abcam

Product datasheet

Human SMAD3 knockout A549 cell line ab263915

4 Images

Overview

Product name	Human SMAD3 knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 1 bp deletion; Frameshift = 100%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS)
Tested applications	**Suitable for:** WB
Biosafety level	1
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 A549 cell line (ab259777). 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:Hams F12 + 5% 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 $2x10^3$-$1x10^4$ 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 $6x10^4$ 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.
Do not exceed $7x10^4$ cells/cm^2.
This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our limited use license and patent pages.

Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Viability	~80%
Adherent /Suspension	Adherent
Tissue	Lung
Cell type	epithelial
Disease	Carcinoma
Gender	Male
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether
Purity	Immunogen affinity purified

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 SMAD3/SMAD4 complex, activates transcription. Also can form a SMAD3/SMAD4/JUN/FOS complex at the AP-1/SMAD site to regulate TGF-beta-mediated transcription. Has an inhibitory effect on wound healing probably by modulating both growth and migration of primary keratinocytes and by altering the TGF-mediated chemotaxis of monocytes. This effect on wound healing appears to be hormone-sensitive. Regulator of chondrogenesis and osteogenesis and inhibits early healing of bone fractures. Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator.
Involvement in disease	Colorectal cancer Loeys-Dietz syndrome 3
Sequence similarities	Belongs to the dwarfin/SMAD family. Contains 1 MH1 (MAD homology 1) domain. Contains 1 MH2 (MAD homology 2) domain.
Domain	The MH1 domain is required for DNA binding. Also binds zinc ions which are necessary for the DNA binding. The MH2 domain is required for both homomeric and heteromeric interactions and for transcriptional regulation. Sufficient for nuclear import. The linker region is required for the TGFbeta-mediated transcriptional activity and acts

synergistically with the MH2 domain.

Post-translational modifications

Phosphorylated on serine and threonine residues. Enhanced phosphorylation in the linker region on Thr-179, Ser-204 and Ser-208 on EGF and TGF-beta treatment. Ser-208 is the main site of MAPK-mediated phosphorylation. CDK-mediated phosphorylation occurs in a cell-cycle dependent manner and inhibits both the transcriptional activity and antiproliferative functions of SMAD3. This phosphorylation is inhibited by flavopiridol. Maximum phosphorylation at the G(1)/S junction. Also phosphorylated on serine residues in the C-terminal SXS motif by TGFBR1 and ACVR1. TGFBR1-mediated phosphorylation at these C-terminal sites is required for interaction with SMAD4, nuclear location and transactivational activity, and appears to be a prerequisite for the TGF-beta mediated phosphorylation in the linker region. Dephosphorylated in the C-terminal SXS motif by PPM1A. This dephosphorylation disrupts the interaction with SMAD4, promotes nuclear export and terminates TGF-beta-mediated signaling. Phosphorylation at Ser-418 by CSNK1G2/CK1 promotes ligand-dependent ubiquitination and subsequent proteasome degradation, thus inhibiting SMAD3-mediated TGF-beta responses. Phosphorylated by PDPK1. Acetylation in the nucleus by EP300 in the MH2 domain regulates positively its transcriptional activity and is enhanced by TGF-beta.
Ubiquitinated. Monoubiquitinated, leading to prevent DNA-binding. Deubiquitination by USP15 alleviates inhibition and promotes activation of TGF-beta target genes.
Poly-ADP-ribosylated by PARP1 and PARP2. ADP-ribosylation negatively regulates SMAD3 transcriptional responses during the course of TGF-beta signaling.

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 (PubMed:15799969). Through the action of the phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus by interaction with RANBP1 (PubMed:16751101, PubMed:19289081). Co-localizes with LEMD3 at the nucleus inner membrane (PubMed:15601644). MAPK-mediated phosphorylation appears to have no effect on nuclear import (PubMed:19218245). PDPK1 prevents its nuclear translocation in response to TGF-beta (PubMed:17327236).

Applications

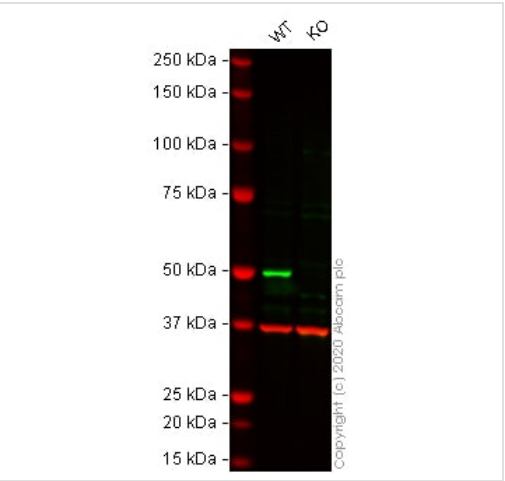
The Abpromise guarantee

Our Abpromise guarantee covers the use of ab263915 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. 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 SMAD3 knockout A549 cell line (ab263915)

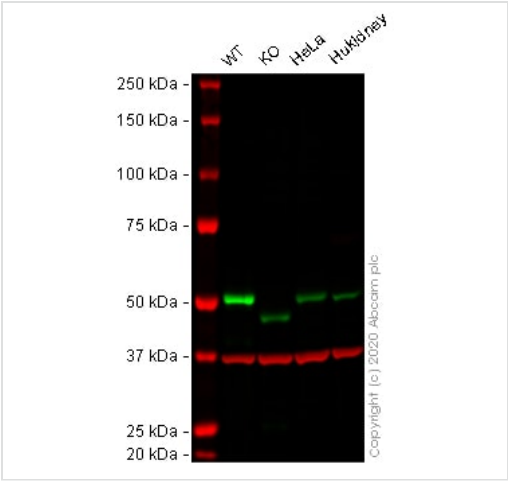
All lanes : Anti-Smad3 antibody (ab84177) at 1 µg/ml

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : SMAD3 knockout A549 (Human lung carcinoma cell line) whole cell lysate

Performed under reducing conditions.

Lanes 1 - 2: Merged signal (red and green). Green - ab84177 observed at 50 kDa. Red - loading control ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab84177 was shown to bind specifically to Smad3. A band was observed at 50 kDa in wild-type cell lysates with no signal at this size in Smad3 knockout cell line ab263915 (knockout cell lysate ab264513). Wild-type and SMAD3 knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab84177 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at 1 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human SMAD3 knockout A549 cell line (ab263915)

All lanes : Anti-Smad3 antibody [EPR19686] - ChIP Grade (ab208182) at 1/1000 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : SMAD3 knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : Human Kidney cell lysate

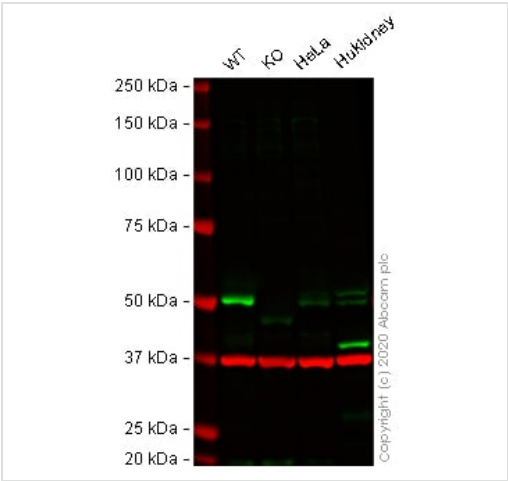
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 50 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab208182 observed at 50 kDa. Red - loading control, ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab208182 was shown to bind specifically to Smad3. A band was observed at 50 kDa in wild-type cell lysates with no signal at this size in Smad3 knockout cell line ab263915 (knockout cell lysate ab264513). Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab208182 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human SMAD3 knockout A549 cell line (ab263915)

All lanes : Anti-Smad3 antibody [EP568Y] (ab40854) at 1/1000 dilution

Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : SMAD3 knockout A549 (Human lung carcinoma cell line) whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : Human Kidney cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 50 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab40854 observed at 48 kDa. Red - loading control, ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab40854 was shown to bind specifically to Smad3. A band was observed at 50 kDa in wild-type cell lysates with no signal at this size in Smad3 knockout cell line ab263915 (knockout cell lysate ab264513). Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab40854 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Next Generation Sequencing - Human SMAD3 knockout A549 cell line (ab263915)

Knockout achieved by CRISPR/Cas9; X = 1 bp deletion; Frameshift = 100%

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