abcam

Product datasheet

Human PDCD6IP knockout HEK-293 cell line ab260864

3 Images

Overview

Product name	Human PDCD6IP knockout HEK-293 cell line
Parental Cell Line	HEK-293
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 1 bp insertion, 1 bp insertion
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Next Generation Sequencing (NGS), Western Blot (WB)
Tested applications	**Suitable for:** ICC, WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type HEK-293 cell line (**ab259776**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^4$ cells/cm^2. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm^2 is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

Cells should be passaged when they have achieved 80-90% confluence.
This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.

Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Viability	~80%
Adherent /Suspension	Adherent
Tissue	Kidney
Cell type	epithelial
Gender	Female
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

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.

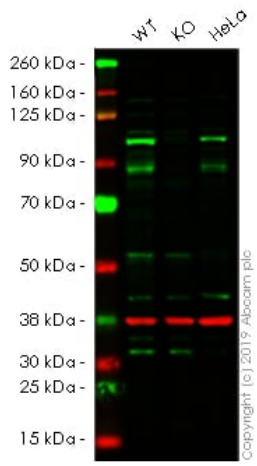
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab260864 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
ICC		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration.

Images



Western blot - Human PDCD6IP knockout HEK-293 cell line (ab260864)

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

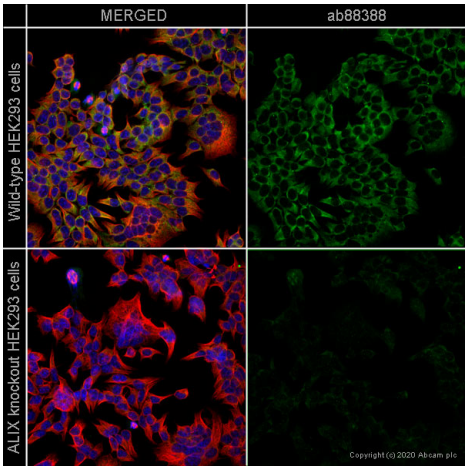
Lane 1 : Wild-type HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 2 : ALIX knockout HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

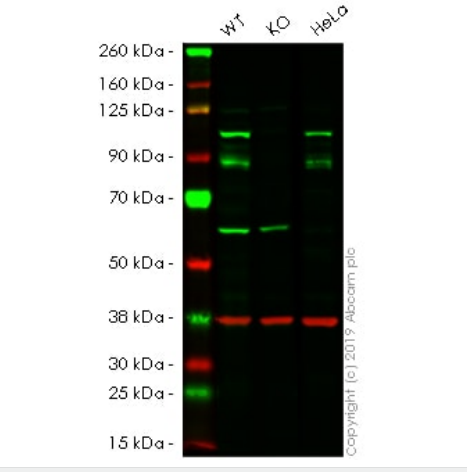
ab88388 was shown to recognize in wild-type HEK-293 cells as signal was lost at the expected MW in ALIX knockout cell line ab260864 (knockout cell lysate **ab261656**). Additional cross-reactive bands were observed in the wild-type and knockout samples. Wild-type and ALIX knockout samples were subjected 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.



Immunocytochemistry - Human PDCD6IP knockout HEK-293 cell line (ab260864)

ab88388 staining ALIX in wild-type HEK-293 cells (top panel) and ALIX knockout HEK-293 cells (ab260864) (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 - Human PDCD6IP knockout HEK-293 cell line (ab260864)

All lanes : Anti-ALIX antibody [EPR15314-33] - N-terminal (**ab186728**) at 1/5000 dilution

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.

Lanes 1 - 3: Merged signal (red and green). Green - **ab186728** observed at 96 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab186728 was shown to recognize in wild-type HEK-293 cells as signal was lost at the expected MW in ALIX knockout cell line ab260864 (knockout cell lysate **ab261656**). Additional cross-reactive bands were observed in the wild-type and knockout samples. Wild-type and ALIX knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% milk. **ab186728** and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/5000 dilution 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.

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