abcam

Product datasheet

Human RANBP2 knockout HeLa cell lysate ab257627

3 Images

Overview

Product name Human RANBP2 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, 211 bp insertion in exon1 and 82 bp insertion in

exon1.

Passage number <20

Knockout validation Sanger Sequencing, Western Blot (WB)

Reconstitution notesTo 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.

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and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the

licenses and patents please refer to our <u>limited use license</u> and <u>patent pages</u>.

Tested applications Suitable for: WB

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Properties

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

Components	1 kit
ab262167 - Human RANBP2 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 E3 SUMO-protein ligase which facilitates SUMO1 and SUMO2 conjugation by UBE2I. Involved in

transport factor (Ran-GTP, karyopherin)-mediated protein import via the F-G repeat-containing domain which acts as a docking site for substrates. Could also have isomerase or chaperone activity and may bind RNA or DNA. Component of the nuclear export pathway. Specific docking

site for the nuclear export factor exportin-1.

Pathway Protein modification; protein sumoylation.

Involvement in diseaseDefects in RANBP2 are the cause of susceptibility to encephalopathy acute necrotizing type 1

(ANE1) [MIM:608033]. A rapidly progressive encephalopathy manifesting in susceptibile individuals with seizures and coma. It can occur within days in otherwise healthy children after common viral infections such as influenza and parainfluenza, without evidence of viral infection of the brain or inflammatory cell infiltration. Brain T2-weighted magnetic resonance imaging reveals

characteristic symmetric lesions present in the thalami, pons and brainstem.

Sequence similaritiesContains 1 PPlase cyclophilin-type domain.

Contains 4 RanBD1 domains.

Contains 8 RanBP2-type zinc fingers.

Contains 1 TPR repeat.

Domain Contains F-X-F-G repeats.

Domain Contains 1 X 1 Cropeats

Post-translational modifications

Polyubiquitinated by PARK2, which leads to proteasomal degradation.

Cellular localization Nucleus > nuclear pore complex. Cytoplasmic filaments.

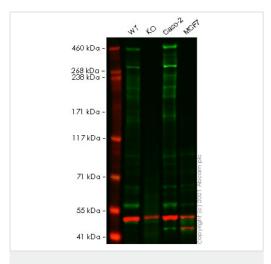
Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab257627 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 RANBP2 knockout HeLa cell lysate (ab257627)

Lane 1: Wild-type HeLa cell lysate 20 µg

Lane 2: RANBP2 knockout HeLa cell lysate 20 µg

Lane 3: Caco2 cell lysate 20 µg

Lane 4: MCF7 cell lysate 20 µg

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab64276</u> observed at 450 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab64276 was shown to react with RanBP2 in wild-type HeLa cells in Western blot with loss of signal observed in RANBP2 knockout cell line ab265618 (RANBP2 knockout cell lysate ab257627). Wild-type HeLa and RANBP2 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab64276 and ab7291 (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1:1000 dilution and 1:20000 dilution respectively. Blots were incubated with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1:20000 dilution for 1 h at room temperature before imaging.

Allele-1: 82 bp insertion in exon1

Mut	AGGCGCAGCAAGGCTGACGTGGAGCGGTACATCGGGCAGTACATCAATGGGCGTGGACAG
Sa	anger Sequencing - Human RANBP2 knockout

HeLa cell lysate (ab257627)

Allele-2: 211 bp insertion in exon1

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