% H₂O₂ in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody 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.

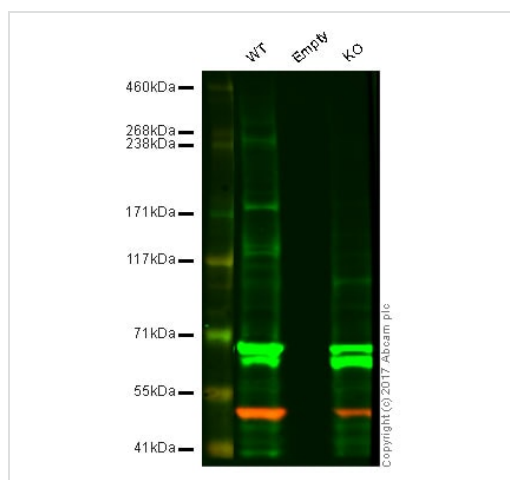


Flow Cytometry - Anti-ABCA1 antibody [HJ1] (ab66217)

Overlay histogram showing HepG2 cells stained with ab66217 (red line). The cells were fixed with methanol (5 min) and incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab66217, 2µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 2µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a decreased signal in HepG2 cells fixed with 4% paraformaldehyde

(10 min) used under the same conditions.

Please note that Abcam does not have data for use of this antibody on non-fixed cells. We welcome any customer feedback.



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.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <https://www.abcam.com/abpromise> or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors