abcam

Product datasheet

Human PDK4 knockout HeLa cell line ab261805

3 Images

Overview

Product name	Human PDK4 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 1 and 1 bp insertion in exon 1
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type HeLa cell line (**ab255928**). 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.
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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	Cervix
Cell type	epithelial
Disease	Adenocarcinoma
Gender	Female
STR Analysis	Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8, 12 CSF1PO: 9, 10
Antibiotic resistance	Puromycin 1.00µg/ml
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	Inhibits the mitochondrial pyruvate dehydrogenase complex by phosphorylation of the E1 alpha subunit, thus contributing to the regulation of glucose metabolism.
Tissue specificity	Ubiquitous; highest levels of expression in heart and skeletal muscle.
Sequence similarities	Belongs to the PDK/BCKDK protein kinase family. Contains 1 histidine kinase domain.
Cellular localization	Mitochondrion matrix.

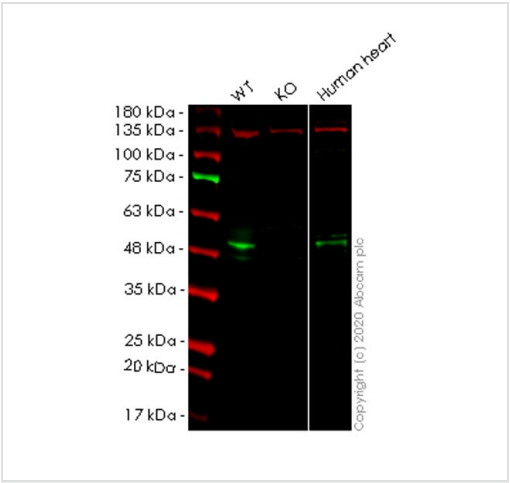
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab261805 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: 46 kDa.

Images



Western blot - Human PDK4 knockout HeLa cell line (ab261805)

All lanes : Anti-PDK4 antibody [1C2BG5] (**ab110336**) at 1/500 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PDK4 knockout HeLa cell lysate
Lane 3 : Human heart tissue lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

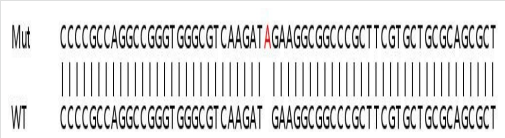
Predicted band size: 46 kDa
Observed band size: 48 kDa

Lanes 1-3: Merged signal (red and green). Green - **ab110336** observed at 48 kDa. Red - loading control, **ab129002** observed at 124 kDa.

ab110336 Anti-PDK4 antibody [1C2BG5] was shown to specifically react with PDK4 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab261805 (knockout cell lysate **ab257217**) was used. Wild-type and PDK4 knockout samples were subjected to SDS-PAGE. **ab110336** and Anti-Vinculin antibody [EPR8185] - Loading Control (**ab129002**) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 1000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

Sanger Sequencing - Human PDK4 knockout HeLa cell line (ab261805)

Allele-1: 1 bp deletion in exon 1.



Sanger Sequencing - Human PDK4 knockout HeLa cell line (ab261805)

Allele-2: 1 bp insertion in exon 1.

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