# abcam

# Product datasheet

# Human SMAD1 knockout HeLa cell line ab265400

## 画像数3

#### 製品の概要

特記事項

製品名 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)

アプリケーション **適用あり**: WB

Biosafety level

ziocaicty icro.

**Recommended 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  $37^{\circ}\text{C}$  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<sup>4</sup> cells/cm<sup>2</sup>. 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<sub>2</sub>. 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<sup>2</sup> 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.

製品の特性

Number of cells 1 x 10<sup>6</sup> 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

保存方法 Shipped on Dry Ice. Store in liquid nitrogen.

バッファー Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

機能 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.

組織特異性 Ubiquitous. Highest expression seen in the heart and skeletal muscle.

配列類似性 Belongs to the dwarfin/SMAD family.

Contains 1 MH1 (MAD homology 1) domain. Contains 1 MH2 (MAD homology 2) domain.

翻訳後修飾 Phosphorylated on serine by BMP type 1 receptor kinase.

Ubiquitin-mediated proteolysis by SMAD-specific E3 ubiquitin ligase SMURF1.

**細胞内局在** 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.

アプリケーション

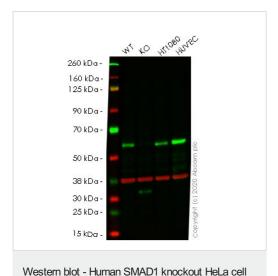
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アプリケーション	Abreviews	特記事項

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 52 kDa.

#### 画像

line (ab265400)



**All lanes :** Anti-Smad1 antibody [EPR5522] (<u>ab126761</u>) 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

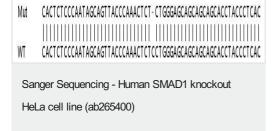
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

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

<u>ab126761</u> 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 <u>ab257686</u>) was used. Wild-type HeLa and SMAD1 knockout HeLa cell lysates were subjected to SDS-PAGE. <u>ab126761</u> and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) 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 (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Allele-1: 1 bp deletion in exon 3.

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

Allele-2: 1 bp insertion in exon 3.

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