abcam

Product datasheet

Human NFATC2 knockout Raji cell lysate ab282940

3 Images

Overview

Product name	Human NFATC2 knockout Raji 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	Raji
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 122 bp deletion and 3 bp insertion 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
ab283084 - Human NFATC2 knockout Raji cell lysate	1 x 100µg
ab277365 - Human wild-type Raji cell lysate	1 x 100µg

Cell type Burkitt's lymphoma

Disease Lymphoma

Gender Male

Target

Function Plays a role in the inducible expression of cytokine genes in T-cells, especially in the induction of the IL-2, IL-3, IL-4, TNF-alpha or GM-CSF.

Tissue specificity Expressed in thymus, spleen, heart, testis, brain, placenta, muscle and pancreas.

Sequence similarities Contains 1 RHD (Rel-like) domain.

Domain Rel Similarity Domain (RSD) allows DNA-binding and cooperative interactions with AP1 factors.

Post-translational modifications In resting cells, phosphorylated by NFATC-kinase on at least 18 sites in the 99-363 region. Upon cell stimulation, all these sites except Ser-243 are dephosphorylated by calcineurin. Dephosphorylation induces a conformational change that simultaneously exposes an NLS and masks an NES, which results in nuclear localization. Simultaneously, Ser-53 or Ser-56 is phosphorylated; which is required for full transcriptional activity.

Cellular localization Cytoplasm. Nucleus. Cytoplasmic for the phosphorylated form and nuclear after activation that is controlled by calcineurin-mediated dephosphorylation. Rapid nuclear exit of NFATC is thought to be one mechanism by which cells distinguish between sustained and transient calcium signals. The subcellular localization of NFATC plays a key role in the regulation of gene transcription.

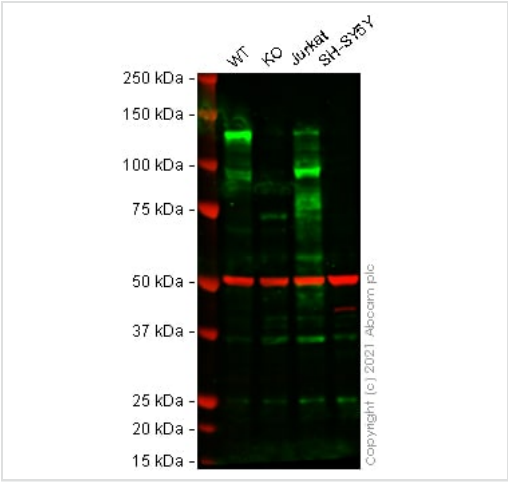
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab282940 in the following tested applications.

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

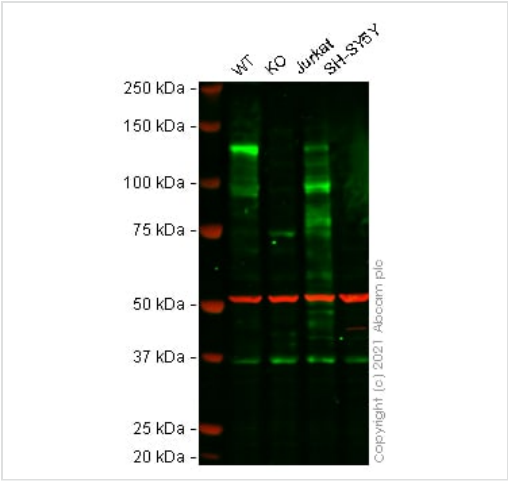
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.

Images



Western blot - Human NFATC2 knockout Raji cell lysate (ab282940)

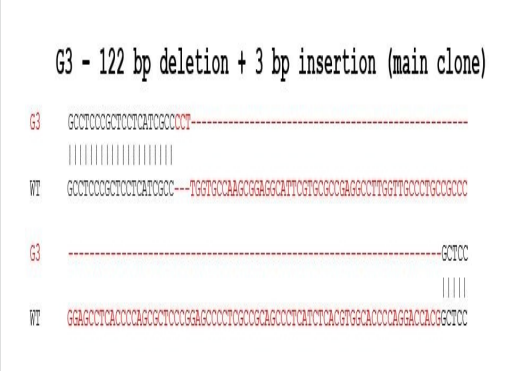
Lane 1: Wild-type Raji cell lysate 20 µg
Lane 2: NFATC2 knockout Raji cell lysate 20 µg
Lane 3: Jurkat cell lysate 20 µg
Lane 4: SH-SY5Y cell lysate 20 µg
False colour image of Western blot: Anti-NFAT1 antibody [EPR24658-149] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab283649** was shown to bind specifically to NFAT1. A band was observed at 100 kDa in wild-type Raji cell lysates with no signal observed at this size in NFATC2 knockout cell line **ab280906** (knockout cell lysate ab282940). The band observed in the knockout lysate lane below 100 kDa is likely to represent a truncated form of NFAT1. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and NFATC2 knockout Raji cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Human NFATC2 knockout Raji cell lysate (ab282940)

Lane 1: Wild-type Raji cell lysate 20 µg
Lane 2: NFATC2 knockout Raji cell lysate 20 µg
Lane 3: Jurkat cell lysate 20 µg
Lane 4: SH-SY5Y cell lysate 20 µg
False colour image of Western blot: Anti-NFAT1 antibody [EPR24658-43] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab283691** was shown to bind specifically to NFAT1. A band was observed at 100 kDa in wild-type Raji cell lysates with no signal observed at this size in NFATC2 knockout cell line **ab280906** (knockout cell lysate ab282940). The band observed in the knockout lysate lane below 100 kDa is likely to represent a truncated form of NFAT1. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and NFATC2 knockout Raji cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto

a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Sanger Sequencing - Human NFATC2 knockout Raji cell lysate

122 bp deletion and 3 bp insertion in exon 2

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors