abcam

Product datasheet

Human RALA knockout HeLa cell lysate ab258165

2 Images

Overview

Product name	Human RALA 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 exon2.
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
ab262328 - Human RALA 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 Multifuntional GTPase involved in a variety of cellular processes including gene expression, cell migration, cell proliferation, oncogenic transformation and membrane trafficking. Accomplishes its multiple functions by interacting with distinct downstream effectors. Acts as a GTP sensor for GTP-dependent exocytosis of dense core vesicles. Plays a role in the early stages of cytokinesis and is required to tether the exocyst to the cytokinetic furrow. The RALA-exocyst complex regulates integrin-dependent membrane raft exocytosis and growth signaling. Key regulator of LPAR1 signaling and competes with ADRBK1 for binding to LPAR1 thus affecting the signaling properties of the receptor. Required for anchorage-independent proliferation of transformed cells.

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

Post-translational modifications Prenylation is essential for membrane localization. The geranylgeranylated form and the farnesylated mutant does not undergo alternative prenylation in response to geranylgeranyltransferase I inhibitors (GGTIs) and farnesyltransferase I inhibitors (FTIs).

Cellular localization Cell surface. Cell membrane. Cleavage furrow. Midbody. Prior to LPA treatment found predominantly at the cell surface and in the presence of LPA co-localizes with LPAR1 and LPAR2 in the endocytic vesicles. During early cytokinesis localizes at the cleavage furrow membrane. Colocalizes with EXOC2 at the early midbody ring and persists there till maturation of the midbody.

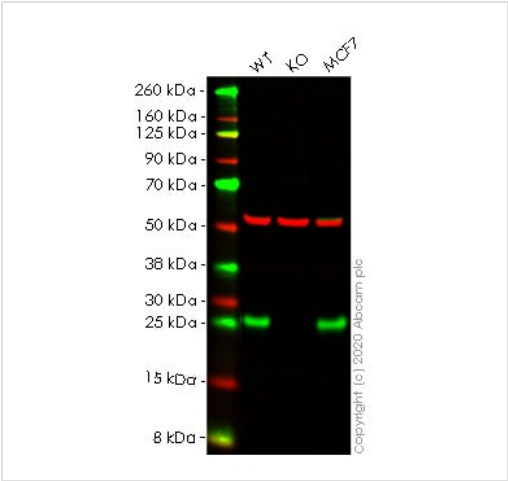
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab258165 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: 24 kDa.

Images



Western blot - Human RALA knockout HeLa cell lysate (ab258165)

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

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

Lane 3: MCF7 cell lysate (20 µg)

Lanes 1-3: Merged signal (red and green). Green - **ab126627** observed at 25 kDa. Red - loading control **ab7291** observed at 50 kDa.

ab126627 Anti-RALA antibody [EPR6468] was shown to specifically react with RALA in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265092** (knockout cell lysate ab258165) was used. Wild-type and RALA knockout samples were subjected to SDS-PAGE. **ab126627** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) 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 20000 dilution for 1 hour at room temperature before imaging.

Sanger Sequencing - Human RALA knockout HeLa cell lysate (ab258165)

Homozygous: 1 bp deletion in exon2

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