abcam

Product datasheet

Human LMNB1 (Lamin B1) knockout HeLa cell line ab255404

4 Images

Overview

Product name Human LMNB1 (Lamin B1) knockout HeLa cell line

Parental Cell Line HeLa
Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, 2 bp deletion in exon 1 and 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 notesRecommended control: Human wild-type HeLa cell line (<u>ab255448</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.

 $\textbf{Cryopreservation cell medium:} \ \ \textbf{Cell Freezing Medium-DMSO Serum free media, contains}$

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.

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

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

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

Adherent Adherent/Suspension **Tissue** Cervix epithelial Cell type

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

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 Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the

inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and

may also interact with chromatin.

Involvement in disease Defects in LMNB1 are the cause of leukodystrophy demyelinating autosomal dominant adult-

onset (ADLD) [MIM:169500]. ADLD is a slowly progressive and fatal demyelinating

leukodystrophy, presenting in the fourth or fifth decade of life. Clinically characterized by early autonomic abnormalities, pyramidal and cerebellar dysfunction, and symmetric demyelination of

the CNS. It differs from multiple sclerosis and other demyelinating disorders in that

neuropathology shows preservation of oligodendroglia in the presence of subtotal demyelination

and lack of astrogliosis.

Sequence similarities Belongs to the intermediate filament family.

Post-translational B-type lamins undergo a series of modifications, such as farnesylation and phosphorylation.

modifications Increased phosphorylation of the lamins occurs before envelope disintegration and probably plays

a role in regulating lamin associations.

Cellular localization Nucleus inner membrane.

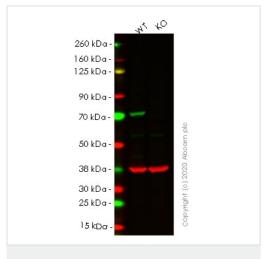
Applications

Our **Abpromise guarantee** covers the use of ab255404 in the following tested applications. The Abpromise guarantee

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: 66 kDa.

Images



Western blot - Human LMNB1 (Lamin B1) knockout HeLa cell line (ab255404)

All lanes : Anti-Lamin B1 antibody [EPR22165-121] (<u>ab229025</u>) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: LMNB1 knockout HeLa cell lysate

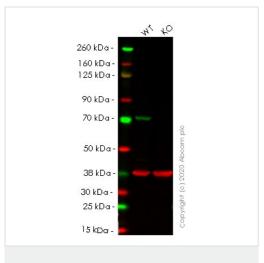
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 66 kDa **Observed band size:** 66-70 kDa

Lanes 1-2: Merged signal (red and green). Green - <u>ab229025</u> observed at 66-70 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) observed at 37 kDa.

ab229025 was shown to react with LMNB1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab255404 (knockout cell lysate ab263825) was used. Wild-type HeLa and LMNB1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab229025 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 dilution and a 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 LMNB1 (Lamin B1) knockout HeLa cell line (ab255404)

All lanes : Anti-Lamin B1 antibody [EPR8985(B)] - Nuclear Envelope Marker (<u>ab133741</u>) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: LMNB1 knockout HeLa cell lysate

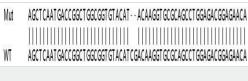
Lysates/proteins at 20 µg per lane.

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<u>ab133741</u> was shown to react with LMNB1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab255404 (knockout cell lysate <u>ab263825</u>) was used. Wild-type HeLa and LMNB1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. <u>ab133741</u> and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) overnight at 4°C at a 1 in 1000 dilution and a 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.



Sanger Sequencing - Human LMNB1 knockout HeLa cell line (ab255404)

Allele-1: 2 bp deletion in exon 1.

Mut AGCTCAATGACCGGCTGGCGGTGTACATACCGACAAGGTGCGCAGCCTGGAGACGGAGAA

WT AGCTCAATGACCGGCTGGCGGTGTACAT CGACAAGGTGCGCAGCCTGGAGACGGAGAA

Sanger Sequencing - Human LMNB1 knockout HeLa

cell line (ab255404)

Allele-2: 2 bp insertion in exon 1.

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