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Product datasheet

Human RHOA knockout HEK-293T cell lysate ab257637

3 Images

Overview

Product name	Human RHOA knockout HEK-293T cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 4 bp insertion in exon 2 and Insertion of the selection cassette in exon 2.
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
ab260309 - Human RHOA knockout HEK293T cell lysate	1 x 100µg
ab255553 - Human wild-type HEK293T cell lysate	1 x 100µg

Cell type epithelial

STR Analysis Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12

Target

Function Regulates a signal transduction pathway linking plasma membrane receptors to the assembly of focal adhesions and actin stress fibers. Serves as a target for the yopT cysteine peptidase from Yersinia pestis, vector of the plague, and Yersinia pseudotuberculosis, which causes gastrointestinal disorders. May be an activator of PLCE1. Activated by ARHGEF2, which promotes the exchange of GDP for GTP.

Sequence similarities Belongs to the small GTPase superfamily. Rho family.

Domain The basic-rich region is essential for yopT recognition and cleavage.

Post-translational modifications Substrate for botulinum ADP-ribosyltransferase.
Cleaved by yopT protease when the cell is infected by some Yersinia pathogens. This removes the lipid attachment, and leads to its displacement from plasma membrane and to subsequent cytoskeleton cleavage.
AMPylation at Tyr-34 and Thr-37 are mediated by bacterial enzymes in case of infection by H.somnus and V.parahaemolyticus, respectively. AMPylation occurs in the effector region and leads to inactivation of the GTPase activity by preventing the interaction with downstream effectors, thereby inhibiting actin assembly in infected cells. It is unclear whether some human enzyme mediates AMPylation; FICD has such ability in vitro but additional experiments remain to be done to confirm results in vivo.
Ubiquitinated by the BCR(BACURD1) and BCR(BACURD2) E3 ubiquitin ligase complexes, leading to its degradation by the proteasome, thereby regulating the actin cytoskeleton and cell migration.

Cellular localization Cell membrane. Cytoplasm > cytoskeleton.

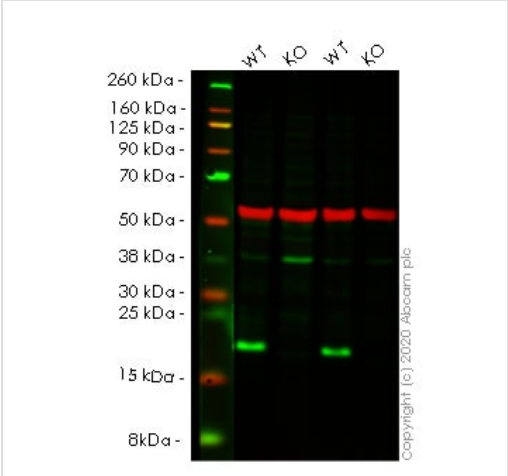
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257637 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: 22 kDa.

Images



Western blot - Human RHOA knockout HEK293T cell lysate (ab257637)

Lane 1: Wild-type HEK-293T cell lysate (20µg)

Lane 2: RHOA knockout HEK-293T cell lysate (20µg)

Lane 3: Wild-type HEK-293T cell lysate (20µg)

Lane 4: RHOA knockout HEK-293T cell lysate (20µg)

Lanes 1- 4: Merged signal (red and green). Green - **ab187027** observed at 21 kDa. Red - loading control **ab7291** observed at 50 kDa.

ab187027 Anti-RhoA antibody [EPR18134] was shown to specifically react with RhoA in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266592** (knockout cell lysate ab257637) was used. Wild-type and RhoA 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. **ab187027** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated overnight at 4°C at 1 in 5000 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 RHOA knockout HEK293T cell lysate (ab257637)

Allele-1: 4 bp insertion in exon 2

Sanger Sequencing - Human RHOA knockout HEK293T cell lysate (ab257637)

Allele-2: Insertion of the selection cassette in exon 2

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