abcam

Product datasheet

Anti-ALIX antibody ab88388

KO VALIDATED

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Overview

Product name	Anti-ALIX antibody
Description	Rabbit polyclonal to ALIX
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat, Orangutan
Immunogen	Synthetic peptide corresponding to Human ALIX aa 300-400 conjugated to keyhole limpet haemocyanin. (Peptide available as **ab89369**)
Positive control	This antibody gave a positive signal in the following whole cell lysates: HeLa; HepG2; HEK-293, SHSY5Y; HUVEC. ICC: Hek293 cell line ICC KO: HEK293 (HEK293-ALIX KO used as a negative cell line)
General notes	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

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab88388 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	★★★★★ (2)	Use a concentration of 1 µg/ml. Detects a band of approximately 100 kDa (predicted molecular weight: 96 kDa).
ICC		Use a concentration of 5 µg/ml.

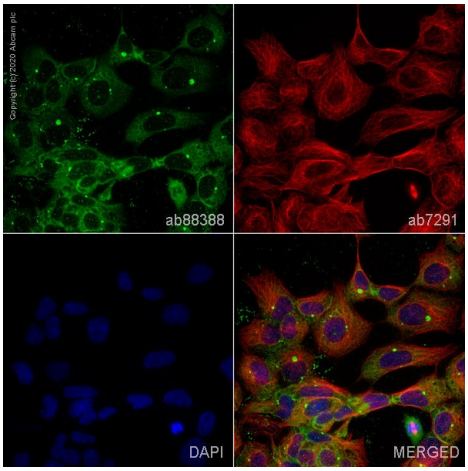
Target

Function Class E VPS protein involved in concentration and sorting of cargo proteins of the multivesicular body (MVB) for incorporation into intralumenal vesicles (ILVs) that are generated by invagination and scission from the limiting membrane of the endosome. Binds to the phospholipid lysobisphosphatidic acid (LBPA) which is abundant in MVBs internal membranes. The MVB pathway appears to require the sequential function of ESCRT-O, -I,-II and -III complexes. The ESCRT machinery also functions in topologically equivalent membrane fission events, such as the terminal stages of cytokinesis and enveloped virus budding (HIV-1 and other lentiviruses). Appears to be an adapter for a subset of ESCRT-III proteins, such as CHMP4, to function at distinct membranes. Required for completion of cytokinesis. Involved in HIV-1 virus budding. Can replace TSG101 it its role of supporting HIV-1 release; this function implies the interaction with CHMP4B. May play a role in the regulation of both apoptosis and cell proliferation.

Sequence similarities Contains 1 BRO1 domain.

Cellular localization Cytoplasm > cytosol. Melanosome. Cytoplasm > cytoskeleton > centrosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV. Colocalized with CEP55 in the midbody during cytokinesis. Colocalized with CEP55 at centrosomes of non-dividing cells.

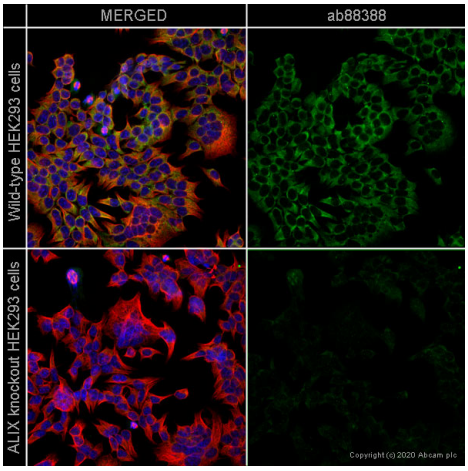
Images



Immunocytochemistry - Anti-ALIX antibody (ab88388)

ab88388 staining ALIX in Hek293 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab88388 at 5µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

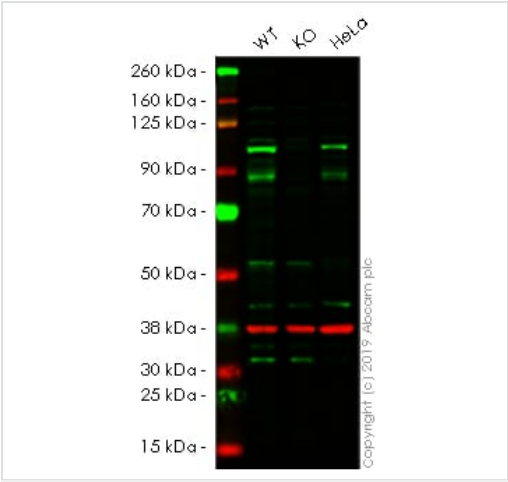
Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Immunocytochemistry - Anti-ALIX antibody (ab88388)

ab88388 staining ALIX in wild-type HEK293 cells (top panel) and ALIX knockout HEK293 cells (bottom panel). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab88388 at 5µg/ml concentration and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a high-content analysis system (Perkin Elmer, Operetta CLS™).



Western blot - Anti-ALIX antibody (ab88388)

All lanes : Anti-ALIX antibody (ab88388) at 1 µg/ml

Lane 1 : Wild-type HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 2 : ALIX knockout HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

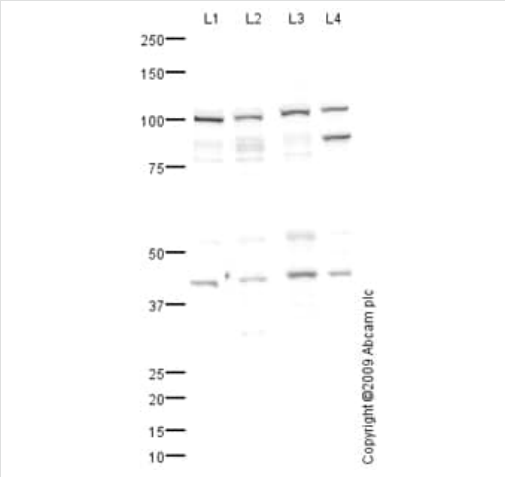
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 96 kDa
Observed band size: 96 kDa

Lanes 1 - 3: Merged signal (red and green). Green - ab88388 observed at 96 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab88388 was shown to recognize in wild-type HEK-293 cells as signal was lost at the expected MW in ALIX knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and ALIX knockout samples were subjected to SDS-PAGE. ab88388 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1 ug/ml and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-ALIX antibody (ab88388)

All lanes : Anti-ALIX antibody (ab88388) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
Lane 3 : SHSY-5Y (Human neuroblastoma cell line) Whole Cell Lysate
Lane 4 : HUVEC (Human Umbilical Vein Endothelial Cell) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 96 kDa
Observed band size: 100 kDa
Additional bands at: 44 kDa, 82 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 1 minute

Programmed cell death 6-interacting protein (PDC6I) contains a number of potential phosphorylation sites (SwissProt) which may explain its migration at a higher molecular weight than predicted.

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