abcam

Product datasheet

Human FHL2 knockout HeLa cell line ab265475

4 Images

Overview

Product name Human FHL2 knockout HeLa cell line

Parental Cell Line HeLa
Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 3 and 1 bp insertion in exon 3

Passage number <20

Knockout validation Sanger Sequencing, Western Blot (WB)

Tested applications Suitable for: WB

Biosafety level

General notesRecommended control: Human wild-type HeLa cell line (<u>ab255928</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. A guide seeding density of $2x10^4$ cells/cm² is recommended.

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

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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

Cell type

Number of cells 1 x 10⁶ cells/vial, 1 mL

Adherent /Suspension Adherent
Tissue Cervix

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.

epithelial

Storage buffer Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function May function as a molecular transmitter linking various signaling pathways to transcriptional

regulation. Negatively regulates the transcriptional repressor E4F1 and may function in cell

growth.

Tissue specificity Expressed in skeletal muscle and heart. **Sequence similarities** Contains 4 LIM zinc-binding domains.

Domain The third LIM zinc-binding mediates interaction with E4F1.

Cellular localization Cytoplasm. Nucleus.

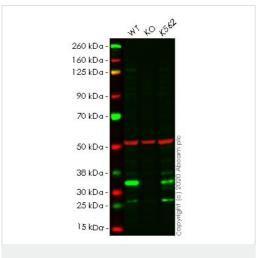
Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab265475 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: 32 kDa.

Images



Western blot - Human FHL2 knockout HeLa cell line (ab265475)

All lanes : Anti-FHL2 antibody [EPR17860-23] (<u>ab202586</u>) at 1/1000 dilution

Lane 1: Wild-type HeLa lysate

Lane 2: FHL2 knockout HeLa lysate

Lane 3: K562 lysate

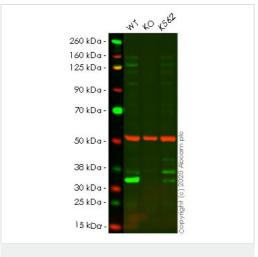
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 32 kDa

Lanes 1-3: Merged signal (red and green). Green - <u>ab202586</u> observed at 32 kDa. Red - loading control <u>ab7291</u> observed at 50 kDa.

<u>ab202586</u> Recombinant Anti-FHL2 antibody [EPR17860-23] was shown to specifically react with FHL2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265475 (knockout cell lysate <u>ab257441</u>) was used. Wild-type and FHL2 knockout samples were subjected to SDS-PAGE. <u>ab202586</u> and Anti-alpha Tubulin antibody [DM1A] - Loading Control (<u>ab7291</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.



Western blot - Human FHL2 knockout HeLa cell line (ab265475)

All lanes: Anti-FHL2 antibody [EPR17860-20] (ab202584) at 1/1000 dilution

Lane 1: Wild-type HeLa lysate

Lane 2: FHL2 knockout HeLa lysate

Lane 3: K562 lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 32 kDa

Lanes 1-3: Merged signal (red and green). Green - <u>ab202584</u> observed at 32 kDa. Red - loading control <u>ab7291</u> observed at 50 kDa.

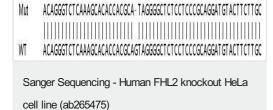
ab202584 Recombinant Anti-FHL2 antibody [EPR17860-20] was shown to specifically react with FHL2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265475 (knockout cell lysate ab257441) was used. Wild-type and FHL2 knockout samples were subjected to SDS-PAGE. ab202584 and Anti-alpha Tubulin antibody [DM1A] - Loading Control?(ab7291) 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.

Mut AACAGGGTCTCAAAGCACACCACGCAAGTAGGGGCTCTCCTCCCGCAGGATGTACTTCTT

WT AACAGGGTCTCAAAGCACACCACGC AGTAGGGGCTCTCCTCCCGCAGGATGTACTTCTT

Sanger Sequencing - Human FHL2 knockout HeLa cell line (ab265475)

Allele-1: 1 bp insertion in exon 3.



Allele-2: 1 bp deletion in exon 3.

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