# abcam

### Product datasheet

## Human PPP2R5D knockout A-431 cell lysate ab270499

### 3 Images

#### Overview

Product name Human PPP2R5D knockout A-431 cell lysate

**Product overview** Knockout cell lysate achieved by CRISPR/Cas9.

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)

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

Storage instructions

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

Components	1 kit
ab280552 - Human PPP2R5D knockout A-431 cell lysate	1 x 100µg
ab263973 - Human wild-type A-431 cell lysate	1 x 100µg

Cell type epithelial

**Disease** Epidermoid Carcinoma

**Gender** Female

#### **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 <u>Abpromise guarantee</u> covers the use of ab270499 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

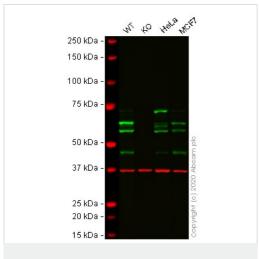
Application	Abreviews	Notes
WB		Use at an assay dependent concentration.

#### **Images**

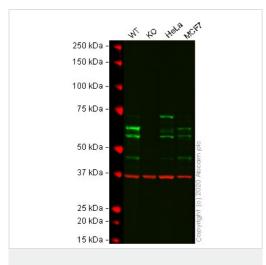
GAGSTGAAGCGGGCAGGACTCAACGAGATGGTGGAGTACATCACCCAT Reference

Next Generation Sequencing - Human PPP2R5D knockout A-431 cell lysate (ab270499)

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



Western blot - Human PPP2R5D knockout A-431 cell lysate (ab270499)



Western blot - Human PPP2R5D knockout A-431 cell lysate (ab270499)

Lane 1: Wild-type A431 cell lysate 20 ug

Lane 2: PPP2R5D knockout A431 cell lysate 20 ug

**Lane 3:** HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate 20 ug

Lane 4: MCF7 (Human breast adenocarcinoma cell line) whole cell lysate 20 ug

**Lanes 1 - 4:** Merged signal (red and green). Green - <u>ab188325</u> observed at 60-65 kDa. Red - loading control <u>ab8245</u> (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 lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Lane 1: Wild-type A431 cell lysate 20 ug

Lane 2: PPP2R5D knockout A431 cell lysate 20 ug

**Lane 3:** HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate 20 ug

**Lane 4:** MCF7 (Human breast adenocarcinoma cell line) whole cell lysate 20 ug

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

<u>ab188323</u> 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 <u>ab270476</u> (knockout cell lysate ab270499). Wildtype and PPP2R5D knockout A-431 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween<sup>®</sup>) before incubation with <u>ab188323</u> and <u>ab8245</u> (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<sup>®</sup> 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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