abcam

Product datasheet

Human ARHGEF2 (GEF H1) knockout HeLa cell lysate ab257223

2 Images

Overview

Product name	Human ARHGEF2 (GEF H1) 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 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, 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
ab260912 - Human ARHGEF2 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 Activates Rho-GTPases by promoting the exchange of GDP for GTP. May be involved in epithelial barrier permeability, cell motility and polarization, dendritic spine morphology, antigen presentation, leukemic cell differentiation, cell cycle regulation, and cancer. Binds Rac-GTPases, but does not seem to promote nucleotide exchange activity toward Rac-GTPases, which was uniquely reported in PubMed:9857026. May stimulate instead the cortical activity of Rac. Inactive toward CDC42, TC10, or Ras-GTPases. Forms an intracellular sensing system along with NOD1 for the detection of microbial effectors during cell invasion by pathogens. Required for RHOA and RIP2 dependent NF-kappaB signaling pathways activation upon S.flexneri cell invasion. Involved not only in sensing peptidoglycan (PGN)-derived muropeptides through NOD1 that is independent of its GEF activity, but also in the activation of NF-kappaB by Shigella effector proteins (IpgB2 and OspB) which requires its GEF activity and the activation of RhoA.

Sequence similarities Contains 1 DH (DBL-homology) domain.
Contains 1 PH domain.
Contains 1 phorbol-ester/DAG-type zinc finger.

Domain The DH (DBL-homology) domain interacts with and promotes loading of GTP on RhoA.
The PH (pleckstrin-homology) domain is involved in microtubule binding and targeting to tight junctions.

Post-translational modifications Phosphorylation of Ser-886 by PAK1 induces binding to protein 14-3-3 zeta, promoting its relocation to microtubules and the inhibition of its activity. Phosphorylated by STK6 and CDK1 during mitosis, which negatively regulates its activity. Phosphorylation by MAPK1 or MAPK3 increases nucleotide exchange activity. Phosphorylation by PAK4 releases GEF-H1 from the microtubules.

Cellular localization Cytoplasm. Cell junction > tight junction. Golgi apparatus. Cytoplasm > cytoskeleton > spindle. Cell projection > ruffle membrane. Localizes to the tips of cortical microtubules of the mitotic spindle during cell division, and is further released upon microtubule depolymerization. Recruited into membrane ruffles induced by S.flexneri at tight junctions of polarized epithelial cells.

Applications

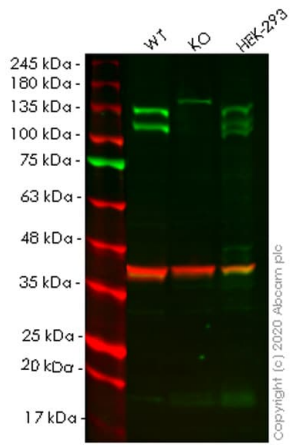
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257223 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: 111 kDa.

Images



Western blot - Human ARHGEF2 knockout HeLa cell lysate (ab257223)

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

Lane 2: ARHGEF2 knockout HeLa cell lysate (20 µg)

Lane 3: HEK293 cell lysate (20 µg)

Lanes 1-3: Merged signal (red and green). Green - **ab201687** observed at 75 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab201687 Anti-GEF H1 antibody [EPR17963] - C-terminal was shown to specifically react with GEF H1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265797** (knockout cell lysate ab257223) was used. Wild-type and GEF H1 knockout samples were subjected to SDS-PAGE. **ab201687** 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 10000 dilution for 1 hour at room temperature before imaging.

Sanger Sequencing - Human ARHGEF2 knockout HeLa cell lysate (ab257223)

Homozygous: 1 bp insertion in exon4

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