

Human RAB29 knockout A549 cell line ab280040

3 Images

Overview

Product name	Human RAB29 knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	Suitable for: WB
Biosafety level	1
General notes	<p>Recommended control: Human wild-type A549 cell line (ab275463). 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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: F-12K + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 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 2×10^3-1×10^4 cells/cm². 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% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods.</p> <p>A guide seeding density of 6×10^4 cells/cm² is recommended.</p> <p>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.</p> <p>Cells should be passaged when they have achieved 80-90% confluence.</p>

Do not exceed 7×10^4 cells/cm².

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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	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 <i>Salmonella enterica</i> 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 <i>Salmonella enterica</i> 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 proteolytic 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 <i>Salmonella enterica</i> 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.

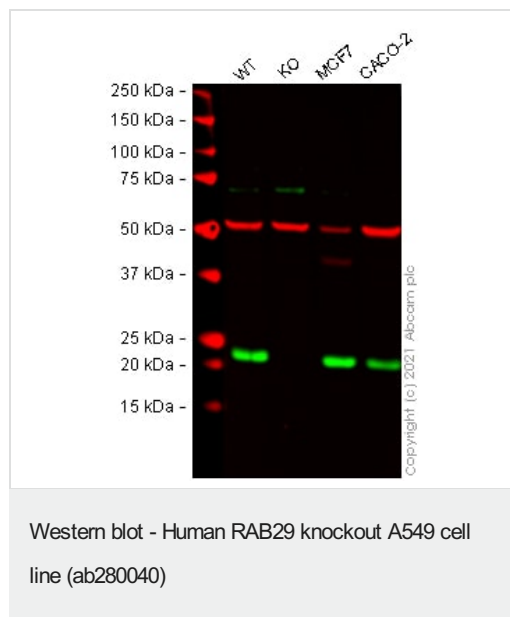
Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab280040 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. Predicted molecular weight: 23 kDa.

Images

All lanes : Anti-RAB29 antibody [MJF-R30-104] ([ab256527](#)) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate

Lane 2 : RAB29 knockout A549 cell lysate

Lane 3 : MCF-7 cell lysate

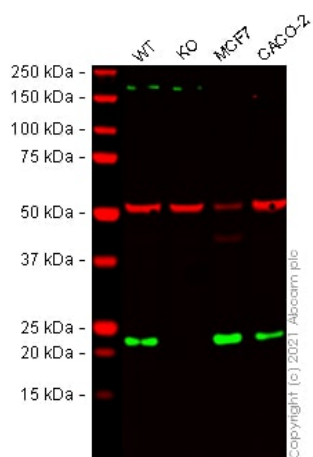
Lane 4 : Caco-2 cell lysate

Performed under reducing conditions.

Predicted band size: 23 kDa

Observed band size: 23 kDa

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.



Western blot - Human RAB29 knockout A549 cell line (ab280040)

All lanes : Anti-RAB29 antibody [MJF-R30-124] ([ab256526](#)) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate

Lane 2 : RAB29 knockout A549 cell lysate

Lane 3 : MCF-7 cell lysate

Lane 4 : Caco-2 cell lysate

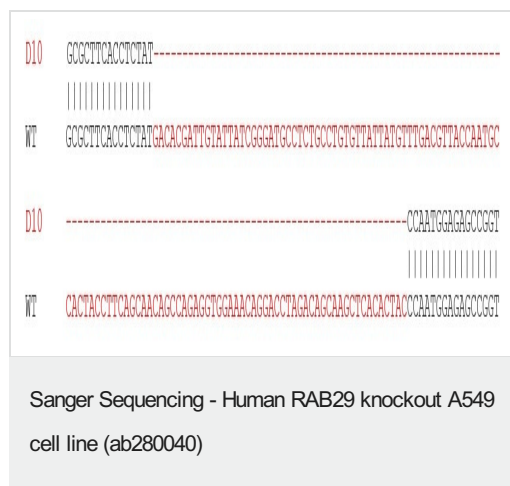
Performed under reducing conditions.

Predicted band size: 23 kDa

Observed band size: 23 kDa

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 (IRDye[®] 800CW) preabsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed ([ab216776](#)) at 1/20000 dilution.

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



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