abcam

Product datasheet

Human NFATC2 knockout Raji cell line ab280906

3 Images

Overview

Product name	Human NFATC2 knockout Raji cell line
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
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images. **Recommended Control:** Human wild-type Raji (**ab275473**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose **Culture Medium:** RPMI + 10% FBS **Initial Handling Guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water for bath approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $4x10^5$ cells/mL. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** • All seeding densities should be based on cell counts gained by established methods.

• A guide seeding density of $4x10^5$ cells/mL is recommended.
•A maximum of $3x10^6$ viable cells/ml is obtainable

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**.

We will provide viable cells that proliferate on revival.

Properties

Number of cells	1×10^6 cells/vial, 1 mL
Adherent /Suspension	Suspension
Tissue	Lymphatic
Cell type	Burkitt's lymphoma
Disease	Lymphoma
Gender	Male
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

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.

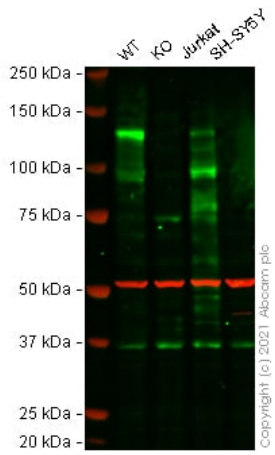
Applications

The Abpromise guarantee	Our **Abpromise guarantee** covers the use of ab280906 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 line (ab280906)

All lanes : Anti-NFAT1 antibody [EPR24658-43] (**ab283691**) at 1/1000 dilution

Lane 1 : Wild-type Raji cell lysate
Lane 2 : NFATC2 knockout Raji cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : SH-SY5Y cell lysate

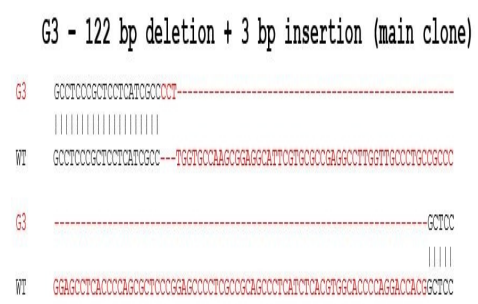
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 100 kDa

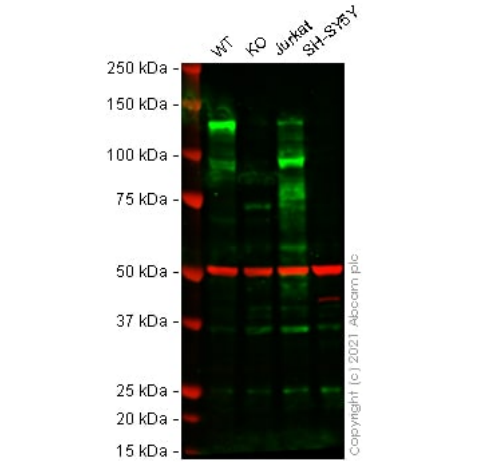
False colour image of Western blot: Anti-NFAT1 antibody [EPR24658-43] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab283691** was shown to bind specifically to NFAT1. A band was 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 line (ab280906)

122 bp deletion and 3 bp insertion in exon 2



Western blot - Human NFATC2 knockout Raji cell line (ab280906)

All lanes : Anti-NFAT1 antibody [EPR24658-149] (**ab283649**) at 1/1000 dilution

Lane 1 : Wild-type Raji cell lysate
Lane 2 : NFATC2 knockout Raji cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : SH-SY5Y cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 100 kDa

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.

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