# abcam

# Product datasheet

# Human PRKCD (PKC delta) knockout HEK-293T cell lysate ab257042

3 Images

Overview

Product name Human PRKCD (PKC delta) knockout HEK-293T cell lysate

**Product overview** 

Knockout cell lysate achieved by CRISPR/Cas9.

Parental Cell Line HEK293T
Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon2 and 2 bp deletion in exon2.

Passage number <20

Knockout validation Sanger Sequencing

**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.

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licenses and patents please refer to our <u>limited use license</u> and <u>patent pages</u>.

Tested applications Suitable for: WB

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#### **Properties**

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

Components	1 kit
ab262820 - Human PRKCD knockout HEK293T cell lysate	1 x 100µg
ab255553 - Human wild-type HEK293T cell lysate	1 x 100µg

**Cell type** epithelial

**STR Analysis** Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01:

7, 9.3 TPOX: 11 CSF1PO: 11, 12

### **Target**

**Function** This is calcium-independent, phospholipid-dependent, serine- and threonine-specific enzyme.

PKC is activated by diacylglycerol which in turn phosphorylates a range of cellular proteins. PKC also serves as the receptor for phorbol esters, a class of tumor promoters. May play a role in antigen-dependent control of B-cell function. Phosphorylates MUC1 in the C-terminal and

regulates the interaction between MUC1 and beta-catenin.

**Sequence similarities**Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family. PKC subfamily.

Contains 1 AGC-kinase C-terminal domain.

Contains 1 C2 domain.

Contains 2 phorbol-ester/DAG-type zinc fingers.

Contains 1 protein kinase domain.

**Domain** The C1 domain, containing the phorbol ester/DAG-type region 1 (C1A) and 2 (C1B), is the

diacylglycerol sensor.

The C2 domain is a non-calcium binding domain. It binds proteins containing phosphotyrosine in

a sequence-specific manner.

Post-translational modifications

 $Phosphory lated\ on\ Thr-507,\ within\ the\ activation\ loop.\ Autophosphory lated\ and/or\ phosphory lated.$ 

Although the Thr-507 phosphorylation occurs it is not a prerequisite for enzymatic activity.

**Cellular localization** Cytoplasm. Membrane.

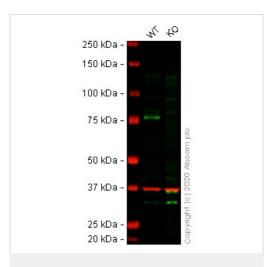
#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab257042 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: 78 kDa.

#### **Images**

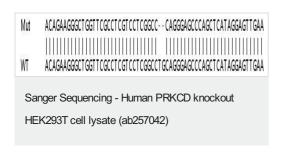


Western blot - Human PRKCD (PKC delta) knockout HEK293T cell lysate (ab257042)

Lane 1: Wild-type HEK-293T cell lysate 20 ug

Lane 2: PRKCD knockout HEK-293T cell lysate 20 ug

Lanes 1 - 2: Merged signal (red and green). Green - ab182126 observed at 78 kDa. Red - loading control, ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.#ab182126 was shown to react with PKC in wild-type HeLa cells in western blot. The bands observed in PRKCD knockout cell line ab266143 (PRKCD knockout cell lysate ab257042) below 78kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and PRKCD knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab182126 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 5000 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 IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Allele-1: 2 bp deletion in exon2

Mut	ACAGAAGGGCTGGTTCGCCTCGTCCTCGGCCCTGCAGGGAGCCCAGCTCATAGGAGTTGA		
WT	ACAGAAGGGCTGGTTCGCCTCGTCCTCGGCC TGCAGGGAGCCCAGCTCATAGGAGTTGA		
Sanger Sequencing - Human PRKCD knockout HEK293T cell lysate (ab257042)			

Allele-2: 1 bp insertion in exon2

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