abcam

Product datasheet

Anti-NFAT2 antibody [7A6] ab2796



Overview

Product name Anti-NFAT2 antibody [7A6]

Description Mouse monoclonal [7A6] to NFAT2

Host species Mouse

Tested applications Suitable for: WB, IHC-P, Flow Cyt (Intra)

Species reactivity Reacts with: Human

Predicted to work with: Mouse, Rat, Hamster, Non human primates

Recombinant fragment. This information is proprietary to Abcam and/or its suppliers. **Immunogen**

Positive control IHC-P: FFPE human Hodgkin's lymphoma tissue sections.

General notes This monoclonal antibody is manufactured by Abcam. If you require this antibody in a particular

buffer formulation or a particular conjugate for your experiments,

please contact orders@abcam.com or you can find more information here.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

Properties

Form Liquid

Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -Storage instructions

80°C. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.05% Sodium azide

Constituent: PBS

Purity Protein G purified

Clonality Monoclonal

Clone number 7A6

lsotype lgG1 **Light chain type** kappa

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab2796 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	★★★★ <u>(2)</u>	Use at an assay dependent concentration.
IHC-P	★★★ ☆☆ (3)	Use at an assay dependent concentration.
Flow Cyt (Intra)		Use 1µg for 10 ⁶ cells. ab170190 - Mouse monoclonal lgG1, is suitable for use as an isotype control with this antibody.

Function

Plays a role in the inducible expression of cytokine genes in T-cells, especially in the induction of the IL-2 or IL-4 gene transcription. Also controls gene expression in embryonic cardiac cells. Could regulate not only the activation and proliferation but also the differentiation and programmed death of T-lymphocytes as well as lymphoid and non-lymphoid cells.

Tissue specificity

Expressed in thymus, peripheral leukocytes as T-cells and spleen. Isoforms A are preferentially expressed in effector T-cells (thymus and peripheral leukocytes) whereas isoforms B and isoforms C are preferentially expressed in naive T-cells (spleen). Isoforms B are expressed in naive T-cells after first antigen exposure and isoforms A are expressed in effector T-cells after second antigen exposure.

Sequence similarities

Contains 1 RHD (Rel-like) domain.

Domain

Rel Similarity Domain (RSD) allows DNA-binding and cooperative interactions with AP1 factors. The N-terminal transactivation domain (TAD-A) binds to and is activated by Cbp/p300. The dephosphorylated form contains two unmasked nuclear localization signals (NLS), which allow translocation of the protein to the nucleus.

Isoforms C have a C-terminal part with an additional trans-activation domain, TAD-B, which acts as a transcriptional activator. Isoforms B have a shorter C-terminal part without complete TAD-B which acts as a transcriptional repressor.

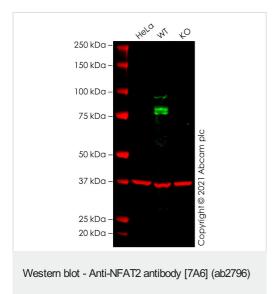
Post-translational modifications

Phosphorylated by NFATC-kinase; dephosphorylated by calcineurin.

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



All lanes: Anti-NFAT2 antibody [7A6] (ab2796) at 5 µg/ml

Lane 1: HeLa cell lysate

Lane 2: Wild-type HAP1 cell lysate

Lane 3: NFATC1 knockout HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

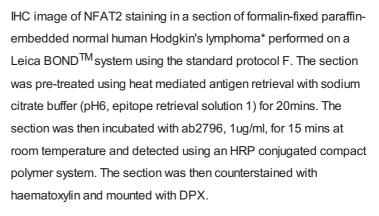
Performed under reducing conditions.

Observed band size: 75,80,90 kDa

False colour image of Western blot: Anti-NFAT2 antibody [7A6] staining at 5 ug/ml, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (ab181602) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab2796 was shown to bind specifically to NFAT2. A band was observed at 75/80/90 kDa in wild-type HAP1 cell lysates with no signal observed at this size in NFATC1 knockout cell line. To generate this image, wild-type and NFATC1 knockout HAP1 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-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) and Goat anti-Rabbit lgG H&L (IRDye® 680RD) preabsorbed (ab216777) at 1/20000 dilution.

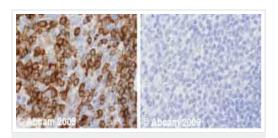


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NFAT2 antibody [7A6] (ab2796)



For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



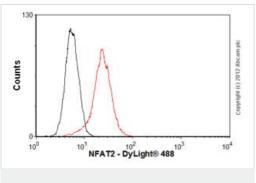
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NFAT2 antibody [7A6] (ab2796)

ab2796 staining human normal tonsil tissue. Staining is localized to cytoplasm and nucleus.

Left panel: with primary antibody at 1 µg/ml. **Right panel:** Isotype control.

Sections were stained using an automated system DAKO Autostainer Plus , at room temperature. Sections were rehydrated and antigen retrieved with the Dako 3-in-1 AR buffer EDTA pH 9.0 in a DAKO PT Link. Slides were peroxidase blocked in $3\%\ H_2O_2$ in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS), then incubated with primary antibody for 20 minutes, and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with diaminobenzidine for 5 minutes. Slides were counterstained with hematoxylin and coverslipped under DePeX.

Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required.



Flow Cytometry (Intracellular) - Anti-NFAT2 antibody [7A6] (ab2796)

Overlay histogram showing Jurkat cells stained with ab2796 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab2796, 1µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight[®] 488 goat anti-mouse lgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse lgG1 [ICIGG1] (ab91353, 2µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in Jurkat cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

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

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