abcam

Product datasheet

Human ROCK2 knockout HeLa cell lysate ab257643

3 Images

Overview

Product name	Human ROCK2 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 exon4 and 59 bp deletion in exon4.
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 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**.
Tested applications	**Suitable for:** WB

Properties

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

Components	1 kit
ab262172 - Human ROCK2 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 Regulates the assembly of the actin cytoskeleton. Promotes formation of stress fibers and of focal adhesion complexes. Plays a role in smooth muscle contraction.

Sequence similarities Belongs to the protein kinase superfamily. AGC Ser/Thr protein kinase family.
Contains 1 AGC-kinase C-terminal domain.
Contains 1 PH domain.
Contains 1 phorbol-ester/DAG-type zinc finger.
Contains 1 protein kinase domain.
Contains 1 REM (Hr1) repeat.

Post-translational modifications Phosphorylated upon DNA damage, probably by ATM or ATR.

Cellular localization Cytoplasm. Cell membrane. Cytoplasmic, and associated with actin microfilaments and the plasma membrane.

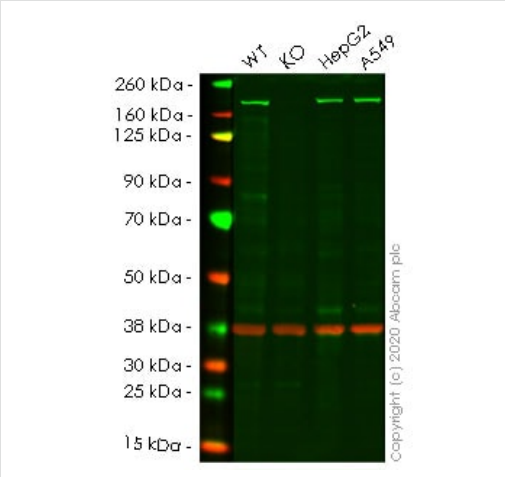
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257643 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: 161 kDa.

Images



Western blot - Human ROCK2 knockout HeLa cell lysate (ab257643)

Lane 1: Wild-type HeLa cell lysate (20µg)

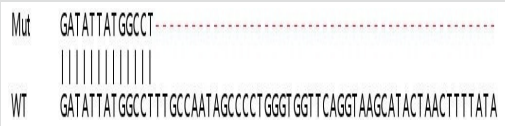
Lane 2: ROCK2 knockout HeLa cell lysate (20µg)

Lane 3: HepG2 cell lysate (20µg)

Lane 4: A549 cell lysate (20µg)

Lanes 1- 4: Merged signal (red and green). Green - **ab125025** observed at 175 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab125025 Anti-ROCK2 antibody [EPR7141(B)] was shown to specifically react with ROCK2 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265679** (knockout cell lysate ab257643) was used. Wild-type and ROCK2 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab125025** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 10000 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 ROCK2 knockout HeLa cell lysate (ab257643)

Allele-1: 59 bp deletion in exon4

Sanger Sequencing - Human ROCK2 knockout HeLa cell lysate (ab257643)

Allele-2: 1 bp deletion in exon4

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