abcam

Product datasheet

Human RAB29 knockout A549 cell lysate ab280099

3 Images

Overview

Product name	Human RAB29 knockout A549 cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	A549
Organism	Human
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
Notes	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.
Tested applications	**Suitable for:** WB

Properties

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Storage instructions	Store at -80°C. Please refer to protocols.

Components	1 kit
ab280142 - Human RAB29 knockout A549 cell lysate	1 x 100µg
ab277305 - Human wild-type A549 cell lysate	1 x 100µg

Cell type	epithelial
Disease	Carcinoma
Gender	Male

Target

Function	Rab GTPase key regulator in vesicle trafficking. Essential for maintaining the integrity of the endosome-trans-Golgi network structure. Together with LRRK2, plays a role in the retrograde trafficking pathway for recycling proteins, such as mannose 6 phosphate receptor (M6PR), between lysosomes and the Golgi apparatus in a retromer-dependent manner. Regulates neuronal process morphology in the intact central nervous system (CNS). May play a role in the formation of typhoid toxin transport intermediates during Salmonella enterica serovar Typhi (S.Typhi) epithelial cell infection.
Tissue specificity	Ubiquitous.
Sequence similarities	Belongs to the small GTPase superfamily. Rab family.
Post-translational modifications	In case of Salmonella enterica serovar Typhimurium (S.Typhimurium) infection, is proteolytically cleaved between Gly-41 and Val-42 by the GtgE viral protease encoded on the Gifsy-2 lysogen bacteriophage, which therefore prevents the recruitment of RAB29 to S.Typhimurium-containing vacuoles. In contrast, no proteolytically cleavage is detected in S.Typhi-infected cells (PubMed:22042847).
Cellular localization	Cell membrane. Cytoplasm. Cytoplasm, perinuclear region. Golgi apparatus. Golgi apparatus, trans-Golgi network. Vacuole. Cytoplasm, cytoskeleton. Colocalizes with LRRK2 along tubular structures emerging from Golgi apparatus (By similarity). Colocalizes with GM130 at the Golgi apparatus. Colocalizes with dynamic tubules emerging from and retracting to the Golgi apparatus. Colocalizes with TGN46 at the trans-Golgi network (TGN). In Salmonella enterica serovar Typhi (S.Typhi) infected epithelial cells, is recruited and colocalized with both S.Typhi-containing vacuoles and dynamic tubules as well as those emerging from the vacuole toward the cell periphery.

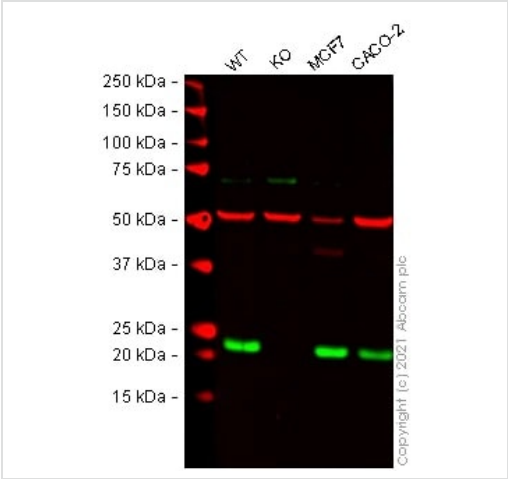
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab280099 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 RAB29 knockout A549 cell line (

ab280040

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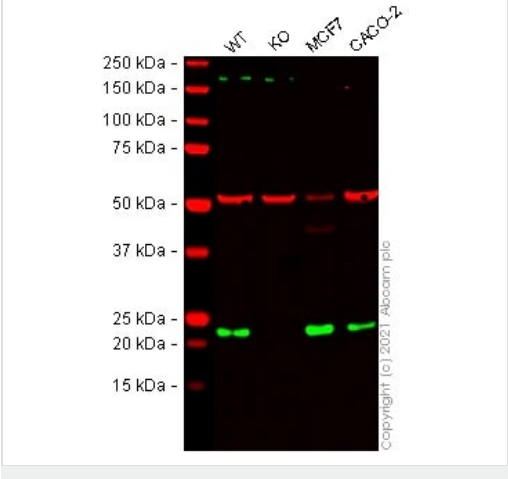
Lane 1: Wild-type A549 lysate 20 µg
Lane 2: RAB29 knockout A549 cell lysate 20 µg
Lane 3: MCF7 cell lysate 20 µg
Lane 4: CACO-2 cell lysate 20 µg

False colour image of Western blot: Anti-RAB29 antibody [MJF-R30-104] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab256527** was shown to bind specifically to RAB29. A band was observed at 23 kDa in wild-type A549 cell lysates with no signal observed at this size in RAB29 knockout cell line **ab280040** (knockout cell lysate ab280099). To generate this image, wild-type and RAB29 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



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Lane 3: MCF7 cell lysate 20 µg
Lane 4: CACO-2 cell lysate 20 µg

False colour image of Western blot: Anti-RAB29 antibody [MJF-R30-124] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab256526** was shown to bind specifically to RAB29. A band was observed at 23 kDa in wild-type A549 cell lysates with no signal observed at this size in RAB29 knockout cell line **ab280040** (knockout cell lysate ab280099). To generate this image, wild-type and RAB29 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged.Secondary antibodies used were Goat anti-Rabbit IgG H&L

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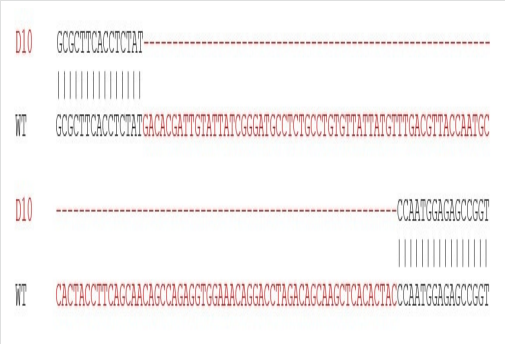

Sanger sequencing

Human RAB29 KO in A549 Cells with 119 bp Deletion in Exon 4

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