abcam

Product datasheet

Human SMAD2 knockout HeLa cell line ab255430

5 Images

Overview

Product name	Human SMAD2 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 2 and 1 bp insertion in exon 2
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
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	Receptor-regulated SMAD (R-SMAD) that is an intracellular signal transducer and transcriptional modulator activated by TGF-beta (transforming growth factor) and activin type 1 receptor kinases. Binds the TRE element in the promoter region of many genes that are regulated by TGF-beta and, on formation of the SMAD2/SMAD4 complex, activates transcription. May act as a tumor suppressor in colorectal carcinoma.
Tissue specificity	Expressed at high levels in skeletal muscle, heart and placenta.
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 one or several of Thr-220, Ser-245, Ser-250, and Ser-255. In response to TGF-beta, phosphorylated on Ser-465/467 by TGF-beta and activin type 1 receptor kinases. Able to interact with SMURF2 when phosphorylated on Ser-465/467, recruiting other proteins, such as SNON, for degradation. In response to decorin, the naturally occurring inhibitor of TGF-beta signaling, phosphorylated on Ser-240 by CaMK2. Phosphorylated by MAPK3 upon EGF stimulation; which increases transcriptional activity and stability, and is blocked by calmodulin. In response to TGF-beta, ubiquitinated by NEDD4L; which promotes its degradation. Acetylated on Lys-19 by coactivators in response to TGF-beta signaling, which increases transcriptional activity. Isoform short: Acetylation increases DNA binding activity in vitro and enhances its association with target promoters in vivo. Acetylation in the nucleus by EP300 is enhanced by TGF-beta.
Cellular localization	Cytoplasm. Nucleus. Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta stimulation, migrates to the nucleus when complexed with SMAD4. On dephosphorylation by phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the

nucleus by interaction with RANBP1.

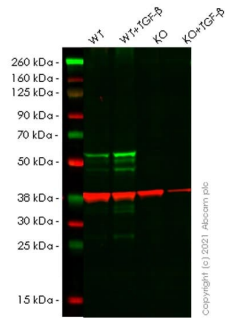
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab255430 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: 52 kDa.

Images



Western blot - Human SMAD2 knockout HeLa cell line (ab255430)

All lanes : Anti-Smad2 (phospho S467) antibody [EPR23681-40] (**ab280888**) at 1/1000 dilution

Lane 1 : Wild-type HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate
Lane 2 : Wild-type HeLa (human cervix adenocarcinoma epithelial cell) treated with 20 ng/ml TGF beta1 for 15 minutes, whole cell lysate
Lane 3 : Smad2 knockout HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate
Lane 4 : Smad2 knockout HeLa (human cervix adenocarcinoma epithelial cell), treated with 20 ng/ml TGF beta1 for 15 minutes, whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (**ab216773**) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (**ab216776**) at 1/20000 dilution

Predicted band size: 52 kDa
Observed band size: 60 kDa

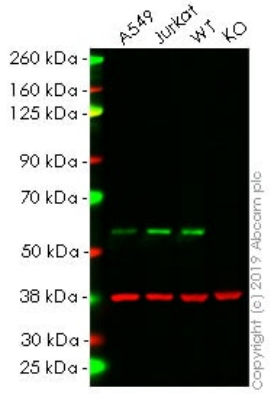
Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS

Lanes 1-4: Merged signal (red and green). Green - **ab280888**

observed at 60 kDa. Red - loading control **ab8245** (Mouse monoclonal [6C5] to GAPDH) observed at 36 kDa.

ab280888 Anti-Smad2 (phospho S467) antibody [EPR23681-40] was shown to specifically react with Smad2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255430 (knockout cell lysate **ab263833**) was used. Wild-type and Smad2 knockout samples were subjected to SDS-PAGE.

ab280888 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at 4? overnight at 1 in 1000 dilution and 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 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Human SMAD2 knockout HeLa cell line (ab255430)

All lanes : Anti-Smad2 antibody [EP784Y] (**ab40855**) at 1/2000 dilution

Lane 1 : A549 cell lysate
Lane 2 : Jurkat cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : SMAD2 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

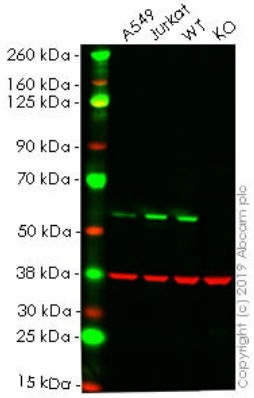
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

Predicted band size: 52 kDa
Additional bands at: 37 kDa (possible Loading Control)

Lanes 1 - 4: Merged signal (red and green). Green - **ab40855** observed at 58 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab40855 was shown to react with Smad2 in wild-type HeLa. Loss of signal was observed when knockout cell line ab255430 (knockout cell lysate **ab263833**) was used. Wild-type and Smad2 knockout samples were subjected to SDS-PAGE. **ab40855** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 2000 dilution and 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 SMAD2 knockout HeLa cell line (ab255430)

All lanes : Anti-Smad2 antibody [EP567Y] (**ab33875**) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : Jurkat cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : SMAD2 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 52 kDa
Additional bands at: 37 kDa (possible Loading Control)

Lanes 1 - 4: Merged signal (red and green). Green - **ab33875** observed at 58 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab33875 was shown to react with Smad2 in wild-type HeLa. Loss of signal was observed when knockout cell line ab255430 (knockout cell lysate **ab263833**) was used. Wild-type and Smad2 knockout samples were subjected to SDS-PAGE. **ab33875** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 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 SMAD2 knockout

HeLa cell line (ab255430)

Allele-1: 1 bp deletion in exon 2.

Sanger Sequencing - Human SMAD2 knockout

HeLa cell line (ab255430)

Allele-2: 1 bp insertion in exon 2.

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