abcam

Product datasheet

Anti-NFAT1 antibody [EPR24658-43] - BSA and Azide free ab283720

リコンビナント RabMAb

画像数 6

製品の概要

製品名	Anti-NFAT1 antibody [EPR24658-43] - BSA and Azide free
製品の詳細	Rabbit monoclonal [EPR24658-43] to NFAT1 - BSA and Azide free
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), IP, WB **適用なし:** ICC/IF or IHC-P
種交差性	**交差種:** Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: Ramos, Raji, and Daudi whole cell lysates Flow cyt-intra: Ramos and Jurkat cells. IP: Ramos whole cell lysate.
特記事項	ab283720 is the carrier-free version of **ab283691**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C.
バッファー	pH: 7.20 Constituent: 100% PBS
キャリア・フリー	はい
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR24658-43
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab283720の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

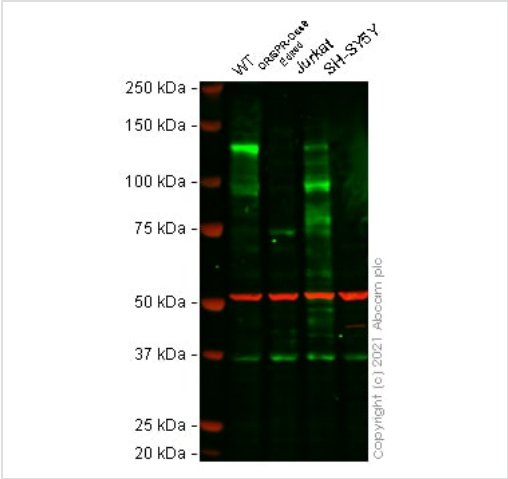
アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 100 kDa.

追加情報 Is unsuitable for ICC/IF or IHC-P.

ターゲット情報

機能	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.
組織特異性	Expressed in thymus, spleen, heart, testis, brain, placenta, muscle and pancreas.
配列類似性	Contains 1 RHD (Rel-like) domain.
ドメイン	Rel Similarity Domain (RSD) allows DNA-binding and cooperative interactions with AP1 factors.
翻訳後修飾	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.
細胞内局在	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.

画像



Western blot - Anti-NFAT1 antibody [EPR24658-43] - BSA and Azide free (ab283720)

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.

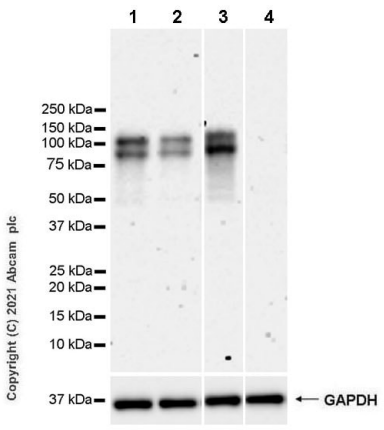
Performed under reducing conditions.

Predicted band size: 100 kDa
Observed band size: 100 kDa

This data was developed using **ab283691**, the same antibody clone in a different buffer formulation.

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] - BSA and Azide free (ab283720)

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

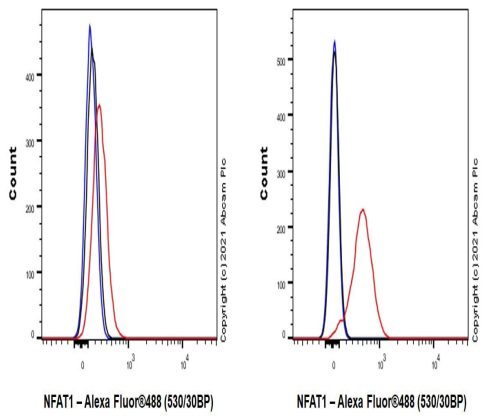
This data was developed using **ab283691**, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: This 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)

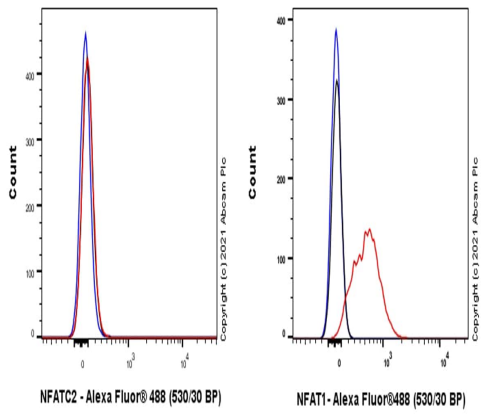
Exposure time: 3 minutes



Flow Cytometry (Intracellular) - Anti-NFAT1 antibody [EPR24658-43] - BSA and Azide free (ab283720)

This data was developed using **ab283691**, the same antibody clone in a different buffer formulation.

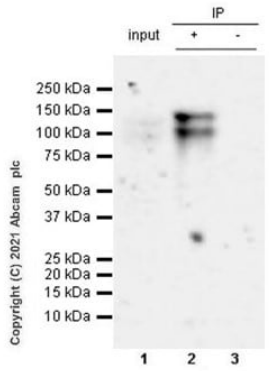
Flow cytometric analysis of HeLa (human cervix adenocarcinoma epithelial cell, Left) / Ramos (Human Burkitt's lymphoma B lymphocyte, Right) cells labelling NFAT1 with **ab283691** at 1/50 dilution (1ug)/ red compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). 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] - BSA and Azide free (ab283720)

This data was developed using **ab283691**, the same antibody clone in a different buffer formulation.

Flow cytometric analysis of 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) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). 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] - BSA and Azide free (ab283720)

This data was developed using **ab283691**, the same antibody clone in a different buffer formulation.

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] - BSA and Azide free (ab283720)

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