abcam

Product datasheet

Human SMAD1 knockout HeLa cell line ab265400

3 Images

Overview

Product name	Human SMAD1 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	2

General notes

Recommended control: Human wild-type HeLa cell line (**ab255928**). 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	Transcriptional modulator activated by BMP (bone morphogenetic proteins) type 1 receptor kinase. SMAD1 is a receptor-regulated SMAD (R-SMAD). SMAD1/OAZ1/PSMB4 complex mediates the degradation of the CREBBP/EP300 repressor SNIP1.
Tissue specificity	Ubiquitous. Highest expression seen in the heart and skeletal muscle.
Sequence similarities	Belongs to the dwarfin/SMAD family. Contains 1 MH1 (MAD homology 1) domain. Contains 1 MH2 (MAD homology 2) domain.
Post-translational modifications	Phosphorylated on serine by BMP type 1 receptor kinase. Ubiquitin-mediated proteolysis by SMAD-specific E3 ubiquitin ligase SMURF1.
Cellular localization	Cytoplasm. Nucleus. Cytoplasmic in the absence of ligand. Migrates to the nucleus when complexed with SMAD4. Co-localizes with LEMD3 at the nucleus inner membrane.

Applications

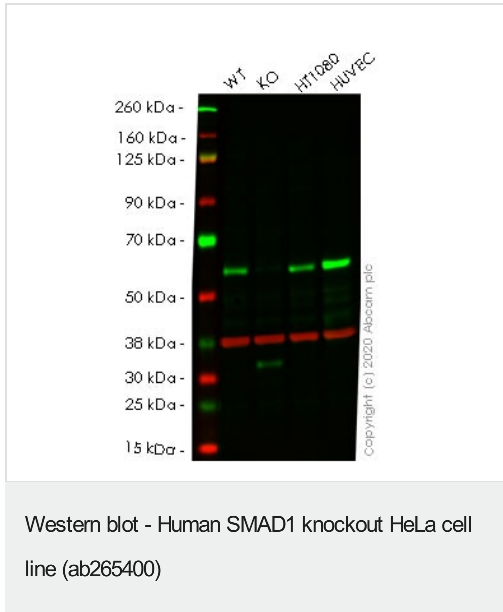
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab265400 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 52 kDa.

Images



Western blot - Human SMAD1 knockout HeLa cell line (ab265400)

All lanes : Anti-Smad1 antibody [EPR5522] (**ab126761**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : SMAD1 knockout HeLa cell lysate
Lane 3 : HT1080 cell lysate
Lane 4 : Huvec cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 52 kDa
Observed band size: 52 kDa

Lanes 1- 4: Merged signal (red and green). Green - **ab126761** observed at 52 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab126761 was shown to react with Smad1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265400 (knockout cell lysate **ab257686**) was used. Wild-type HeLa and SMAD1 knockout HeLa cell lysates were subjected to SDS-PAGE. **ab126761** 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.

Mut CACTCTCCCAATAGCAGTTACCCAAACTCT-CTGGGAGCAGCAGCAGCACCTACCCTCAC

WT CACTCTCCCAATAGCAGTTACCCAAACTCTCCTGGGAGCAGCAGCAGCACCTACCCTCAC

Sanger Sequencing - Human SMAD1 knockout HeLa cell line (ab265400)

Allele-1: 1 bp deletion in exon 3.

Mut CACTCTCCCAATAGCAGTTACCCAAACTCTTCCTGGGAGCAGCAGCAGCACCTACCCTCA

WT CACTCTCCCAATAGCAGTTACCCAAACTCT CCTGGGAGCAGCAGCAGCACCTACCCTCA

Sanger Sequencing - Human SMAD1 knockout HeLa cell line (ab265400)

Allele-2: 1 bp insertion in exon 3.

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