abcam

Product datasheet

Human DAPK3 (ZIP Kinase) knockout HEK-293T cell line ab266755

3 Images

Overview

Product name	Human DAPK3 (ZIP Kinase) knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 1
Passage number	<20
Knockout validation	Sanger Sequencing
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images. **Recommended control:** Human wild-type HEK293T cell line (**ab255449**). 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. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **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. 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 $2x10^4$ cells/cm^2. 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% CO_2. Cultures should be monitored daily. **Subculture guidelines:**

All seeding densities should be based on cell counts gained by established methods.
A guide seeding density of $2x10^4$ cells/cm^2 is recommended.
A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.
Cells should be passaged when they have achieved 80-90% confluence.
This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and 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	Kidney
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
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	Serine/threonine kinase which acts as a positive regulator of apoptosis. Phosphorylates histone H3 on 'Thr-11' at centromeres during mitosis. Regulates myosin light chain phosphatase through phosphorylation of MYPT1 thereby regulating the assembly of the actin cytoskeleton, cell migration, invasiveness of tumor cells, smooth muscle contraction and neurite outgrowth. Involved in the formation of promyelocytic leukemia protein nuclear body (PML-NB), one of many subnuclear domains in the eukaryotic cell nucleus, and which is involved in oncogenesis and viral infection.
Sequence similarities	Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. DAP kinase subfamily. Contains 1 protein kinase domain.
Post-translational modifications	Ubiquitinated. Ubiquitination mediated by the UBE2D3 E3 ligase does not lead to proteasomal degradation, but influences promyelocytic leukemia protein nuclear bodies (PML-NBs) formation in the nucleus. Autophosphorylated. Phosphorylated by ROCK1.
Cellular localization	Nucleus. Cytoplasm. Nucleus > PML body. Relocates to the cytoplasm on binding PAWR where the complex appears to interact with actin filaments (By similarity). Localizes to promyelocytic leukemia protein nuclear bodies (PML-NBs). Associates to centromeres from prophase to anaphase.

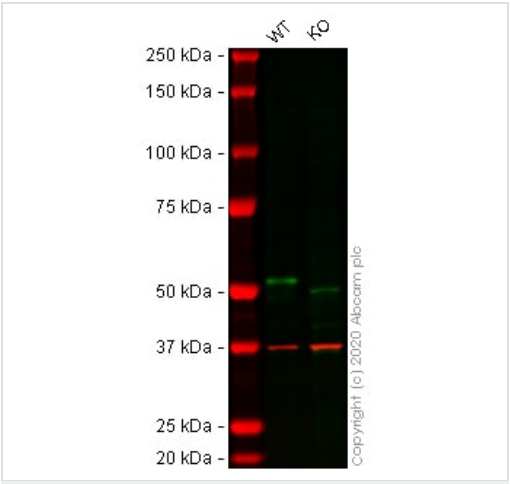
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab266755 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: 53 kDa. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.

Images



Western blot - Human DAPK3 (ZIP Kinase) knockout HEK293T cell line (ab266755)

All lanes : Anti-ZIP Kinase antibody [EPR1636Y] (**ab51602**) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate

Lane 2 : DAPK3 knockout HEK293T cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

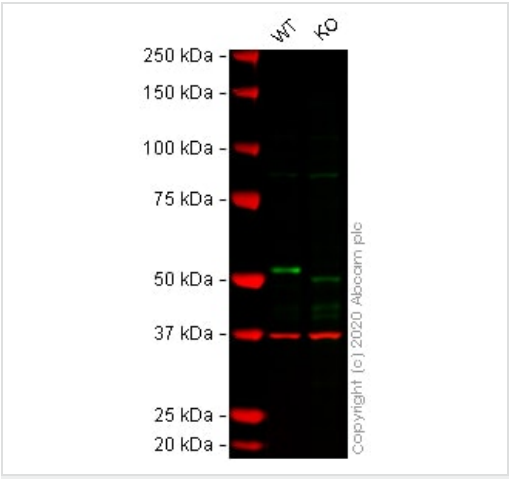
Predicted band size: 53 kDa

Observed band size: 53 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab51602** observed at 53 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab51602 was shown to react with ZIP Kinase in wild-type HEK-293T cells in western blot. The band observed in knockout cell line ab266755 (knockout cell lysate **ab257407**) lane below 53kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HEK-293T and DAPK3 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab51602** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary

antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human DAPK3 (ZIP Kinase) knockout HEK293T cell line (ab266755)

All lanes : Anti-ZIP Kinase antibody [EPR1635] (**ab79422**) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : DAPK3 knockout HEK293T cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 53 kDa
Observed band size: 53 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab79422** observed at 53 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab79422 was shown to react with ZIP Kinase in wild-type HEK-293T cells in western blot. The band observed in knockout cell line ab266755 (knockout cell lysate **ab257407**) lane below 53kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HEK-293T and DAPK3 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab79422** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 5000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Sanger Sequencing - Human DAPK3 knockout HEK293T cell line (ab266755)

Homozygous: 1 bp insertion in exon 1

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

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