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.

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Tested applications

Suitable for: WB

Properties

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

<table>
<thead>
<tr>
<th>Components</th>
<th>1 kit</th>
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<tbody>
<tr>
<td>ab260311 - Human RNF2 knockout HeLa cell lysate</td>
<td>1 x 100µg</td>
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<tr>
<td>ab255552 - Human wild-type HeLa cell lysate</td>
<td>1 x 100µg</td>
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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.
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.

Allele-1: 8 bp deletion in exon 2

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>WB</td>
<td></td>
<td>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.</td>
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Sanger Sequencing - Human RNF2 knockout HeLa cell lysate (ab257640)

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

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Mud: ATGCTCAAGCTGACAAAAAAAACCTCAAACCTTAAG

WT: ATGCTCAAGCTGACAAAAAAAACCTCAAACCTTAAG
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