

Human PPP2R5D knockout A-431 cell line ab270476

4 Images

Overview

Product name	Human PPP2R5D knockout A-431 cell line
Parental Cell Line	A431
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 7 bp deletion; Frameshift = 99.91%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), Western Blot (WB)
Tested applications	Suitable for: WB, Next Generation Sequencing
Biosafety level	1
General notes	<p>Recommended control: Human wild-type A-431 cell line (ab263975). 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: DMEM (High Glucose) + 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 2×10^4 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. A guide seeding density of 2×10^4 cells/cm² is recommended.</p> <p>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.</p>

Cells should be passaged when they have achieved 80-90% confluence.
 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](#).

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	Skin
Cell type	epithelial
Disease	Epidermoid Carcinoma
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

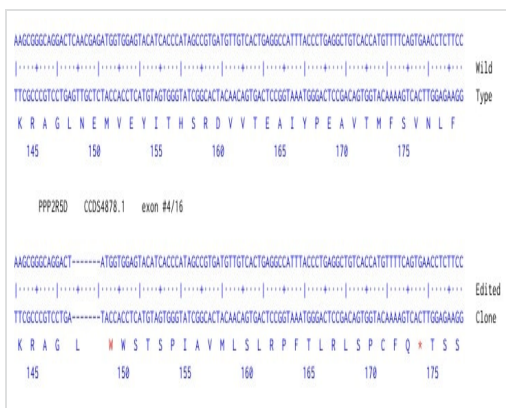
Function	The B regulatory subunit might modulate substrate selectivity and catalytic activity, and also might direct the localization of the catalytic enzyme to a particular subcellular compartment.
Tissue specificity	Isoform Delta-2 is widely expressed. Isoform Delta-1 is highly expressed in brain.
Sequence similarities	Belongs to the phosphatase 2A regulatory subunit B56 family.
Cellular localization	Cytoplasm. Nucleus. Nuclear in interphase, nuclear during mitosis.

Applications

The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab270476 in the following tested applications.
 The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

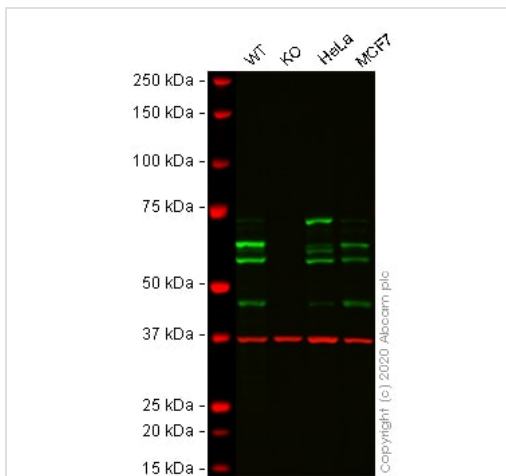
Application	Abreviews	Notes
WB		Use at an assay dependent concentration.
Next Generation Sequencing		Use at an assay dependent concentration.

Images



Next Generation Sequencing - Human PPP2R5D
knockout A-431 cell line (ab270476)

7 bp deletion after Gly147 of the WT protein



Western blot - Human PPP2R5D knockout A-431
cell line (ab270476)

All lanes : Anti-PPP2R5D antibody [EPR15617-50] (**ab188325**) at 1/10000 dilution

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 2 : PPP2R5D knockout A-431 (Human epidermoid 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: 60-65 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab188325** observed at 60-65 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab188325 was shown to react with PPP2R5D in wild-type A-431 cells in western blot with loss of signal observed in PPP2R5D knockout cell line ab270476 (knockout cell lysate **ab270499**). Wild-type and PPP2R5D knockout A-431 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab188325** and **ab8245** (Mouse

anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 hour at room temperature before imaging.

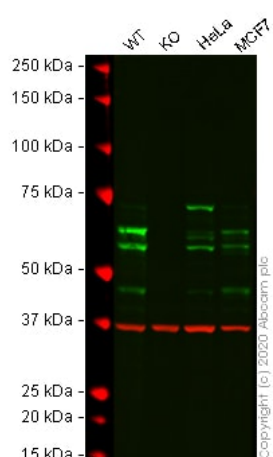
Knockout achieved by CRISPR/Cas9; X = 7 bp deletion; Frameshift = 99.91%

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GAGGTGAAGCGGCGAGGACTCAACGAGTGGTGGAGTACATCACCCT Reference
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GAGGTGAAGCGGCGAGGACT-----ATGGTGGAGTACATCACCCT Deletion, 25997 reads, 49.35%

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Next Generation Sequencing - Human PPP2R5D
knockout A-431 cell line (ab270476)



Western blot - Human PPP2R5D knockout A-431
cell line (ab270476)

All lanes : Anti-PPP2R5D antibody [EPR15617] (**ab188323**) at 1/10000 dilution

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 2 : PPP2R5D knockout A-431 (Human epidermoid 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: 60-65 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab188323** observed at 60-65 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab188323 was shown to react with PPP2R5D in wild-type A-431 cells in western blot with loss of signal observed in PPP2R5D knockout cell line ab270476 (knockout cell lysate **ab270499**). Wild-

type and PPP2R5D knockout A-431 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab188323** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 hour at room temperature before imaging.

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