abcam

Product datasheet

Human CHMP2B knockout A549 cell line ab261874

3 Images

Overview

Product name	Human CHMP2B knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 1 bp insertion; Frameshift = 99%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	1
General notes	**Recommended control:** Human wild-type A549 cell line (**ab259777**). 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:Hams F12 + 5% 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 $2x10^3$-$1x10^4$ 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 $6x10^4$ 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.
Do not exceed $7x10^4$ cells/cm^2.
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We will provide viable cells that proliferate on revival.

Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Lung
Cell type	epithelial
Disease	Carcinoma
Gender	Male
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	Probable core component of the endosomal sorting required for transport complex III (ESCRT-III) which is involved in multivesicular bodies (MVBs) formation and sorting of endosomal cargo proteins into MVBs. MVBs contain intraluminal vesicles (ILVs) that are generated by invagination and scission from the limiting membrane of the endosome and mostly are delivered to lysosomes enabling degradation of membrane proteins, such as stimulated growth factor receptors, lysosomal enzymes and lipids. The MVB pathway appears to require the sequential function of ESCRT-O, -I,-II and -III complexes. ESCRT-III proteins mostly dissociate from the invaginating membrane before the ILV is released. The ESCRT machinery also functions in topologically equivalent membrane fission events, such as the terminal stages of cytokinesis and the budding of enveloped viruses (HIV-1 and other lentiviruses). ESCRT-III proteins are believed to mediate the necessary vesicle extrusion and/or membrane fission activities, possibly in conjunction with the AAA ATPase VPS4.
Tissue specificity	Widely expressed. Expressed in brain, heart, skeletal muscle, spleen, kidney, liver, small intestine, pancreas, lung, placenta and leukocytes. In brain, it is expressed in cerebellum, cerebral cortex, medulla, spinal chord, occipital lobe, frontal lobe, temporal lobe and putamen.
Involvement in disease	Defects in CHMP2B are the cause of frontotemporal dementia, chromosome 3-linked (FTD3) [MIM:600795]. FTD3 is characterized by an onset of dementia in the late 50's initially characterized by behavioral and personality changes including apathy, restlessness, disinhibition and hyperorality, progressing to stereotyped behaviors, non-fluent aphasia, mutism and dystonia, with a marked lack of insight. The brains of individuals with FTD3 have no distinctive neuropathological features. They show global cortical and central atrophy, but no beta-amyloid deposits.
Sequence similarities	Belongs to the SNF7 family.
Domain	The acidic C-terminus and the basic N-termminus are thought to render the protein in a closed,

soluble and inactive conformation through an autoinhibitory intramolecular interaction. The open and active conformation, which enables membrane binding and oligomerization, is achieved by interaction with other cellular binding partners, probably including other ESCRT components.

Cellular localization Cytoplasm > cytosol. Late endosome membrane.

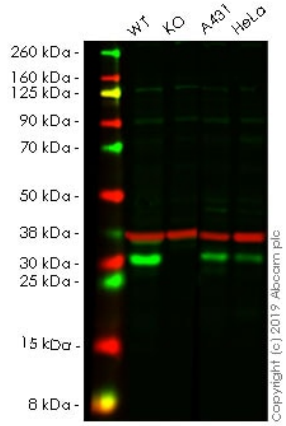
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab261874 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		Use at an assay dependent concentration.

Images



Western blot - Human CHMP2B knockout A549 cell line (ab261874)

All lanes : Anti-CHMP2B antibody (**ab33174**) at 1 µg/ml

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : CHMP2B knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 4 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

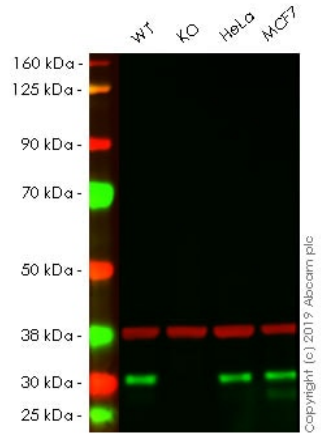
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Lanes 1 - 4: Merged signal (red and green). Green - **ab33174** observed at 30 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab33174 was shown to recognize CHMP2B in wild-type A549 cells as signal was lost at the expected MW in CHMP2B knockout cell line ab261874 (knockout cell lysate **ab261683**). Additional cross-reactive bands were observed in the wild-type and knockout samples. Wild-type and CHMP2B knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% milk. Ab33174 and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1 µg/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 - Human CHMP2B knockout A549 cell line (ab261874)

All lanes : Anti-CHMP2B antibody [EPR10807(B)] (**ab157208**) at 1/1000 dilution

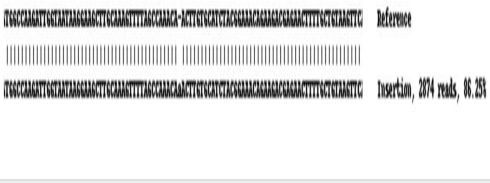
Lane 1 : Wild-type A549 whole cell lysate
Lane 2 : CHMP2B knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Lanes 1 - 4: Merged signal (red and green). Green - **ab157208** observed at 45 kDa. Red - loading control, **ab130007**, observed at 130 kDa.

ab157208 was shown to specifically react with CHMP2B in wild-type A549 cells as signal was lost in CHMP2B knockout cell line ab261874 (knockout cell lysate **ab261683**). Wild-type and CHMP2B knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% milk. Ab157208 and **ab130007** (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/1000 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.



Next Generation Sequencing - Human CHMP2B knockout A549 cell line (ab261874)

X = 1 bp insertion

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