abcam

Product datasheet

# Human PINK1 knockout HEK-293T cell line ab266393

1 References 6 Images

## Overview

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|---|---|
| **Product name** | Human PINK1 knockout HEK-293T cell line |
| **Parental Cell Line** | HEK293T |
| **Organism** | Human |
| **Mutation description** | Knockout achieved by using CRISPR/Cas9, Homozygous: 10 bp deletion in exon 1 |
| **Passage number** | <20 |
| **Knockout validation** | Sanger Sequencing |
| **Tested applications** | **Suitable for:** WB, Sanger Sequencing |
| **Biosafety level** | 2 |
| **General notes** | **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.<br><br>**Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.<br><br>**Culture medium:** DMEM (High Glucose) + 10% FBS<br><br>**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.<br><br>1. Thaw the vial in 37°C water bath for approximately 1-2 minutes.<br>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.<br>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.<br>4. Incubate the culture at 37°C incubator with 5% $CO_2$. Cultures should be monitored daily.<br><br>**Subculture guidelines:**<br>All seeding densities should be based on cell counts gained by established methods.<br>A guide seeding density of $2x10^4$ cells/$cm^2$ is recommended.<br>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 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

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|---|---|
| **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 |
| **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

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| **Function** | Protects against mitochondrial dysfunction during cellular stress, potentially by phosphorylating mitochondrial proteins. Involved in the clearance of damaged mitochondria via selective autophagy (mitophagy). It is necessary for PARK2 recruitement to dysfunctional mitochondria to initiate their degradation. |
| **Tissue specificity** | Highly expressed in heart, skeletal muscle and testis, and at lower levels in brain, placenta, liver, kidney, pancreas, prostate, ovary and small intestine. Present in the embryonic testis from an early stage of development. |
| **Involvement in disease** | Defects in PINK1 are the cause of Parkinson disease type 6 (PARK6) [MIM:605909]. A neurodegenerative disorder characterized by parkinsonian signs such as rigidity, resting tremor and bradykinesia. A subset of patients manifest additional symptoms including hyperreflexia, autonomic instability, dementia and psychiatric disturbances. Symptoms show diurnal fluctuation and can improve after sleep. |
| **Sequence similarities** | Belongs to the protein kinase superfamily. Ser/Thr protein kinase family.<br>Contains 1 protein kinase domain. |
| **Post-translational modifications** | Autophosphorylated. |
| **Cellular localization** | Mitochondrion outer membrane. Cytoplasm > cytosol. |

## Applications

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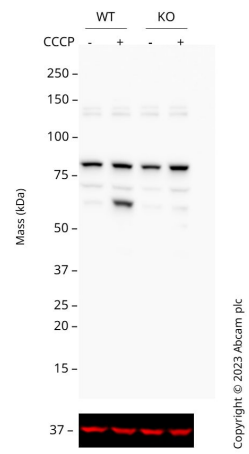
| **The Abpromise guarantee** | Our **Abpromise guarantee** covers the use of ab266393 in the following tested applications. |

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: 63 kDa. |
| **Sanger Sequencing** | | Use at an assay dependent concentration. |

## Images




Western blot - Human PINK1 knockout HEK-293T cell line (ab266393)

**All lanes :** Anti-PINK1 antibody [MJF-R32-7] (**ab300623**) at 1/1000 dilution

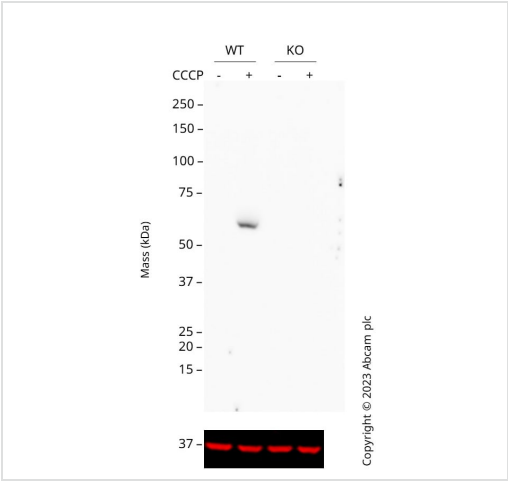
**Lane 1 :** Wild-type HEK-293 Vehicle Control CCCP, **ab141229** (0 µM, 24 h) cell lysate
**Lane 2 :** Wild-type HEK-293 Treated CCCP, **ab141229** (10 µM, 24 h) cell lysate
**Lane 3 :** PINK1 knockout HEK-293 Vehicle Control CCCP, **ab141229** (0 µM, 24 h) cell lysate
**Lane 4 :** PINK1 knockout HEK-293 Treated CCCP, **ab141229** (10 µM, 24 h) cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 63 kDa

Anti-PINK1 antibody [MJF-R32-7] (**ab300623**) staining at 1/1000 dilution, shown in black; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. **ab300623** was shown to bind specifically to PINK1. A band was observed at 60 kDa in wild-type HEK-293 cell lysates with no signal observed at this size in PINK1 knockout cell line ab266393 (knockout cell lysate **ab257030**). To generate this image, wild-type and PINK1 knockout HEK-293 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times before development with Optiblot (ECL reagent **ab133456**) and imaged with 4 minutes exposure time.




Western blot - Human PINK1 knockout HEK-293T cell line (ab266393)

Secondary antibodies used were HRP conjugated Goat anti-Rabbit (H+L) and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

**All lanes :** Anti-PINK1 antibody [EPR20730] (**ab216144**) at 1/200 dilution

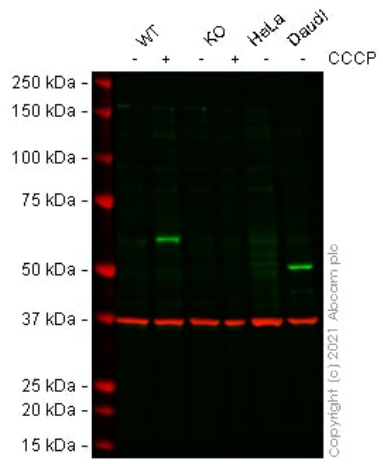
**Lane 1 :** Wild-type HEK-293 Vehicle Control CCCP, **ab141229** (0 µM, 24h) cell lysate
**Lane 2 :** Wild-type HEK-293 Treated CCCP, **ab141229** (10 µM, 24 h) cell lysate
**Lane 3 :** PINK1 knockout HEK-293 Vehicle Control CCCP, **ab141229** (0 µM, 24 h) cell lysate
**Lane 4 :** PINK1 knockout HEK-293 Treated CCCP, **ab141229** (10 µM, 24 h) cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 63 kDa

Anti-PINK1 antibody [EPR20730] (**ab216144**) staining at 1/200 dilution shown in black; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution 44 shown in red. **ab216144** was shown to bind specifically to PINK1. A band was observed at 60 kDa in wild-type HEK-293 cell lysates with no signal observed at this size in PINK1 knockout cell line ab266393 (knockout cell lysate **ab257030**). Membranes were blocked in 5 % milk in TBS-0.1 % Tween 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T and incubated with secondary antibodies for 1 h at room temperature, washed again four times before development with Optiblot (ECL reagent **ab133456**) and imaged with 20 minutes exposure time. Secondary antibodies used were HRP conjugated Goat anti-Rabbit (H+L) and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.




Western blot - Human PINK1 knockout HEK-293T cell line (ab266393)

**All lanes :** Anti-PINK1 antibody at 1/1000 dilution

**Lane 1 :** Wild-type HEK-293T Vehicle Control CCCP (0 µM, 24 h) cell lysate
**Lane 2 :** Wild-type HEK-293T Treated CCCP (10 µM, 24 h) cell lysate
**Lane 3 :** PINK1 knockout HEK-293T Vehicle Control CCCP (0 µM, 24 h) cell lysate
**Lane 4 :** PINK1 knockout HEK-293T Treated CCCP (10 µM, 24 h) cell lysate
**Lane 5 :** HeLa cell lysate
**Lane 6 :** Daudi cell lysate

Lysates/proteins at 20 µg per lane.

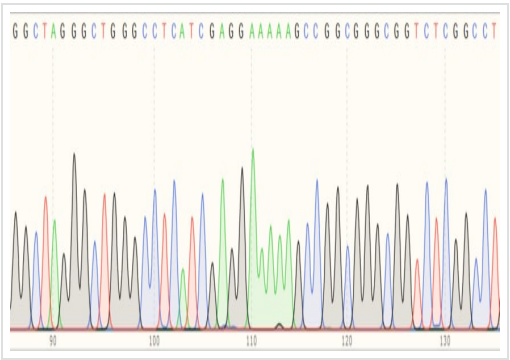
Performed under reducing conditions.

**Predicted band size:** 63 kDa
**Observed band size:** 63 kDa

False colour image of Western blot: Anti-PINK1 antibody staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, the antibody was shown to bind specifically to PINK1. A band was observed at 63 kDa in wild-type cell lysates with no signal observed at this size in PINK1 knockout cell line ab266393 (knockout cell lysate **ab257030**). To generate this image, wild-type and PINK1 knockout cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.

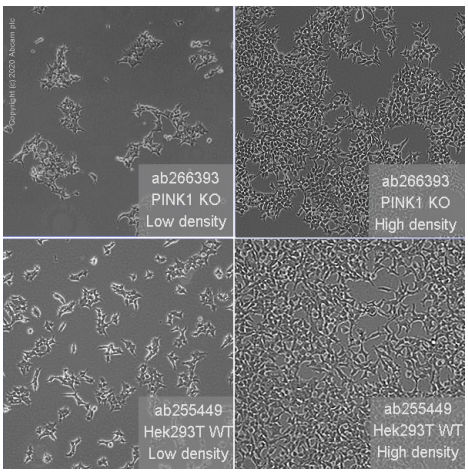

Sanger Sequencing - Human PINK1 knockout HEK293T cell line (ab266393)

Homozygous: 10 bp deletion in exon1




Sanger Sequencing - Human PINK1 knockout HEK-293T cell line (ab266393)

Sequencing chromatogram displaying sequence edit in exon 1




Cell Culture - Human PINK1 knockout HEK-293T cell line (ab266393)

Representative images PINK1 knockout HEK293T cells, low and high confluency examples (top left and right respectively) and wild-type HEK293T cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using a EVOS M5000 microscope.



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