abcam

ab2722

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

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	Preservative: 0.05% Sodium azide Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Synthetic Peptide within Mouse Nfatc2 aa 50-100. The exact immunogen used to generate this antibody is proprietary information. Database link Q60591
Clone number	25A10.D6.D2
Purification technique	Affinity purification Protein G
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.
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Flow Cyt

Tested

Species	Human
Dilution info	-

Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

WB

Tested

Species	Human
Dilution info	1 µg/mL
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/50 - 1/200
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100 - 1/1000
Notes	-

Target data

See full target information NFATC2

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 or GM-CSF (PubMed:15790681). Promotes invasive migration through the activation of GPC6 expression and WNT5A signaling pathway (PubMed:21871017). Is involved in the negative regulation of chondrogenesis (PubMed:35789258). Recruited by AKAP5 to ORAI1 pore-forming subunit of CRAC channels in Ca(2+) signaling microdomains where store-operated Ca(2+) influx is coupled to calmodulin and calcineurin signaling and activation of NFAT-dependent transcriptional responses.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	NFAT1 also known as NFATc2 is a transcription factor involved in gene regulation with a molecular weight of approximately 100 kDa. It belongs to the NFAT (nuclear factor of activated T-cells) family and is expressed in a variety of tissues including the immune system and the nervous system. NFAT1 plays an essential role in activating specific gene expressions by translocating into the nucleus upon cell stimulation. Researchers frequently use NFAT molecular weight to confirm its presence in protein analyses such as NFAT western blot techniques.
Biological function summary	NFAT1 influences immune response and development. It acts as part of a large complex with other transcription factors to bind DNA and regulate transcription of cytokines and other critical immune molecules. In activated T-cells NFAT1 controls the expression of genes important for immune function including interleukins. In neuronal tissues it contributes to neural growth and survival highlighting its multifunctional nature in different cell types.
Pathways	NFAT1 integrates into the calcineurin signaling pathway and plays a significant role in T-cell activation. It acts downstream of the calcium signaling cascade which leads to dephosphorylation and activation by the phosphatase calcineurin. NFAT1 works closely with proteins like AP-1 and GATA to facilitate the transcription of immune-related genes. These interactions highlight NFAT1's importance in immune signaling pathways which modulate immune surveillance and homeostasis.
Associated diseases and disorders	NFAT1 associates with autoimmune diseases and certain cancers. In autoimmune conditions such as rheumatoid arthritis aberrant NFAT1 function leads to excessive cytokine production exacerbating inflammation. The protein also links to cancer development particularly in lymphoid malignancies where dysregulated NFAT1 activity contributes to uncontrolled cell proliferation. It interacts with oncogenic kinases and other dysregulated proteins in these disorders influencing both their progression and possible therapeutic targeting.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

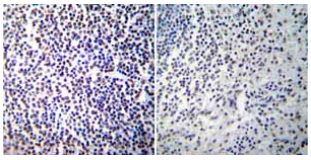
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

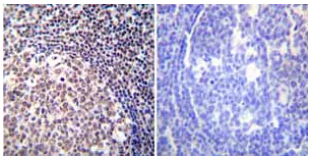
Full details and terms and conditions can be found here:
Terms & Conditions.

10 product images



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

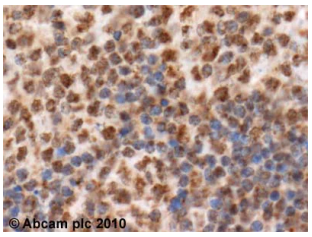
Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized Human spleen tissue tissues. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked 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.



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

NFAT1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-NFAT1 antibody

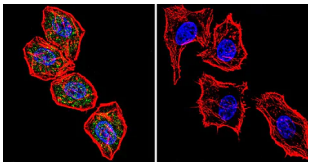
Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized Human tonsil tissue tissues. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked 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.



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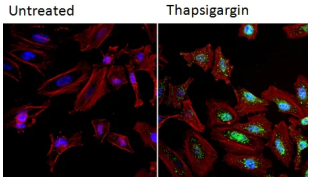
ab2722 (4µg/ml) staining NFAT in human tonsil, using an automated system (DAKO Autostainer Plus). Using this protocol there is strong nuclear and weak cytoplasmic staining.
Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer EDTA pH 9.0 in a DAKO PT link. Slides were peroxidase 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)

NFAT1 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-NFAT1 antibody

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.

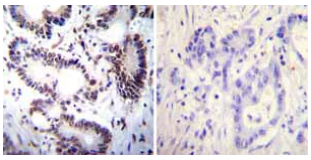


Immunocytochemistry/ Immunofluorescence - Anti-NFAT1 antibody [25A10.D6.D2] (ab2722)

NFAT1 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-NFAT1 antibody

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

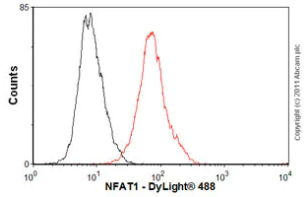
temperature. F-Actin (red) was stained with DyLight 554 Phalloidin and nuclei (blue) were stained with Hoechst 33342 dye. Images were taken at 20X magnification.



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

NFAT1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-NFAT1 antibody

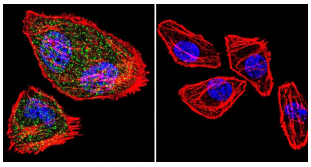
Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized Human colon carcinoma tissues. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were 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.



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

NFAT1 Flow Cytometry staining using mouse Anti-NFAT1 antibody

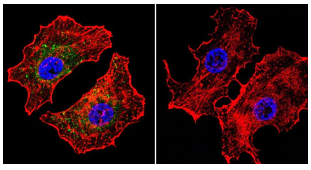
Overlay histogram showing Jurkat cells stained with ab2722 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab2722, 2µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. 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.



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

NFAT1 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-NFAT1 antibody

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.

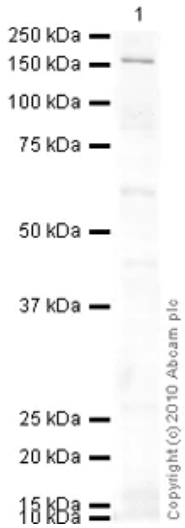


Immunocytochemistry/ Immunofluorescence - Anti-NFAT1 antibody [25A10.D6.D2] (ab2722)

NFAT1 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-NFAT1 antibody

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.



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

NFAT1 Western blot staining of Human spleen tissue lysate - total protein (ab29699) using mouse Anti-NFAT1 antibody

NFAT1 contains an exstensive number of potential phosphorylation sites (SwissProt) which may explain its migration at a higher molecular weight than predicted.
All lanes:
Western blot - Anti-NFAT1 antibody [25A10.D6.D2] (ab2722) at 1 µg/mL

All lanes:
Human spleen tissue lysate - total protein (ab29699) at 10 µg

Secondary

All lanes:
Western blot - 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: 100 kDa

Observed band size: 150 kDa, 62 kDa

Exposure time: 4min

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.