abcam

ab66217

Anti-ABCA1 antibody [HJ1]

KO Validated

Key facts

Isotype	IgG2b
Host species	Mouse
Storage buffer	pH: 7.4 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	HJ1
Purification technique	Affinity purification
Light chain type	kappa
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	0.2 µg/mL

Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	2 µg for 10^6 Cells
Notes	(methanol fixed cells) ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1 µg/mL
Notes	Abcam recommends using 3% Milk as the blocking agent.

Species	Rat
Dilution info	1 µg/mL
Notes	Abcam recommends using 3% Milk as the blocking agent.

Species	Human

Dilution info	1 µg/mL
Notes	Abcam recommends using 3% Milk as the blocking agent.

Target data

See full target information ABCA1

Function	Catalyzes the translocation of specific phospholipids from the cytoplasmic to the extracellular/lumenal leaflet of membrane coupled to the hydrolysis of ATP (PubMed:24097981, PubMed:35974019). Thereby, participates in phospholipid transfer to apolipoproteins to form nascent high density lipoproteins/HDLs (PubMed:14754908). Transports preferentially phosphatidylcholine over phosphatidylserine (PubMed:24097981). May play a similar role in the efflux of intracellular cholesterol to apolipoproteins and the formation of nascent high density lipoproteins/HDLs (PubMed:10533863, PubMed:14754908, PubMed:24097981, PubMed:35974019). Translocates phospholipids from the outer face of the plasma membrane and forces it through its gateway and annulus into an elongated hydrophobic tunnel in its extracellular domain (PubMed:35974019).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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.

Want a custom formulation?
This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The ABCA1 protein also known as ATP-binding cassette transporter A1 plays an important role in lipid transport processes. It helps in the export of cholesterol and phospholipids to apolipoprotein A-I which is essential for high-density lipoprotein (HDL) formation. The ABCA1 molecular weight is approximately 220 kDa. This protein is widely expressed in various tissues including the liver lungs and intestines indicating its systemic role in lipid metabolism. Researchers often study ABCA1 using techniques like ABCA1 ELISA and H10 flow cytometry.
Biological function summary	ABCA1 functions as a cholesterol efflux pump in the cellular membrane. It facilitates the transfer of cholesterol to lipid-poor apolipoproteins such as apoA-I which then forms HDL particles. This active process helps maintain cellular cholesterol homeostasis and reduces cholesterol accumulation. ABCA1 is a critical component of the HDL synthesis pathway and does not form part of a larger protein complex acting more as an individual unit in this function.
Pathways	Many regulatory mechanisms control the activity of ABCA1. It plays a significant role in the reverse cholesterol transport pathway thereby promoting the movement of cholesterol away from peripheral tissues back to the liver for excretion. This pathway helps maintain systemic lipid balance. The LXR/RXR pathway regulates ABCA1 expression aligning it closely with other proteins such as LXR and ABCG1 which also deal with cholesterol metabolism.
Associated diseases and disorders	ABCA1 is intimately connected with atherosclerosis and Tangier disease. Dysfunction or mutations in the ABCA1 gene lead to impaired cholesterol efflux contributing to cholesterol build-up and atherosclerotic plaque formation. Tangier disease a rare genetic disorder arises from severe mutations in ABCA1 resulting in very low levels of HDL cholesterol. This protein's role in disease is often studied alongside proteins such as apoA-I and LDL receptors which influence cholesterol transportation and storage.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

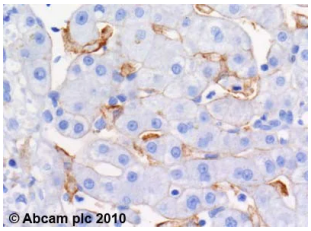
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

7 product images

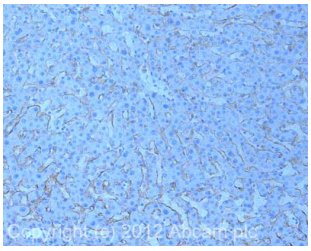


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ABCA1 antibody [HJ1] (ab66217)

ABCA1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-ABCA1 antibody

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.

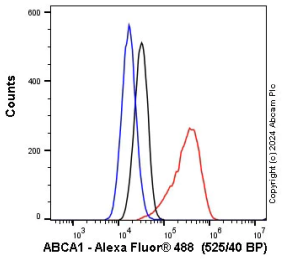


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ABCA1 antibody [HJ1] (ab66217)

ABCA1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-ABCA1 antibody

IHC image of ABCA1 staining in human liver formalin fixed paraffin embedded tissue section, performed on a Leica Bond^{TM} 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 (Intracellular) - Anti-ABCA1 antibody [HJ1] (ab66217)

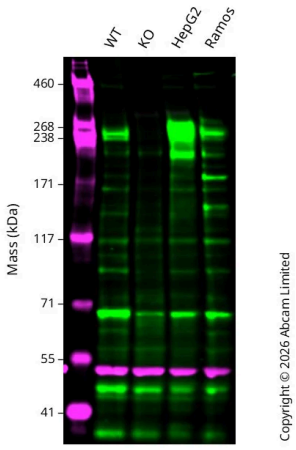
ABCA1 Flow Cytometry (Intracellular) staining using mouse Anti-ABCA1 antibody

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 106 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)

ABCA1 Western blot staining using mouse Anti-ABCA1 antibody

Western blot: Anti-ABCA1 antibody [HJ1] ab66217 staining at 1/1000 dilution, shown in green; Rabbit anti alpha Tubulin ab52866 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 254 kDa in Wild-type HAP1 UNBOILED cell lysates with no signal observed at this size in ABCA1 knockout HAP1 UNBOILED cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% 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-Mouse 800CW and Goat anti-Rabbit 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-ABCA1 antibody [HJ1] (ab66217) at 1/1000 dilution

Lane 1:
Wild-type HAP1 UNBOILED at 20 µg

Lane 2:
ABCA1 knockout HAP1 UNBOILED at 20 µg

Lane 3:
HepG2 UNBOILED at 20 µg

Lane 4:
Ramos UNBOILED at 20 µg

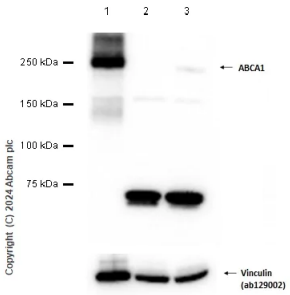
Secondary

All lanes:
Goat anti-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 254 kDa

Observed band size: 254 kDa



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

ABCA1 Western blot staining using mouse Anti-ABCA1 antibody

Blocking and diluting buffer: 5% NFDM/TBST.

ab129002 was used as a loading control at 1/10000 dilution, followed by a secondary antibody ab97051 at 1/20000 dilution.

Negative control: 293T (PMID:11896206), SH-SY5Y (PMID: 23600914).

Samples are non-boiled as boiling may cause protein aggregates.

All lanes:
Western blot - Anti-ABCA1 antibody [HJ1] (ab66217) at 1/1000 dilution

Lane 1:
U-87 MG (human glioblastoma-astrocytoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 3:
SH-SY5Y (human neuroblastoma epithelial cell) whole cell lysate at 20 µg

Secondary

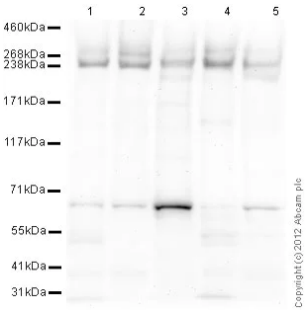
Lanes 1 - 3:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Lanes 1 - 3:
Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/5000 dilution

Predicted band size: 254 kDa

Observed band size: 254 kDa

Exposure time: 10s



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

ABCA1 Western blot staining using mouse Anti-ABCA1 antibody

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.
All lanes:
Western blot - Anti-ABCA1 antibody [HJ1] (ab66217) at 1 µg/mL

Lane 1:
Brain (Mouse) Tissue Lysate at 20 µg

Lane 2:
Brain (Rat) Tissue Lysate at 20 µg

Lane 3:
HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate at 20 µg

Lane 4:
Liver (Mouse) Tissue Lysate at 20 µg

Lane 5:
Liver (Rat) Tissue Lysate at 20 µg

Secondary

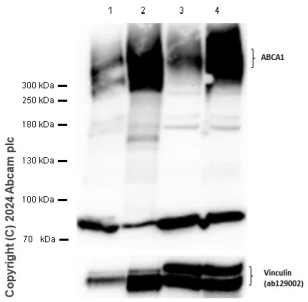
All lanes:
Goat polyclonal Secondary Antibody to Mouse IgG - H&L (HRP), pre-adsorbed at 1/5000 dilution

Performed under reducing conditions.

Predicted band size: 254 kDa

Observed band size: 254 kDa, 70 kDa

Exposure time: 4min



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

ABCA1 Western blot staining using mouse Anti-ABCA1 antibody

Blocking and diluting buffer: 5% NFDM/TBST.

ab129002 was used as a loading control at 1/10000 dilution, followed by a secondary antibody ab97051 at 1/20000 dilution.

The higher band is probably glycosylated form (PMID: 16873719).

All lanes:
Western blot - Anti-ABCA1 antibody [HJ1] (ab66217) at 1/1000 dilution

Lane 1:
Untreated THP-1 (Human monocytic leukemia monocyte) whole cell lysate at 20 µg

Lane 2:
THP-1 treated first with 100ng/ml PAM for 72 hours and then replaced with 100ng/ml LPS for 5 hours, 300ng/ml BFA was then added for additional 3 hours whole cell lysate at 20 µg

Lane 3:
Untreated HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 4:
HepG2 treated with 20uM T0901317 for 16 hours whole cell lysate at 20 µg

Secondary

Lanes 1 - 3:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Lanes 1 - 3:
Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/5000 dilution

Predicted band size: 254 kDa

Observed band size: 254 kDa, 400 kDa

Exposure time: 20s

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