abcam

Product datasheet

Human SMURF2 knockout HeLa cell lysate ab257691

3 Images

Overview

Product name	Human SMURF2 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 insertion in exon5 and 2 bp deletion in exon5.
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
ab261041 - Human SMURF2 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 E3 ubiquitin-protein ligase which accepts ubiquitin from an E2 ubiquitin-conjugating enzyme in the form of a thioester and then directly transfers the ubiquitin to targeted substrates. Interacts with SMAD1 and SMAD7 in order to trigger their ubiquitination and proteasome-dependent degradation. In addition, interaction with SMAD7 activates autocatalytic degradation, which is prevented by interaction with SCYE1. Forms a stable complex with the TGF-beta receptor-mediated phosphorylated SMAD2 and SMAD3. In this way, SMAD2 may recruit substrates, such as SNON, for ubiquitin-mediated degradation. Enhances the inhibitory activity of SMAD7 and reduces the transcriptional activity of SMAD2. Coexpression of SMURF2 with SMAD1 results in considerable decrease in steady-state level of SMAD1 protein and a smaller decrease of SMAD2 level.

Tissue specificity Widely expressed.

Pathway Protein modification; protein ubiquitination.

Sequence similarities Contains 1 C2 domain.
Contains 1 HECT (E6AP-type E3 ubiquitin-protein ligase) domain.
Contains 3 WW domains.

Domain The second and third WW domains are responsible for interaction with the PY-motif of R-SMAD (SMAD1, SMAD2 and SMAD3).
The C2 domain is involved in autoinhibition of the catalytic activity by interacting with the HECT domain.

Post-translational modifications Auto-ubiquitinated and ubiquitinated in the presence of RNF11 and UBE2D1.

Cellular localization Nucleus. Cytoplasm. Cell membrane. Membrane raft. Cytoplasmic in the presence of SMAD7. Co-localizes with CAV1, SMAD7 and TGF-beta receptor in membrane rafts.

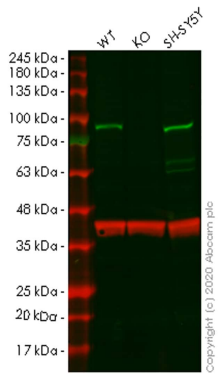
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257691 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: 86 kDa.

Images



Western blot - Human SMURF2 knockout HeLa cell lysate (ab257691)

Lane 1: Wild-type HeLa cell lysate (20 ug)
Lane 2: SMURF2 knockout HeLa cell lysate (20 ug)
Lane 3: SH-SY5Y cell lysate (20 ug)

ab53316 was shown to specifically react with SMURF 2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265231** (knockout cell lysate ab257691) was used. Wild-type and SMURF 2 knockout samples were subjected to SDS-PAGE. **ab53316** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4^{o}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 SMURF2 knockout HeLa cell lysate (ab257691)

Allele-1: 2 bp deletion in exon5

Sanger Sequencing - Human SMURF2 knockout HeLa cell lysate (ab257691)

Allele-2: 1 bp insertion in exon5

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