abcam

Product datasheet

Anti-NFAT1 antibody [25A10.D6.D2] ab2722

★★★★★ 7 Abreviews 53 References 10 Images

Overview

Product name	Anti-NFAT1 antibody [25A10.D6.D2]
Description	Mouse monoclonal [25A10.D6.D2] to NFAT1
Host species	Mouse
Specificity	Ab2722 detects nuclear factor of activated T-cells (NFAT) from mouse, rat and human tissues (endogenously expressed). This antibody does not cross react with NFAT2 (NFATc, NFATc1). This antibody detects both forms NFAT1 - a ~140 kDa protein representing phosphorylated NFAT1 in resting immune cells, and a ~120 kDa protein in stimulated cells that represents fully-dephosphorylated NFAT1.
Tested applications	**Suitable for:** Flow Cyt, WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide corresponding to Mouse NFAT1 aa 50-150. Database link: **Q60591** Run BLAST with Run BLAST with
Positive control	WB: resting immune cells and ionomycin stimulated immune cells (see Shaw et al reference: "1uM for T cells and B cells, 10uM for macrophages,and 0.3uM for mast cells. Treatment was for 20 min.")
General notes	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
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.05% Sodium azide Constituent: PBS

Purity	Protein G purified
Clonality	Monoclonal
Clone number	25A10.D6.D2
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab2722 in the following tested applications.

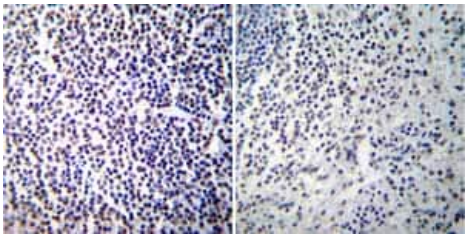
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt		Use at an assay dependent concentration. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ (4)	Use a concentration of 1 µg/ml. Detects a band of approximately 120, 140 kDa (predicted molecular weight: 115 kDa).
IHC-P	★★★★★ (1)	1/50 - 1/200.
ICC/IF		1/100 - 1/1000.

Target

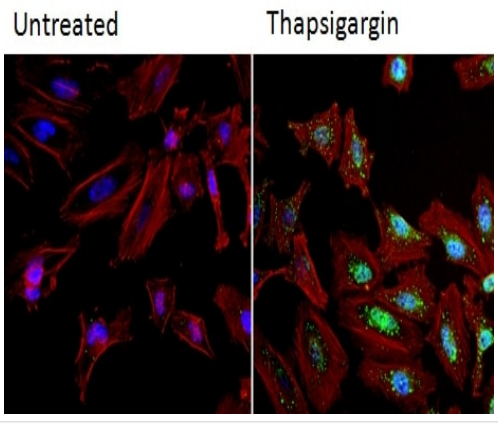
Function	Plays a role in the inducible expression of cytokine genes in T-cells, especially in the induction of the IL-2, IL-3, IL-4, TNF-alpha or GM-CSF.
Tissue specificity	Expressed in thymus, spleen, heart, testis, brain, placenta, muscle and pancreas.
Sequence similarities	Contains 1 RHD (Rel-like) 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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NFAT1 antibody [25A10.D6.D2] (ab2722)

Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized Human spleen tissue tissues. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:100 with a mouse monoclonal antibody recognizing NFATc2 ab2722 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



Immunocytochemistry/ Immunofluorescence - Anti-NFAT1 antibody [25A10.D6.D2] (ab2722)

Immunocytochemistry/Immunofluorescence analysis of NFAT1 (green) in HeLa cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% BSA for 15 minutes at room temperature. Cells were left untreated (left panel) or treated with 1uM staurosporine (right panel) for 3 hours and incubated with ab2722 (1:100) for at least 1 hour at room temperature, washed with PBS, and incubated with a DyLight 488 conjugated goat anti-mouse IgG secondary antibody (1:400) for 30 minutes at room temperature. F-Actin (red) was stained with DyLight 554 Phalloidin and nuclei (blue) were stained with Hoechst 33342 dye. Images were taken at 20X magnification.

Western blot - Anti-NFAT1 antibody [25A10.D6.D2] (ab2722)

Anti-NFAT1 antibody [25A10.D6.D2] (ab2722) at 1 µg/ml + Human spleen tissue lysate - total protein (**ab29699**) at 10 µg

Secondary

Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/5000 dilution ( )

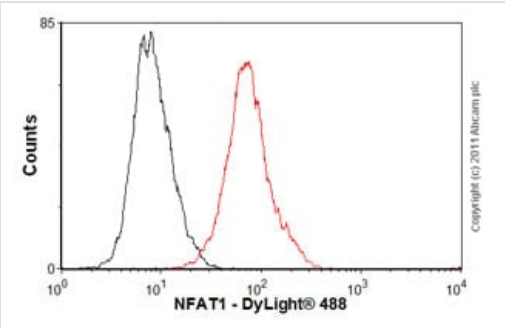
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 115 kDa
Observed band size: 150 kDa
Additional bands at: 62 kDa. We are unsure as to the identity of these extra bands.

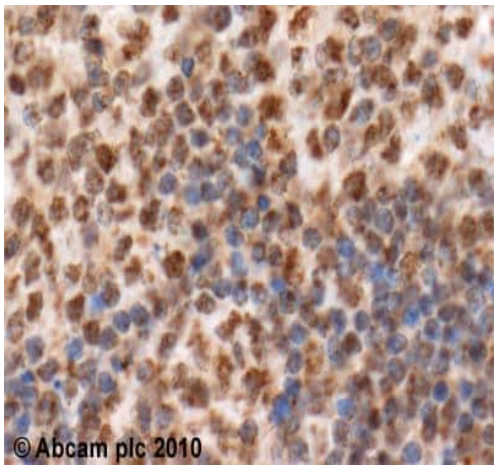
Exposure time: 4 minutes

NFAT1 contains an exstensive number of potential phosphorylation sites (SwissProt) which may explain its migration at a higher molecular weight than predicted.



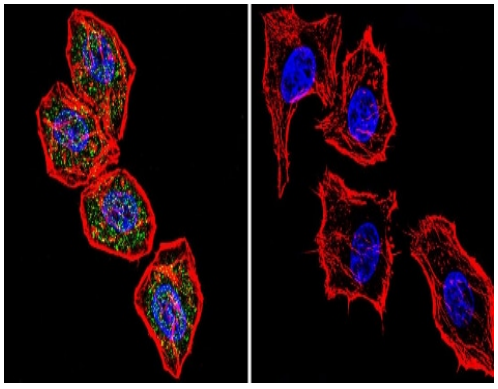
Flow Cytometry - Anti-NFAT1 antibody [25A10.D6.D2] (ab2722)

Overlay histogram showing Jurkat cells stained with ab2722 (red line). The cells were fixed with 80% methanol (5 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 (ab2722, 2µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



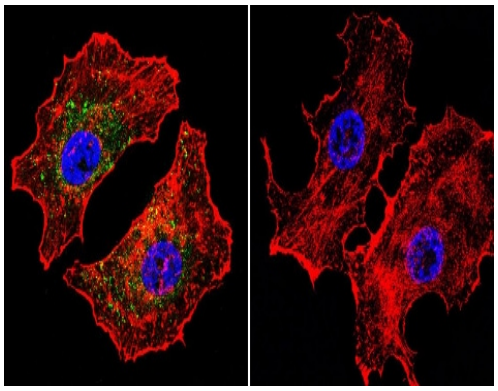
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NFAT1 antibody [25A10.D6.D2] (ab2722)

ab2722 (4µg/ml) staining NFAT in human tonsil, using an automated system (DAKO Autostainer Plus). Using this protocol there is strong nuclear and weak cytoplasmic staining.
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% H2O2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako envision flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.



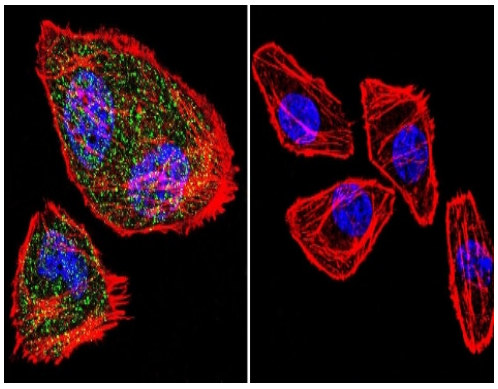
Immunocytochemistry/ Immunofluorescence - Anti-NFAT1 antibody [25A10.D6.D2] (ab2722)

Immunocytochemistry/Immunofluorescence analysis of NFAT1 (green) shows staining in HeLa cells. F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were inbuated without (control) or with ab2722 (1:20) over night at 4°C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Images were taken at 60X magnification.



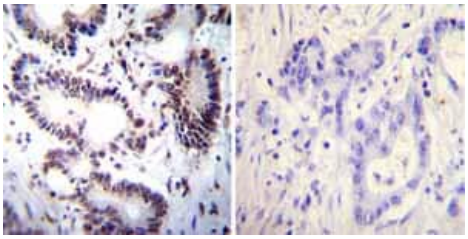
Immunocytochemistry/ Immunofluorescence - Anti-NFAT1 antibody [25A10.D6.D2] (ab2722)

Immunocytochemistry/Immunofluorescence analysis of NFAT1 (green) shows staining in MCF-7 cells. F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were inbuated without (control) or with ab2722 (1:20) over night at 4°C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Images were taken at 60X magnification.



Immunocytochemistry/ Immunofluorescence - Anti-NFAT1 antibody [25A10.D6.D2] (ab2722)

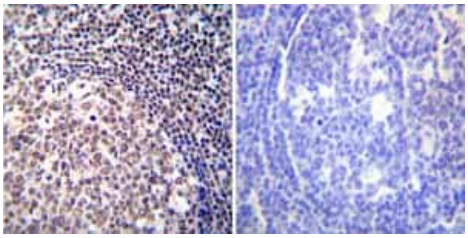
Immunocytochemistry/Immunofluorescence analysis of NFAT1 (green) shows staining in U251 Cells. F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were inbuated without (control) or with ab2722 (1:20) over night at 4°C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Images were taken at 60X magnification.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NFAT1 antibody [25A10.D6.D2] (ab2722)

Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized Human colon carcinoma tissues. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:100 with a mouse monoclonal antibody recognizing NFATc2 ab2722 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase

activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NFAT1 antibody [25A10.D6.D2] (ab2722)

Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized Human tonsil tissue tissues. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:100 with a mouse monoclonal antibody recognizing NFATc2 ab2722 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.