abcam

Product datasheet

Human CD27A knockout Raji cell lysate ab281360

3 Images

Overview

Product name Human CD27A knockout Raji cell lysate

Product overview Knockout cell lysate achieved by CRISPR/Cas9.

Parental Cell Line Raji

Organism Human

Mutation description Knockout achieved by CRISPR/Cas9; X = 10 bp deletion; Frameshift: 100%

Passage number <20

Knockout validation Next Generation Sequencing (NGS), 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

Authorisation, and any other relevant authorisations, for their intended uses.

This product is subject to limited use licenses from The Broad Institute and ERS Genomics

Limited, and is developed with patented technology. For full details of the limited use licenses and

relevant 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
ab281412 - Human CD27A knockout Raji cell lysate	1 x 100µg
ab269600 - Human wild-type Raji cell lysate	1 x 100µg

Cell type Burkitt's lymphoma

Disease Lymphoma

Gender Male

Target

Function Receptor for CD70/CD27L. May play a role in survival of activated T-cells. May play a role in

apoptosis through association with SIVA1.

Tissue specificity Found in most T-lymphocytes.

Sequence similarities Contains 3 TNFR-Cys repeats.

Post-translational

Phosphorylated and O-glycosylated.

modifications

Cellular localization Membrane.

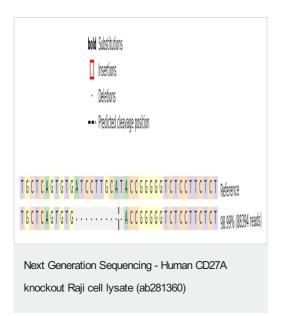
Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab281360 in the following tested applications.

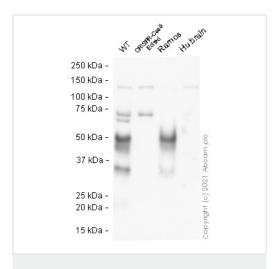
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration.

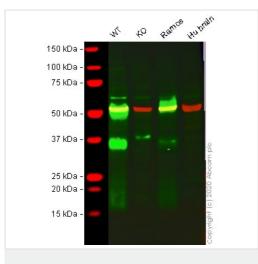
Images



Knockout achieved by CRISPR/Cas9; X = 10 bp deletion; Frameshift: 100%



Western blot - Human CD27A knockout Raji cell lysate



Western blot - Human CD27A knockout Raji cell lysate (ab281360) Lane 1: Wild-type Raji cell lysate (20 ug)

Lane 2: CD27A knockout Raji cell lysate (20 ug)

Lane 3: Ramos cell lysate (20 ug)

Lane 4: Human brain cell lysate (20 ug)

<u>ab133761</u> was shown to react with CD27 in wild-type Raji cells in western blot. Loss of signal was observed when CD27 knockout cell line <u>ab274910</u> (knockout cell lysate ab281360) was used. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween[®]) before incubation with <u>ab133761</u> overnight at 4 °C at a 1 in 1000 dilution. Blots were incubated with HRP conjugated Goat anti-Rabbit (H+L) secondary antibody at 1 in 5000 for 1 hour at room temperature before development with Optiblot ECL reagent (<u>ab133456</u>) and imaging.

Lane 1: Wild-type Raji (Human Burkitt's lymphoma cell line) whole cell lysate 20 ug

Lane 2: CD27 knockout Raji (Human Burkitt's lymphoma cell line) whole cell lysate 20 ug

Lane 3: Ramos (Human Burkitt's lymphoma cell line) whole cell lysate 20 ug

Lane 4: Human brain tissue lysate 20 ug

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab131254</u> observed at 35 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab131254 was shown to react with CD27 in wild-type Raji cells in western blot with loss of signal observed in CD27 knockout cell line ab274910 (knockout cell lysate ab281360). Wild-type and CD27 knockout Raji cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab131254 and ab7291 (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 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 in 20000 dilution for 1 hour at room temperature before imaging.

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