abcam

Product datasheet

Human PARK7 (DJ1) knockout HEK-293T cell line ab266338

4 Images

Overview

Product name Human PARK7 (DJ1) knockout HEK-293T cell line

Parental Cell Line HEK293T
Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, Homozygous: Insertion of the selection cassette in

exon 2

Passage number <20

Knockout validation Sanger Sequencing, Western Blot (WB)

Tested applications Suitable for: WB

Biosafety level 2

General notesRecommended control: Human wild-type HEK293T cell line (<u>ab255449</u>). 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 2x10⁴ cells/cm². 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% CO₂. Cultures should be monitored daily.

Subculture guidelines:

All seeding densities should be based on cell counts gained by established methods.

1

A guide seeding density of 2x10⁴ cells/cm² 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 <u>limited use license</u> and <u>patent pages</u>.

We will provide viable cells that proliferate on revival.

Properties

Number of cells 1 x 10⁶ 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

Protects cells against oxidative stress and cell death. Plays a role in regulating expression or stability of the mitochondrial uncoupling proteins SLC25A14 and SLC25A27 in dopaminergic neurons of the substantia nigra pars compacta and attenuates the oxidative stress induced by calcium entry into the neurons via L-type channels during pacemaking. Eliminates hydrogen peroxide and protects cells against hydrogen peroxide-induced cell death. May act as an atypical peroxiredoxin-like peroxidase that scavenges hydrogen peroxide. Following removal of a Cterminal peptide, displays protease activity and enhanced cytoprotective action against oxidative stress-induced apoptosis. Stabilizes NFE2L2 by preventing its association with KEAP1 and its subsequent ubiquitination. Binds to OTUD7B and inhibits its deubiquitinating activity. Enhances RELA nuclear translocation. Binds to a number of mRNAs containing multiple copies of GG or CC motifs and partially inhibits their translation but dissociates following oxidative stress. Required for correct mitochondrial morphology and function and for autophagy of dysfunctional mitochondria. Regulates astrocyte inflammatory responses. Acts as a positive regulator of androgen receptordependent transcription. Prevents aggregation of SNCA. Plays a role in fertilization. Has no proteolytic activity. Has cell-growth promoting activity and transforming activity. May function as a redox-sensitive chaperone.

Tissue specificity

Highly expressed in pancreas, kidney, skeletal muscle, liver, testis and heart. Detected at slightly lower levels in placenta and brain. Detected in astrocytes, Sertoli cells, spermatogonia, spermatids and spermatozoa.

Involvement in disease

Defects in PARK7 are the cause of Parkinson disease type 7 (PARK7) [MIM:606324]. A neurodegenerative disorder characterized by resting tremor, postural tremor, bradykinesia, muscular rigidity, anxiety and psychotic episodes. PARK7 has onset before 40 years, slow progression and initial good response to levodopa. Some patients may show traits reminiscent of

amyotrophic lateral sclerosis-parkinsonism/dementia complex (Guam disease).

Sequence similarities

Belongs to the peptidase C56 family.

Post-translational modifications

 $Sum oy lated \ on \ Lys-130 \ by \ PIAS2 \ or \ PIAS4; \ which \ is \ enhanced \ after \ ultraviolet \ irradiation \ and$

essential for cell-growth promoting activity and transforming activity.

Cys-106 is easily oxidized to sulfinic acid.

Undergoes cleavage of a C-terminal peptide and subsequent activation of protease activity in

response to oxidative stress.

Cellular localization

Cytoplasm. Nucleus. Mitochondrion. Under normal conditions, located predominantly in the cytoplasm and, to a lesser extent, in the nucleus and mitochondrion. Translocates to the mitochondrion and subsequently to the nucleus in response to oxidative stress and exerts an increased cytoprotective effect against oxidative damage. Detected in tau inclusions in brains

from neurodegenerative disease patients.

Applications

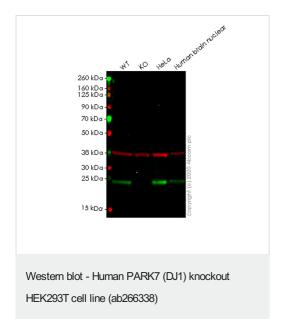
The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab266338 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: 20 kDa.

Images



All lanes : Anti-PARK7/DJ1 antibody [EP2816Y] (ab76241) at 1/1000 dilution

Lane 1: Wild-type HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lane 2: PARK7 knockout HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

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

Lane 4: Human brain nuclear fraction tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/10000 dilution

Predicted band size: 20 kDa

Observed band size: 24 kDa

Lanes 1-4: Merged signal (red and green). Green - <u>ab76241</u> observed at 24 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

ab76241 Anti-PARK7/DJ1 antibody [EP2816Y] was shown to specifically react with PARK7/DJ1 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266338 (knockout cell lysate ab257016) was used. Wild-type and PARK7/DJ1 knockout samples were subjected to SDS-PAGE. ab76241 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

260 kDa - 160 kDa - 125 kDa - 30 kDa - 30 kDa - 25 kDa - 15 kDa -

Western blot - Human PARK7 (DJ1) knockout HEK293T cell line (ab266338)

All lanes : Anti-PARK7/DJ1 antibody [EP2815Y] (<u>ab76008</u>) at 1/1000 dilution

Lane 1: Wild-type HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lane 2: PARK7 knockout HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

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

Lane 4: Human brain nuclear fraction tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/10000 dilution

Predicted band size: 20 kDa Observed band size: 24 kDa

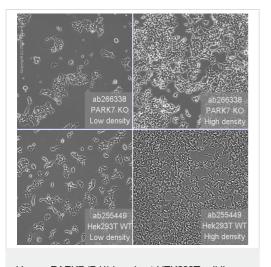
Lanes 1-4: Merged signal (red and green). Green - ab76008

observed at 24 kDa. Red - loading control **ab8245** observed at 36 kDa.

<u>ab76008</u> Anti-PARK7/DJ1 antibody [EP2815Y] was shown to specifically react with PARK7/DJ1 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266338 (knockout cell lysate <u>ab257016</u>) was used. Wild-type and PARK7/DJ1 knockout samples were subjected to SDS-PAGE. <u>ab76008</u> and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Homozygous: Insertion of the selection cassette in exon 2

Sanger Sequencing - Human PARK7 knockout HEK293T cell line (ab266338)



Human PARK7 (DJ1) knockout HEK293T cell line (ab266338)

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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery

- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors