abcam

Product datasheet

Human BMI1 knockout MCF7 cell lysate ab256851

4 Images

Overview

Product name Human BMI1 knockout MCF7 cell lysate

Product overview

Knockout cell lysate achieved by CRISPR/Cas9.

Parental Cell Line MCF7

Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon2 and 1 bp insertion 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

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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 limited use license and patent pages.

Tested applications Suitable for: WB

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Properties

Storage instructions

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

Components	1 kit
ab263505 - Human BMI1 knockout MCF7 cell lysate	1 x 100µg
ab263912 - Human wild-type MCF7 cell lysate	1 x 100µg

Cell type

epithelial

Disease

Adenocarcinoma

Target

Function

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 acts via chromatin remodeling and modification of histones; it mediates monoubiquitination of histone H2A 'Lys-119', rendering chromatin heritably changed in its expressibility. In the PRC1 complex, it is required to stimulate the E3 ubiquitin-protein ligase activity of RNF2/RING2.

Sequence similarities

Contains 1 RING-type zinc finger.

Post-translational modifications

Monoubiquitinated (By similarity). May be polyubiquitinated; which does not lead to proteasomal

degradation.

Cellular localization

Nucleus. Cytoplasm.

Applications

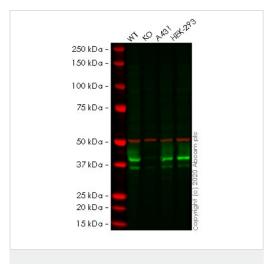
The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab256851 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: 36 kDa.

Images



Western blot - Human BMI1 knockout MCF7 cell lysate (ab256851)

Lane 1: Wild-type MCF7 cell lysate (20µg)

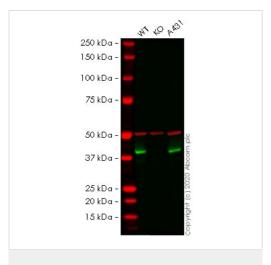
Lane 2: BMI1 knockout MCF7 cell lysate (20µg)

Lane 3: A431 cell lysate (20µg)

Lane 4: HEK-293 cell lysate (20µg)

Lanes 1-4: Merged signal (red and green). Green - <u>ab269678</u> observed at 36 kDa. Red - loading control <u>ab52866</u> observed at 50 kDa.

ab269678 Mouse monoclonal [BMI1/2823] to Bmi1 was shown to specifically react with Bmi1 in wild-type MCF7 cells in western blot. Loss of signal was observed when knockout cell line ab262319 (knockout cell lysate ab256851) was used. Wild-type and Bmi1 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% nonfat dried milk. ab269678 and Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) were incubated overnight at 4°C at 2 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human BMI1 knockout MCF7 cell lysate (ab256851)

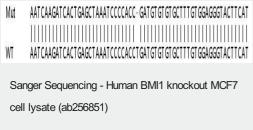
Lane 1: Wild-type MCF7 cell lysate (20µg)

Lane 2: BMI1 knockout MCF7 cell lysate (20µg)

Lane 3: A431 cell lysate (20µg)

Lanes 1-3: Merged signal (red and green). Green - ab126783 observed at 37 kDa. Red - loading control ab7291 observed at 50 kDa.

ab126783 Rabbit monoclonal [EPR3745(2)] to Bmi1 was shown to specifically react with Bmi1 in wild-type MCF7 cells in western blot. Loss of signal was observed when knockout cell line ab262319 (knockout cell lysate ab256851) was used. Wild-type and Bmi1 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% nonfat dried milk. ab126783 and Anti-alpha Tubulin antibody [DM1A] -Loading Control (ab7291) were incubated overnight at 4 °C at 1 in 10000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG 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: 1 bp deletion in exon2

Mut	AATCAAGATCACTGAGCTAAATCCCCACC <mark>G</mark> TGATGTGTGTGCTTTGTGGAGGGTACTTCA			
WT	AATCAAGATCACTGAGCTAAATCCCCACC TGATGTGTGTGCTTTGTGGAGGGTACTTCA			
Sanger Sequencing - Human BMI1 knockout MCF7				
cell lysate (ab256851)				

Allele-2: 1 bp insertion in exon2

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