abcam

Product datasheet

Human HRAS knockout HEK-293 cell lysate ab261650

1 Image

Overview

Product name	Human HRAS knockout HEK-293 cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HEK-293
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 1 bp insertion
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), 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

Properties

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

Components	1 kit
ab280400 - Human HRAS knockout HEK293 cell lysate	1 x 100µg
ab259780 - Human wild-type HEK-293 cell lysate	1 x 100µg

Cell type epithelial

Gender Female

Target

Function Ras proteins bind GDP/GTP and possess intrinsic GTPase activity.

Tissue specificity Widely expressed.

Involvement in disease Costello syndrome
Congenital myopathy with excess of muscle spindles
Thyroid cancer, non-medullary, 2
Mutations which change positions 12, 13 or 61 activate the potential of HRAS to transform cultured cells and are implicated in a variety of human tumors.
Bladder cancer
Schimmelpenning-Feuerstein-Mims syndrome

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

Post-translational modifications Palmitoylated by the ZDHHC9-GOLGA7 complex. A continuous cycle of de- and re-palmitoylation regulates rapid exchange between plasma membrane and Golgi.
S-nitrosylated; critical for redox regulation. Important for stimulating guanine nucleotide exchange. No structural perturbation on nitrosylation.
The covalent modification of cysteine by 15-deoxy-Delta12,14-prostaglandin-J2 is autocatalytic and reversible. It may occur as an alternative to other cysteine modifications, such as S-nitrosylation and S-palmitoylation.
Acetylation at Lys-104 prevents interaction with guanine nucleotide exchange factors (GEFs).

Cellular localization Cell membrane. Cell membrane. Golgi apparatus. Golgi apparatus membrane. The active GTP-bound form is localized most strongly to membranes than the inactive GDP-bound form (By similarity). Shuttles between the plasma membrane and the Golgi apparatus and Nucleus. Cytoplasm. Cytoplasm, perinuclear region. Colocalizes with RACK1 to the perinuclear region.

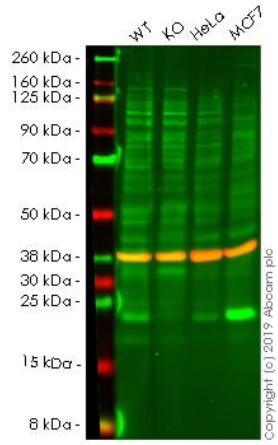
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab261650 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.

Images



Western blot - Human HRAS knockout HEK293 cell lysate (ab261650)

Lane 1: Wild-type HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate 20 ug/ml

Lane 2: HRAS knockout HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate 20 ug/ml

Lane 3: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate 20 ug/ml

Lane 4: MCF7 (Human breast adenocarcinoma cell line) whole cell lysate 20 ug/ml

Lanes 1 - 4: Merged signal (red and green). Green - **ab32417** observed at 21 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab32417 was shown to recognize HRAS in wild-type HEK-293 cells as signal was lost at the expected MW in HRAS knockout cell line **ab260858** (knockout cell lysate ab261650). Additional cross-reactive bands were observed in the wild-type and knockout samples. Wild-type and HRAS knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% milk. Ab32417 and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/500 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

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