abcam

Product datasheet

Human RNF2 (RING2 / RING1B) knockout HeLa cell lysate ab257640

3 Images

Overview

Product name	Human RNF2 (RING2 / RING1B) knockout HeLa cell lysate
Product overview	Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 8 bp deletion in exon 2 and Insertion of the selection cassette in exon 2.
Passage number	<20
Knockout validation	Sanger Sequencing
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
ab260311 - Human RNF2 knockout HeLa cell lysate	1 x 100µg
ab255552 - 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 ubiquitin-protein ligase that mediates monoubiquitination of 'Lys-119' of histone H2A, thereby playing a central role in histone code and gene regulation. H2A 'Lys-119' ubiquitination gives a specific tag for epigenetic transcriptional repression and participates in X chromosome inactivation of female mammals. May be involved in the initiation of both imprinted and random X inactivation. Essential component of the Polycomb group (PcG) multiprotein PRC1 complex, a complex required to maintain the transcriptionally repressive state of many genes, including Hox genes, throughout development. PcG PRC1 complex act via chromatin remodeling and modification of histones, rendering chromatin heritably changed in its expressibility. E3 ubiquitin-protein ligase activity is enhanced by BMI1/PCGF4. Acts as the main E3 ubiquitin ligase on histone H2A of the PRC1 complex, while RING1 may rather act as a modulator of RNF2/RING2 activity.

Pathway Protein modification; protein ubiquitination.

Sequence similarities Contains 1 RING-type zinc finger.

Post-translational modifications Polyubiquitinated in the presence of UBE2D3 (in vitro).
Monoubiquitinated, by auto-ubiquitination.

Cellular localization Nucleus. Chromosome. Enriched on inactive X chromosome (Xi) in female trophoblast stem (TS) cells as well as differentiating embryonic stem (ES) cells. The enrichment on Xi is transient during TS and ES cell differentiation. The association with Xi is mitotically stable in non-differentiated TS cells.

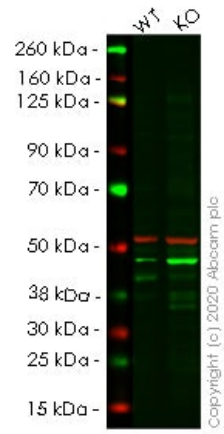
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257640 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: 37 kDa. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.

Images



Western blot - Human RNF2 (RING2 / RING1B) knockout HeLa cell lysate (ab257640)

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

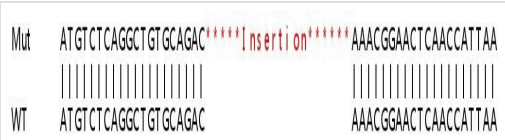
Lane 2: RNF2 knockout HeLa cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab181140** observed at 42 kDa. Red - loading control, **ab7291** observed at 50 kDa.

ab181140 Anti-RING2 / RING1B / RNF2 antibody [EPR12245] was shown to specifically react with RING2 / RING1B / RNF2 in wild-type HeLa cells in western blot. The band observed in the knockout cell line **ab264845** (knockout cell lysate ab257640) lane below 42kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type and RING2 / RING1B / RNF2 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. **ab181140** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated overnight at 4°C at 1 in 1000 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 RNF2 knockout HeLa cell lysate (ab257640)

Allele-1: 8 bp deletion in exon 2



Sanger Sequencing - Human RNF2 knockout HeLa cell lysate (ab257640)

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

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