abcam

Product datasheet

Human RAB10 knockout A549 cell line ab261868

画像数 5

製品の概要

製品名	Human RAB10 knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 1 bp insertion; Frameshift = 100%
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Next Generation Sequencing (NGS), Western Blot (WB)
アプリケーション	**適用あり:** WB, ICC
Biosafety level	1
特記事項	**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.
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**.

We will provide viable cells that proliferate on revival.

製品の特性

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
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

機能	The small GTPases Rab are key regulators of intracellular membrane trafficking, from the formation of transport vesicles to their fusion with membranes. Rabs cycle between an inactive GDP-bound form and an active GTP-bound form that is able to recruit to membranes different set of downstream effectors directly responsible for vesicle formation, movement, tethering and fusion (By similarity). That Rab is mainly involved in the biosynthetic transport of proteins from the Golgi to the plasma membrane. Regulates, for instance, SLC2A4/GLUT4 glucose transporter-enriched vesicles delivery to the plasma membrane. In parallel, it regulates the transport of TLR4, a toll-like receptor to the plasma membrane and therefore may be important for innate immune response. Plays also a specific role in asymmetric protein transport to the plasma membrane within the polarized neuron and epithelial cells. In neurons, it is involved in axonogenesis through regulation of vesicular membrane trafficking toward the axonal plasma membrane while in epithelial cells, it regulates transport from the Golgi to the basolateral membrane. Moreover, may play a role in the basolateral recycling pathway and in phagosome maturation. According to PubMed:23263280, may play a role in endoplasmic reticulum dynamics and morphology controlling tubulation along microtubules and tubules fusion.
配列類似性	Belongs to the small GTPase superfamily. Rab family.
細胞内局在	Cytoplasmic vesicle membrane. Golgi apparatus membrane. Golgi apparatus, trans-Golgi network membrane. Endosome membrane. Recycling endosome membrane. Cytoplasmic vesicle, phagosome membrane. Cell projection, cilium. Endoplasmic reticulum membrane. Associates with SLC2A4/GLUT4 storage vesicles (PubMed:22908308). Localizes to the base of the cilium (PubMed:20576682). Transiently associates with phagosomes (By similarity). Localizes to the endoplasmic reticulum at domains of new tubule growth (PubMed:23263280).

アプリケーション

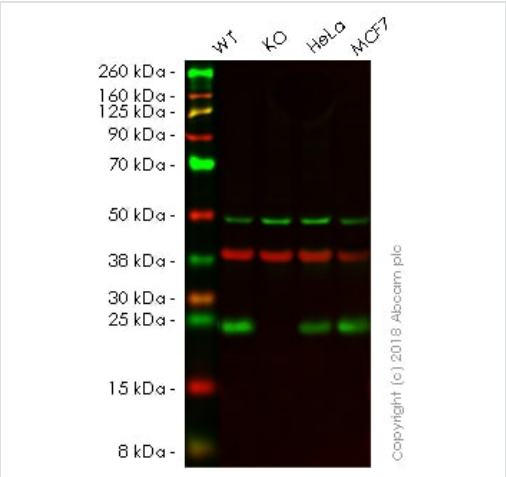
アプリケーション

The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab261868の使用に適用されます**

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration.
ICC		Use at an assay dependent concentration.

画像



Western blot - Human RAB10 knockout A549 cell line (ab261868)

All lanes : Anti-RAB10 antibody [MJF-R23] (**ab237703**) at 1/1000 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : RAB10 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.

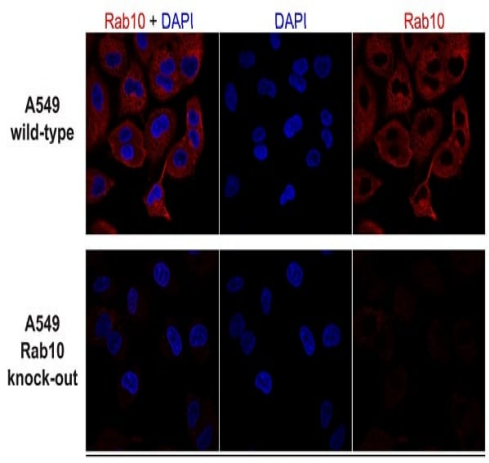
Performed under reducing conditions.

Observed band size: 25 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab237703** observed at 25 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

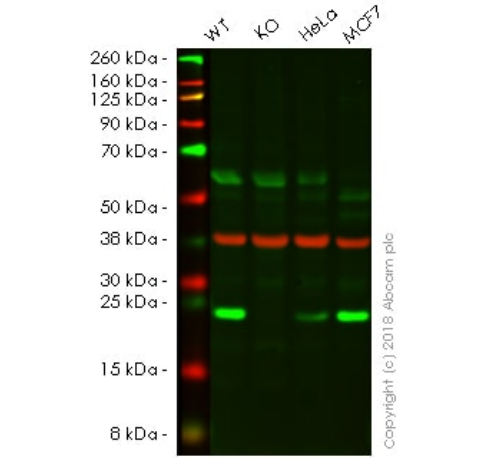
ab237703 was shown to recognize RAB10 in wild-type A549 cells as signal was lost at the expected MW in RAB10 knockout cell line ab261868 (knockout cell lysate **ab261677**). Additional cross-reactive bands were observed in the wild-type and knockout samples. Wild-type and RAB10 knockout samples were subjected to SDS-PAGE. Ab237703 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 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.



Immunocytochemistry/ Immunofluorescence - Human RAB10 knockout A549 cell line (ab261868)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% saponin permeabilized A549 wild-type and knockout cells (ab261868) labeling RAB10 (red) with **ab237703** at 0.5 µg/ml, followed by anti-Rabbit secondary at 1/1000 dilution. The nuclear counter stain is DAPI (blue).



Western blot - Human RAB10 knockout A549 cell line (ab261868)

All lanes : Anti-RAB10 antibody [EPR13242] (**ab181367**) at 1/1000 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : RAB10 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.

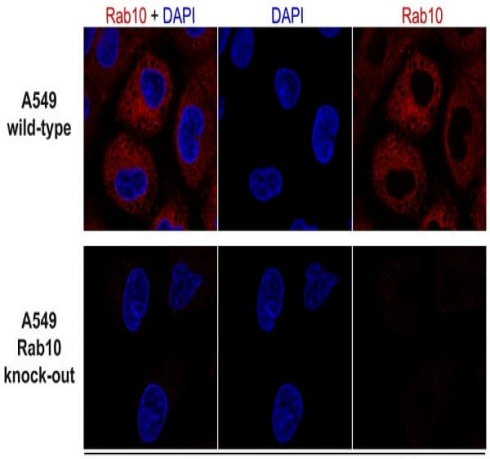
Performed under reducing conditions.

Observed band size: 25 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab181367** observed at 25 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

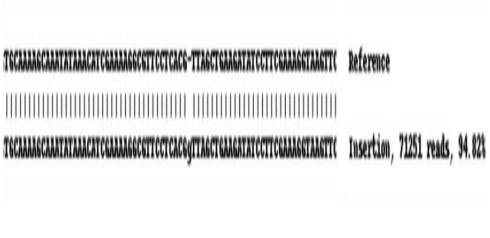
ab181367 was shown to specifically react with RAB10 in wild-type A549 cells as signal was lost in RAB10 knockout cell line

ab261868 (knockout cell lysate **ab261677**). Wild-type and RAB10 knockout samples were subjected to SDS-PAGE. Ab181367 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 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.



Immunocytochemistry/ Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% saponin permeabilized A549 wild-type and knockout cells (ab261868) labeling RAB10 (red) with **ab237703** at 0.5 µg/ml, followed by anti-Rabbit secondary at 1/1000 dilution. The nuclear counter stain is DAPI (blue).

Immunocytochemistry/ Immunofluorescence - Human RAB10 knockout A549 cell line (ab261868)



X = 1 bp insertion

Next Generation Sequencing - Human RAB10 knockout A549 cell line (ab261868)

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