abcam

Product datasheet

Anti-ALIX antibody [EPR23653-32] - BSA and Azide free ab275387

KO VALIDATED Recombinant RabMAb

10 Images

Overview

Product name	Anti-ALIX antibody [EPR23653-32] - BSA and Azide free
Description	Rabbit monoclonal [EPR23653-32] to ALIX - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IP, ICC/IF **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: C6, RAW 264.7, PC-12, NIH/3T3, K-562, HEK-293, HeLa, HCT116, MCF7 and Jurkat whole cell lysates; Human brain tissue lysate; Mouse brain tissue lysate; Rat brain tissue lysate. ICC/IF: NIH/3T3 and HeLa cells. Flow Cyt (intra): NIH/3T3 and HeLa cells. IP: K562 whole cell lysate.
General notes	ab275387 is the carrier-free version of **ab275377**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C.
Storage buffer	Constituent: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR23653-32
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab275387 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)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 80, 90, 100 kDa (predicted molecular weight: 96 kDa).
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.

Application notes Is unsuitable for IHC-P.

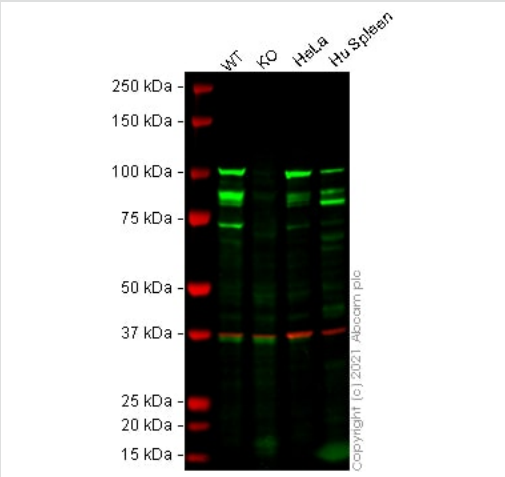
Target

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



Western blot - Anti-ALIX antibody [EPR23653-32] - BSA and Azide free (ab275387)

All lanes : Anti-ALIX antibody [EPR23653-32] (**ab275377**) at 1/1000 dilution

Lane 1 : Wild-type HEK-293 cell lysate
Lane 2 : PDCD6IP knockout HEK-293 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Human Spleen tissue lysate

Lysates/proteins at 20 µg per lane.

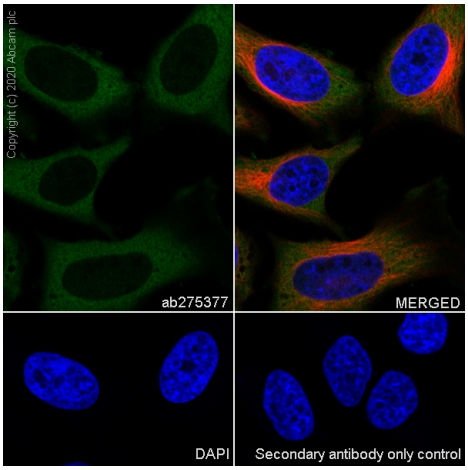
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab275377**).

Lanes 1 - 4: Merged signal (red and green). Green - **ab275377** observed at 96 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab275377 was shown to react with ALIX in wild-type HEK-293T cells in Western blot with loss of signal observed in PDCD6IP knockout cell line **ab260864** (PDCD6IP knockout cell lysate **ab261656**). Wild-type HEK-293T and PDCD6IP knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5 % milk in TBS-T (0.1 % Tween®) before incubation with **ab275377** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 h at room temperature before imaging.

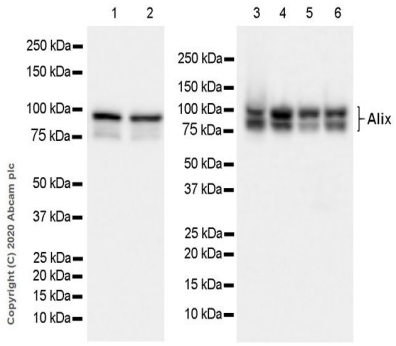


Immunocytochemistry/ Immunofluorescence - Anti-ALIX antibody [EPR23653-32] - BSA and Azide free (ab275387)

This data was developed using **ab275377**, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labelling ALIX with **ab275377** at 1/5000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488, **ab150077**) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in HeLa cells is observed. Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594, **ab195889**) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488, **ab150077**) at 1/1000 dilution.



Western blot - Anti-ALIX antibody [EPR23653-32] - BSA and Azide free (ab275387)

All lanes : Anti-ALIX antibody [EPR23653-32] (**ab275377**) at 1/1000 dilution

Lane 1 : Mouse brain tissue lysate
Lane 2 : Rat brain tissue lysate
Lane 3 : C6 (rat glial tumor glial cell) whole cell lysate
Lane 4 : RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 5 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate
Lane 6 : NIH/3T3 (mouse embryonic fibroblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

Predicted band size: 96 kDa
Observed band size: 100,80,90 kDa

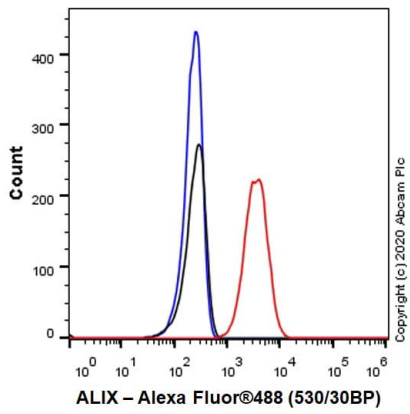
This data was developed using **ab275377**, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The expression pattern is consistent with what has been described

in the literature (PMID: 24834918, 26935291, 28322231).

Exposure time: Lane1-2: 10 seconds Lane3-6: 8 seconds.

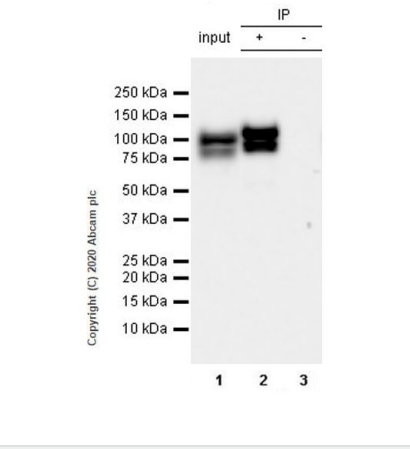


Flow Cytometry (Intracellular) - Anti-ALIX antibody [EPR23653-32] - BSA and Azide free (ab275387)

This data was developed using **ab275377**, the same antibody clone in a different buffer formulation.

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell line) cells labelling ALIX with **ab275377** at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-ALIX antibody [EPR23653-32] - BSA and Azide free (ab275387)

This data was developed using **ab275377**, the same antibody clone in a different buffer formulation.

ALIX was immunoprecipitated from 0.35 mg K-562 (human chronic myelogenous leukemia lymphoblast cell line) whole cell lysate with **ab275377** at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using **ab275377** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

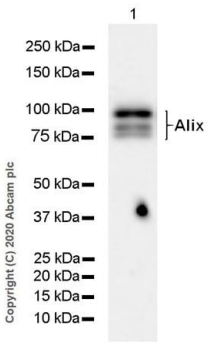
Lane 1: K-562 whole cell lysate 10 ug

Lane 2: **ab275377** IP in K-562 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab275377** in K-562 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 seconds



Western blot - Anti-ALIX antibody [EPR23653-32] - BSA and Azide free (ab275387)

Anti-ALIX antibody [EPR23653-32] (**ab275377**) at 1/1000 dilution + Human brain tissue lysate at 20 µg

Secondary

VeriBlot for IP secondary antibody(HRP)(**ab131366**) at 1/1000 dilution

Predicted band size: 96 kDa

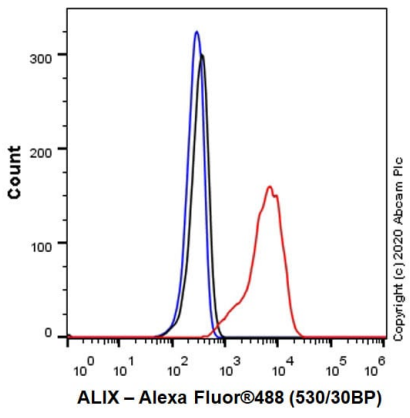
Observed band size: 100,80,90 kDa

This data was developed using **ab275377**, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The expression pattern is consistent with what has been described in the literature (PMID: 24834918, 26935291, 28322231).

Exposure time: 10 seconds.

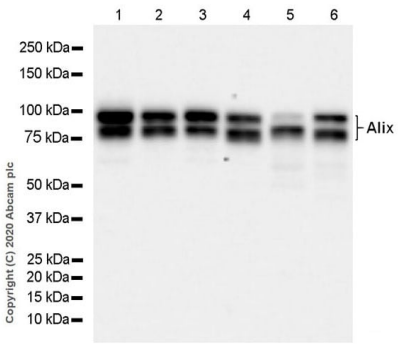


Flow Cytometry (Intracellular) - Anti-ALIX antibody [EPR23653-32] - BSA and Azide free (ab275387)

This data was developed using **ab275377**, the same antibody clone in a different buffer formulation.

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized NIH/3T3 (mouse embryonic fibroblast cell line) cells labelling ALIX with **ab275377** at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (**ab172730**) isotype control (Black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

A Goat anti rabbit IgG (Alexa Fluor®488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-ALIX antibody [EPR23653-32] - BSA and Azide free (ab275387)

All lanes : Anti-ALIX antibody [EPR23653-32] (**ab275377**) at 1/1000 dilution

Lane 1 : K-562 (human chronic myelogenous leukemia lymphoblast) whole cell lysate
Lane 2 : HEK-293 (human embryonic kidney epithelial cell) whole cell lysate
Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 4 : HCT116 (human colorectal carcinoma epithelial cell) whole cell lysate
Lane 5 : MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 6 : Jurkat (human T cell leukemia T lymphocyte) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

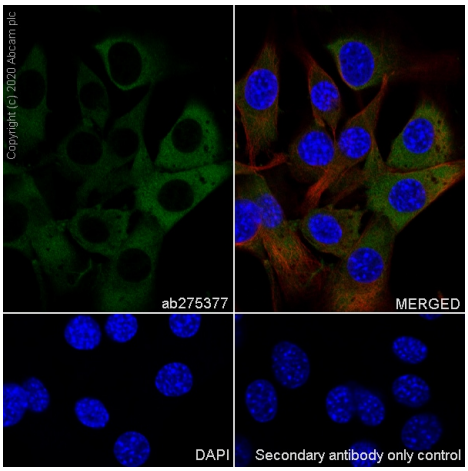
Predicted band size: 96 kDa
Observed band size: 90-100 kDa

This data was developed using **ab275377**, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The expression pattern is consistent with what has been described in the literature (PMID: 24834918, 26935291, 28322231).

Exposure time: 10 seconds.



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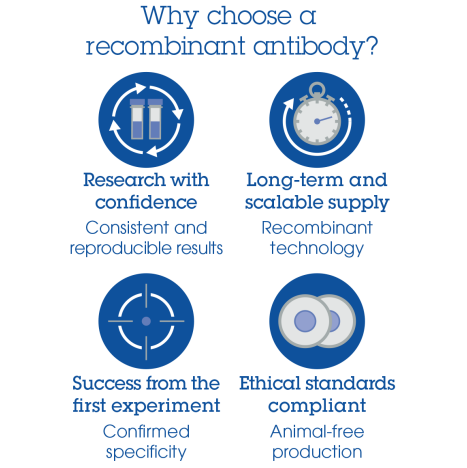
Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Anti-ALIX antibody [EPR23653-32] - BSA and Azide free (ab275387)

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