abcam

Product datasheet

Human SUZ12 knockout HeLa cell line ab264983

3 Images

Overview

Product name	Human SUZ12 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 23 bp deletion in exon 1
Passage number	<20
Knockout validation	Sanger Sequencing
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images. **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	Polycomb group (PcG) protein. Component of the PRC2/EED-EZH2 complex, which methylates 'Lys-9' (H3K9me) and 'Lys-27' (H3K27me) of histone H3, leading to transcriptional repression of the affected target gene. The PRC2/EED-EZH2 complex may also serve as a recruiting platform for DNA methyltransferases, thereby linking two epigenetic repression systems. Genes repressed by the PRC2/EED-EZH2 complex include HOXC8, HOXA9, MYT1 and CDKN2A.
Tissue specificity	Overexpressed in breast and colon cancer.
Involvement in disease	Note=A chromosomal aberration involving SUZ12 may be a cause of endometrial stromal tumors. Translocation t(7;17)(p15;q21) with JAZF1. The translocation generates the JAZF1-SUZ12 oncogene consisting of the N-terminus part of JAZF1 and the C-terminus part of SUZ12. It is frequently found in all cases of endometrial stromal tumors, except in endometrial stromal sarcomas, where it is rarer.
Sequence similarities	Belongs to the VEFS (VRN2-EMF2-FIS2-SU(Z)12) family. Contains 1 C2H2-type zinc finger.
Developmental stage	Expressed at low levels in quiescent cells. Expression rises at the G1/S phase transition.
Cellular localization	Nucleus.

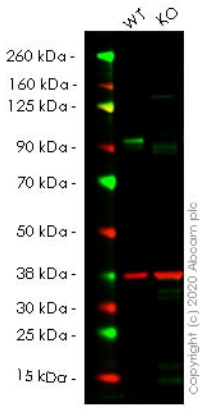
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab264983 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: 83 kDa. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.

Images



Western blot - Human SUZ12 knockout HeLa cell line (ab264983)

All lanes : Anti-SUZ12 antibody [EPR5234(N)] - ChIP Grade (**ab175187**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : SUZ12 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

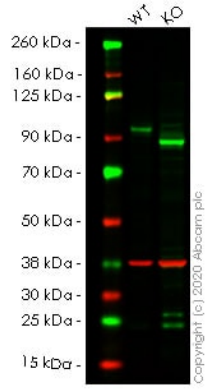
Performed under reducing conditions.

Predicted band size: 83 kDa
Observed band size: 100 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab175187** observed at 100 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab175187 was shown to react with SUZ12 in wild-type HeLa cells in western blot. The band observed in knockout cell line ab264983 (knockout cell lysate **ab257721**) lane below 100kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and SUZ12 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. **ab175187** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated 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 SUZ12 knockout HeLa cell line (ab264983)

All lanes : Anti-SUZ12 antibody (**ab12073**)

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : SUZ12 knockout HeLa cell lysate

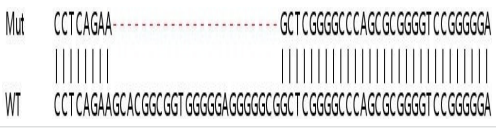
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 83 kDa
Observed band size: 95 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab12073** observed at 95 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab12073 was shown to react with SUZ12 in wild-type HeLa cells in western blot. The band observed in knockout cell line ab264983 (knockout cell lysate **ab257721**) lane below 95kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and SUZ12 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. **ab12073** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 µg/ml 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.



Sanger Sequencing - Human SUZ12 knockout HeLa cell line (ab264983)

Homozygous: 23 bp deletion in exon 1.

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