abcam

Product datasheet

Human SMAD2 knockout HeLa cell lysate ab263833

5 Images

Overview

Product name	Human SMAD2 knockout HeLa cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon2 and 1 bp insertion in exon2.
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
Notes	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.
Tested applications	**Suitable for:** WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab255534 - Human SMAD2 knockout HeLa cell lysate	1 x 100µg
ab255929 - Human wild-type HeLa cell lysate	1 x 100µg

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

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.

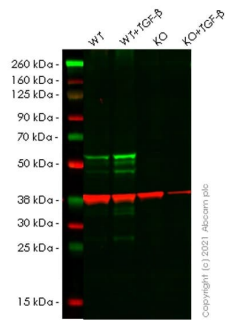
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab263833 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.

Images



Western blot - Human SMAD2 knockout HeLa cell lysate

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

Lane 1: Wild-type HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate, 20 ug

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

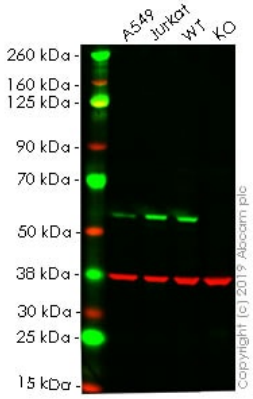
Lane 3: Smad2 knockout HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate, 20 ug

Lane 4: Smad2 knockout HeLa (human cervix adenocarcinoma epithelial cell), treated with 20 ng/ml TGF beta1 for 15 minutes, whole cell lysate, 20 ug

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 lysate (ab263833)

Lane 1: A549 cell lysate (20 µg)

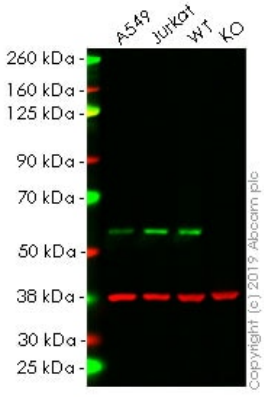
Lane 2: Jurkat cell lysate (20 µg)

Lane 3: Wild-type HeLa cell lysate (20 µg)

Lane 4: SMAD2 knockout HeLa cell lysate (20 µg)

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.



Western blot - Human SMAD2 knockout HeLa cell lysate (ab263833)

Lane 1: A549 cell lysate (20 µg)

Lane 2: Jurkat cell lysate (20 µg)

Lane 3: Wild-type HeLa cell lysate (20 µg)

Lane 4: SMAD2 knockout HeLa cell lysate (20 µg)

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.

Sanger Sequencing - Human SMAD2 knockout HeLa cell lysate (ab263833)

Allele-1: 1 bp deletion in exon2

Sanger Sequencing - Human SMAD2 knockout HeLa cell lysate (ab263833)

Allele-2: 1 bp insertion in exon2

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