abcam

ab275387

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

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR23653-32
Purification technique	Affinity purification Protein A
Concentration	1.037 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human, Mouse
Dilution info	-

Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

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

WB

Tested

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse, Human
Dilution info	-
Notes	-

Expected

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

Target data

See full target information PDCD6IP

Function

Multifunctional protein involved in endocytosis, multivesicular body biogenesis, membrane repair, cytokinesis, apoptosis and maintenance of tight junction integrity. 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 requires the sequential function of ESCRT-O, -I,-II and -III complexes (PubMed:14739459). The ESCRT machinery also functions in topologically equivalent membrane fission events, such as the terminal stages of cytokinesis (PubMed:17556548, PubMed:17853893). Adapter for a subset of ESCRT-III proteins, such as CHMP4, to function at distinct membranes. Required for completion of cytokinesis (PubMed:17556548, PubMed:17853893, PubMed:18641129). May play a role in the regulation of both apoptosis and cell proliferation. Regulates exosome biogenesis in concert with SDC1/4 and SDCBP (PubMed:22660413). By interacting with F-actin, PARD3 and TJP1 secures the proper assembly and positioning of actomyosin-tight junction complex at the apical sides of adjacent epithelial cells that defines a spatial membrane domain essential for the maintenance of epithelial cell polarity and barrier (By similarity). (Microbial infection) Involved in HIV-1 virus budding. Can replace TSG101 it its role of supporting HIV-1 release; this function requires the interaction with CHMP4B. The ESCRT machinery also functions in topologically equivalent membrane fission events, such as enveloped virus budding (HIV-1 and other lentiviruses).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C

Notes

ab275387 is the carrier-free version of ab275377.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Supplementary info

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

Activity summary	ALIX also known as PDCD6IP or ALIX G is an adapter protein involved in various cellular processes. It holds a molecular weight of approximately 95-100 kDa and is widely expressed in different tissue types including HEK 293 cells. ALIX plays a significant role as a component of the endosomal sorting complexes required for transport (ESCRT) machinery contributing to membrane dynamics and intracellular trafficking.

Biological function summary	ALIX acts as an important mediator in the regulation of cellular membrane remodeling. It associates with several protein complexes influencing the budding and fission of vesicles and endosomes. The protein also interacts with ubiquitin and other factors guiding protein sorting and maintaining cellular homeostasis. This adapter characteristic makes ALIX essential for recycling receptors and maintaining cell surface receptor expression levels.
Pathways	ALIX is involved in the ESCRT pathway and the apoptotic pathways. It works closely with proteins like TSG101 and CHMP4 to facilitate vesicle formation and dispatch impacting cellular processes like signaling and waste disposal. Furthermore ALIX regulates apoptosis by interacting with proteins such as ALG-2 and to some extent with apoptosis-related membranes to mediate programmed cell death.
Associated diseases and disorders	ALIX has established links to cancer progression and neurodegenerative disorders. Its participation in membrane remodeling and protein sorting correlates with tumor development with evidence showing that altered expression affects oncogenic pathways. Additionally dysregulation of ALIX-associated pathways has implications in neurodegenerative diseases where ALIX and proteins such as those in the ESCRT complex could play a role in the degradation of misfolded proteins and endocytic dysfunctions contributing to disease pathology.

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

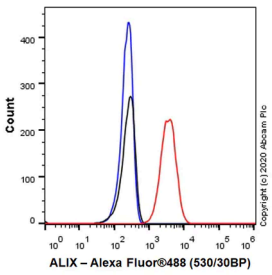
We do not recommend this combination. It is not covered by our product promise.

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.

9 product images

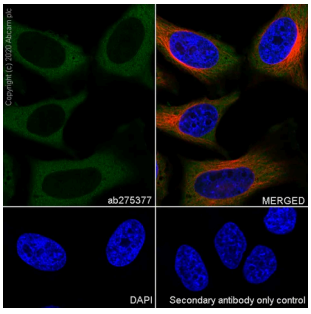


Flow Cytometry (Intracellular) - Anti-ALIX antibody [EPR23653-32] - BSA and Azide free (ab275387)

ALIX Flow Cytometry (Intracellular) staining using rabbit Anti-ALIX antibody

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.

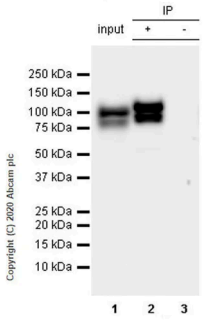


Immunocytochemistry/ Immunofluorescence - Anti-ALIX antibody [EPR23653-32] - BSA and Azide free (ab275387)

ALIX Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ALIX antibody

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.



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

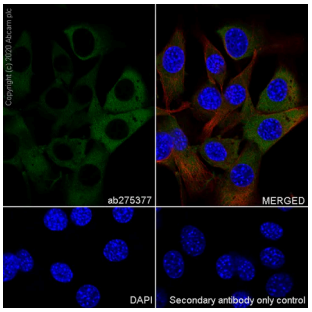
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab275377 in K-562 whole cell lysate

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

Exposure time: 3 seconds
All lanes:
Immunoprecipitation - Anti-ALIX antibody [EPR23653-32] (ab275377)

Predicted band size: 96 kDa

Observed band size: 90-100 kDa

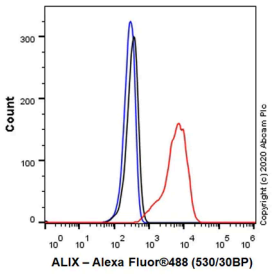


Immunocytochemistry/ Immunofluorescence - Anti-ALIX antibody [EPR23653-32] - BSA and Azide free (ab275387)

ALIX Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ALIX antibody

This data was developed using ab275377, the same antibody clone in a different buffer formulation.
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryonic fibroblast cell line) 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 NIH/3T3 cells. 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 ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.

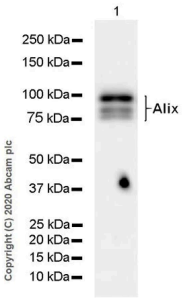


Flow Cytometry (Intracellular) - Anti-ALIX antibody [EPR23653-32] - BSA and Azide free (ab275387)

ALIX Flow Cytometry (Intracellular) staining using rabbit Anti-ALIX antibody

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)

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.

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

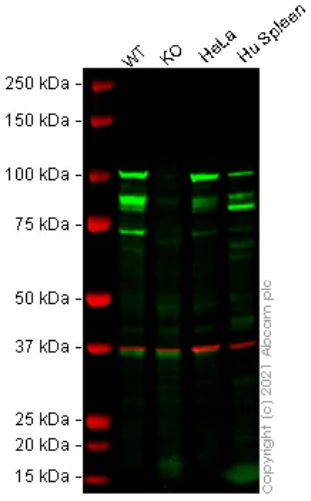
All lanes:
Human brain tissue lysate at 20 µg

Secondary

All lanes:
VeriBlot for IP secondary antibody(HRP)(ab131366) at 1/1000 dilution

Predicted band size: 96 kDa

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



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

ALIX Western blot staining using rabbit Anti-ALIX antibody

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.

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

Lane 1:
Wild-type HEK-293 cell lysate at 20 µg

Lane 2:
PDCD6IP knockout HEK-293 cell lysate at 20 µg

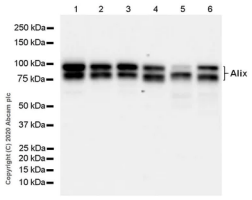
Lane 3:
HeLa cell lysate at 20 µg

Lane 4:
Human Spleen tissue lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 96 kDa

Observed band size: 96 kDa



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

ALIX Western blot staining using rabbit Anti-ALIX antibody

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.

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

Lane 1:
K-562 (human chronic myelogenous leukemia lymphoblast) whole cell lysate at 20 µg

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

Lane 3:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 4:
HCT116 (human colorectal carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 5:
MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

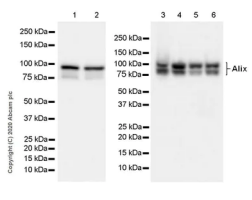
Lane 6:
Jurkat (human T cell leukemia T lymphocyte) whole cell lysate at 20 µg

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



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

Lane 1:
Mouse brain tissue lysate at 20 µg

Lane 2:
Rat brain tissue lysate at 20 µg

Lane 3:
C6 (rat glial tumor glial cell) whole cell lysate at 20 µg

Lane 4:
RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 5:
PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate at 20 µg

Lane 6:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

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 kDa, 80 kDa, 90 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.