abcam

Product datasheet

Human DDIT3 knockout SW480 cell lysate ab270708

2 Images

Overview

Product name Human DDIT3 knockout SW480 cell lysate

Product overview Knockout cell lysate achieved by CRISPR/Cas9.

Parental Cell Line SW480
Organism Human

Mutation description Knockout achieved by CRISPR/Cas9; X = 1 bp deletion; Frameshift: 98.84%

Passage number <20

Knockout validation Next Generation Sequencing (NGS), 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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relevant patents please refer to our <u>limited use license</u> and <u>patent pages</u>.

Tested applications Suitable for: WB

Dranartica

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Properties

Storage instructions

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

Components	1 kit
ab280589 - Human DDIT3 knockout SW480 cell lysate	1 x 100μg
ab269601 - Human wild-type SW480 cell lysate	1 x 100μg

Cell type epithelial

Disease Adenocarcinoma

Gender Male

Target

Function Inhibits the DNA-binding activity of C/EBP and LAP by forming heterodimers that cannot bind

DNA.

Involvement in disease Note=A chromosomal aberration involving DDIT3 is found in a patient with malignant myxoid

liposarcoma. Translocation t(12;16)(q13;p11) with FUS.

Sequence similarities Belongs to the bZIP family.

Contains 1 bZIP domain.

Cellular localization Nucleus.

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab270708 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: 19 kDa.

Images

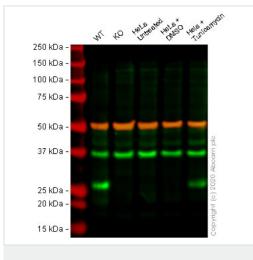
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GARACTETCCAGCTGGAAGCTGGAAGCCTGGT-TGAGGACCTGCAAGAGGTCCTGTCTTCAGATGAAAATGGG Deletim, 42962 reads, 44.53%

Next Generation Sequencing - Human DDIT3 knockout SW480 cell lysate (ab270708)

Knockout achieved by CRISPR/Cas9; X = 1 bp deletion;

Frameshift: 98.84%



Western blot - Human DDIT3 knockout SW480 cell lysate (ab270708)

Lane 1: Wild-type SW480 cell lysate 20 ug

Lane 2: DDIT3 knockout SW480 cell lysate 20 ug

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

Lane 4: HeLa + DMSO control cell lysate 20 ug

Lane 5: HeLa + tunicamycin (20ug/mL,4 hours) cell lysate 20 ug

Lanes 1 - 5: Merged signal (red and green). Green - <u>ab11419</u> observed at 26 kDa. Red - loading control <u>ab52866</u> (Rabbit antialpha Tubulin antibody [EP1332Y]) observed at 55kDa.

ab11419 was shown to react with DDIT3 in wild-type SW480 cells in western blot with loss of signal observed in DDIT3 knockout cell line ab269585 (knockout cell lysate ab270708). Wild-type and DDIT3 knockout SW480 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween[®]) before incubation with ab11419 and ab52866 (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4°C at 5 μg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse lgG H&L (IRDye[®] 800CW) preabsorbed (ab216772) and Goat anti-Rabbit lgG H&L (IRDye[®] 680RD) preabsorbed (ab216777) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

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