abcam

Product datasheet

Anti-NFAT1 antibody [EPR24658-43] ab283691

Recombinant RabMAb

6 Images

Overview

Product name	Anti-NFAT1 antibody [EPR24658-43]
Description	Rabbit monoclonal [EPR24658-43] to NFAT1
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), IP, WB **Unsuitable for:** ICC/IF or IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Ramos, Raji, and Daudi whole cell lysates Flow cyt-intra: Ramos and Jurkat cells. IP: Ramos whole cell lysate.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR24658-43

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab283691 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
Flow Cyt (Intra)		1/50.
IP		1/1000.
WB		1/1000. Predicted molecular weight: 100 kDa.

Application notes Is unsuitable for ICC/IF or IHC-P.

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.

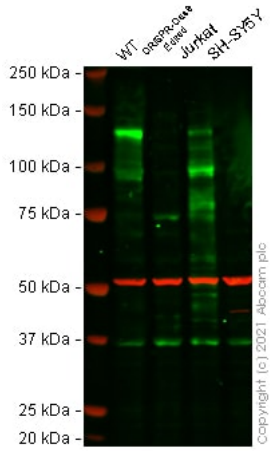
Sequence similarities Contains 1 RHD (Rel-like) domain.

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.

Images



Western blot - Anti-NFAT1 antibody [EPR24658-43] (ab283691)

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

Lane 1 : Wild-type Raji cell lysate
Lane 2 : NFATC2 CRISPR-Cas9 edited Raji cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : SH-SY5Y cell lysate

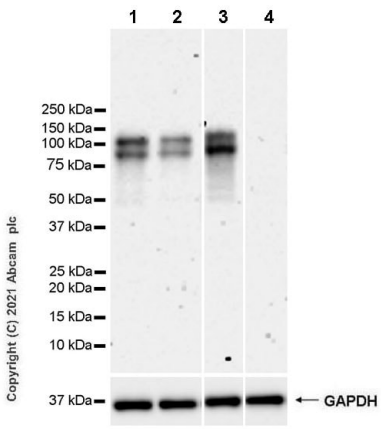
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 100 kDa
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 CRISPR-Cas9 edited cell line **ab280906** (CRISPR-Cas9 edited cell lysate **ab282940**). The band observed in the CRISPR-Cas9 edited 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 CRISPR-Cas9 edited 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 - Anti-NFAT1 antibody [EPR24658-43] (ab283691)

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

Lane 1 : Ramos (human Burkitt's lymphoma B lymphocyte), whole cell lysate
Lane 2 : Raji (human Burkitts lymphoma B lymphocyte), whole cell lysate
Lane 3 : Daudi (human Burkitts lymphoma lymphoblast), whole cell lysate
Lane 4 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

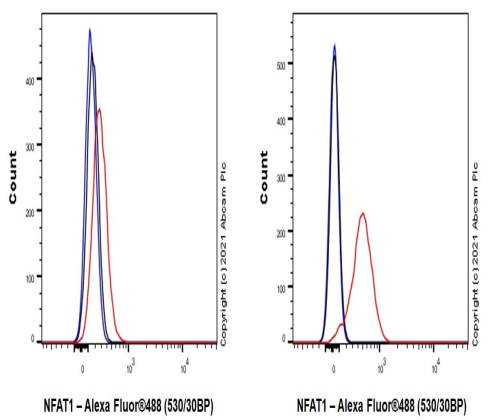
Predicted band size: 100 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBSTThis blot was developed using a higher-sensitivity ECL substrate.

The molecular weight observed is consistent with what has been described in the literature (PMID:21078663, PMID:25696812).

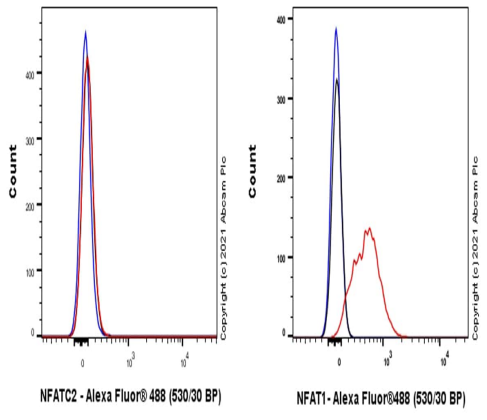
Negative control: Hela (PMID:21078663)

Exposure time: 3 minutes



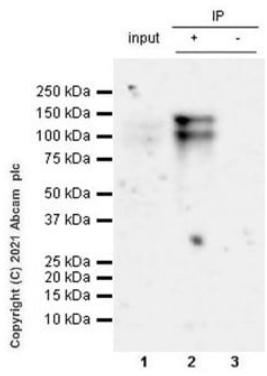
Flow Cytometry (Intracellular) - Anti-NFAT1 antibody [EPR24658-43] (ab283691)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell, Left) / Ramos (Human Burkitt's lymphoma B lymphocyte, Right) cells labelling NFAT1 with ab283691 at 1/50 dilution (1ug)/ red (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody. Negative control: Hela (PMID:21078663).



Flow Cytometry (Intracellular) - Anti-NFAT1 antibody [EPR24658-43] (ab283691)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized LNCaP (Human prostate carcinoma epithelial cell, Left) / Jurkat (Human T cell leukemia T lymphocyte, Right) cells labelling NFAT1 with ab283691 at 1/50 dilution (1ug) (Red) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody. Negative control: LNCaP.



Immunoprecipitation - Anti-NFAT1 antibody [EPR24658-43] (ab283691)

NFAT1 was immunoprecipitated from Ramos (Human Burkitt's lymphoma B lymphocyte), whole cell lysate with ab283691 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab283691 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1 (Input): Ramos (Human Burkitt's lymphoma B lymphocyte), whole cell lysate, 10 µg

Lane 2 (+): Ramos whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab283691 in Ramos whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Anti-NFAT1 antibody [EPR24658-43] (ab283691)

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