abcam

Product datasheet

Human PPIB (Cyclophilin B) knockout HeLa cell line ab261746

3 Images

Overview

Product name	Human PPIB (Cyclophilin B) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 2 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 (**ab255448**). 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	PPIases accelerate the folding of proteins. It catalyzes the cis-trans isomerization of proline imidic peptide bonds in oligopeptides.
Involvement in disease	Defects in PPIB are the cause of osteogenesis imperfecta type 9 (OI9) [MIM:259440]. OI9 is a connective tissue disorder characterized by bone fragility, low bone mass and bowing of limbs due to multiple fractures. Short limb dwarfism and blue sclerae are observed in some but not all patients.
Sequence similarities	Belongs to the cyclophilin-type PPIase family. PPIase B subfamily. Contains 1 PPIase cyclophilin-type domain.
Cellular localization	Endoplasmic reticulum lumen. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

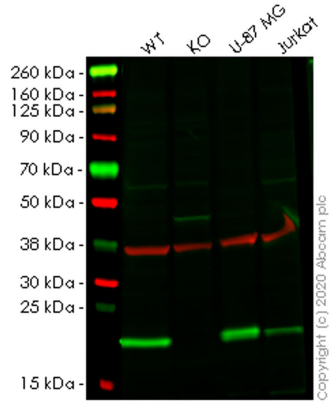
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab261746 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: 24 kDa.

Images



Western blot - Human PPIB (Cyclophilin B) knockout HeLa cell line (ab261746)

All lanes : Anti-Cyclophilin B antibody [CL3901] (**ab236760**) at 1/1000 dilution

Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : PPIB knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : U-87 MG (Human glioblastoma-astrocytoma epithelial cell line) whole cell lysate
Lane 4 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lysates/proteins at 20 µg per lane.

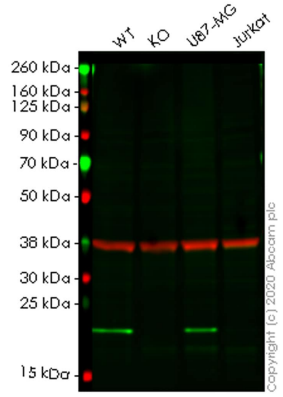
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

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

Lanes 1-4: Merged signal (red and green). Green - **ab236760** observed at 24 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab236760 Anti-Cyclophilin B antibody [CL3901] was shown to specifically react with Cyclophilin B in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab261746 (knockout cell lysate **ab257037**) was used. Wild-type and Cyclophilin B knockout samples were subjected to SDS-PAGE. **ab236760** 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 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 PPIB (Cyclophilin B) knockout HeLa cell line (ab261746)

All lanes : Anti-Cyclophilin B antibody [EPR12703(B)] - Loading Control (**ab178397**) at 1/1000 dilution

Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : PPIB knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : U-87 MG (Human glioblastoma-astrocytoma epithelial cell line) whole cell lysate
Lane 4 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

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

Lanes 1-4: Merged signal (red and green). Green - **ab178397** observed at 24 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab178397 Anti-Cyclophilin B antibody [EPR12703(B)] was shown to specifically react with Cyclophilin B in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab261746 (knockout cell lysate **ab257037**) was used. Wild-type and Cyclophilin B knockout samples were subjected to SDS-PAGE. **ab178397** 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 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 PPIB knockout HeLa cell line (ab261746)

Homozygous: 2 bp insertion in exon 1.

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