# abcam

# Product datasheet

# Anti-NFAT5 antibody ab3446

★★★★★ 1 Abreviews 31 References 画像数 8

#### 製品の概要

製品名 Anti-NFAT5 antibody

製品の詳細 Rabbit polyclonal to NFAT5

由来種 Rabbit

特異性 Detects Nuclear Factor of Activated T-cells 5 (NFAT 5).

アプリケーション 適用あり: IHC-P, ICC/IF, WB, IP

**種交差性** 交差種: Mouse, Human, African green monkey

免疫原 Synthetic peptide corresponding to Human NFAT5 aa 1439-1455 (C terminal).

Sequence:

DLLVSLQNQGNNLTGSF

(Peptide available as ab4978)

Run BLAST with
Run BLAST with

ポジティブ・コントロール WB: MCF7, Jurkat, Raji, Ramos, HepG2, U2OS, HeLa, COS7, EL4, C2C12 and NRK cell

lysates. IHC-P: Human brain and skeletal muscle tissues. ICC/IF: NIH/3T3, MCF7 and HeLa cells.

IP: U2OS cells.

特記事項

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

### 製品の特性

製品の状態 Liquid

保存方法 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.

パッファー Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 99% PBS

精製度 Immunogen affinity purified

1

アイソタイプ

ΙgG

# アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab3446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
IHC-P		1/20. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/100.
WB	<b>★★★★</b> (1)	1/1000. Detects a band of approximately 170 kDa (predicted molecular weight: 160 kDa). Can be blocked with <b>NFAT5 peptide</b> (ab4978).
IP		Use at an assay dependent concentration. 3 µg

### ターゲット情報

機能 Plays a role in the inducible expression of genes. Regulates hypertonicity-induced cellular

accumulation of osmolytes.

組織特異性 Highest levels in skeletal muscle, brain, heart and peripheral blood leukocytes. Also expressed in

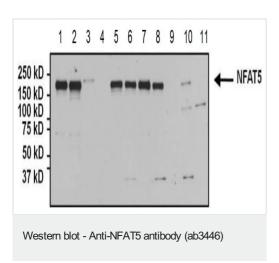
placenta, lung, liver, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine and

colon.

配列類似性 Contains 1 RHD (Rel-like) domain.

細胞内局在 Nucleus.

#### 画像



All lanes: Anti-NFAT5 antibody (ab3446) at 1/1000 dilution

Lane 1 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

**Lane 2 :** Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

 $\textbf{Lane 3:} \ \mathsf{Raji} \ (\mathsf{Human \, Burkitt's \, lymphoma \, cell \, line}) \ \mathsf{whole \, cell \, lysate}$ 

Lane 4 : Ramos (Human Burkitt's lymphoma cell line) whole cell lysate

**Lane 5**: HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

Lane 6: U-2 OS (Human bone osteosarcoma epithelial cell line)

whole cell lysate

Lane 7 : HeLa (Human epithelial adenocarcinoma cell line) whole cell lysate

Lane 8 : COS-7 (African green monkey kidney fibroblast-like cell line) whole cell lysate

Lane 9: EL4 (Mouse thymic lymphoma cell line) whole cell lysateLane 10: C2C12 (Mouse myoblast cell line) whole cell lysateLane 11: NRK (Rat kidney normal tissue) whole cell lysate

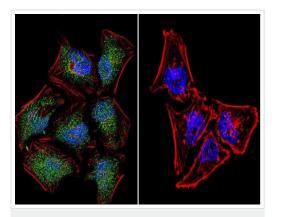
Lysates/proteins at 25 µg per lane.

# **Secondary**

**All lanes :** Goat anti-rabbit-HRP secondary antibody at 1/20000 dilution

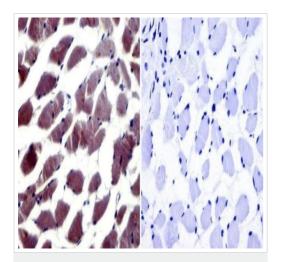
Predicted band size: 160 kDa

Western blot analysis of NFAT5 was performed by loading samples onto a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked with 5% Milk/TBST for at least 1 hour. Membranes were incubated with ab3446 overnight at 4°C on a rocking platform. Membranes were washed in TBS-0.1%Tween 20 and probed with a secondary antibody for at least one hour. Membranes were washed and chemiluminescent detection performed.



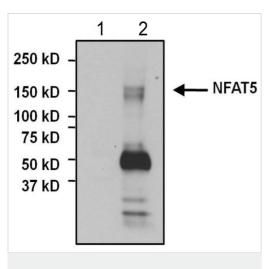
Immunocytochemistry/ Immunofluorescence - Anti-NFAT5 antibody (ab3446)

ICC analysis of NFAT5 in HeLa (Human epithelial adenocarcinoma cell line) cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control-right) or with ab3446 at a dilution of 1:20 overnight at 4 C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. NFAT5 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NFAT5 antibody (ab3446)

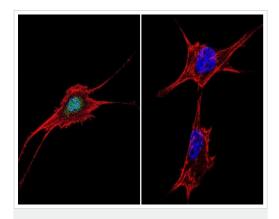
Immunohistochemistry was performed on normal biopsies of deparaffinized human skeletal muscle tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1/20 duution with ab3446 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.



Immunoprecipitation - Anti-NFAT5 antibody (ab3446)

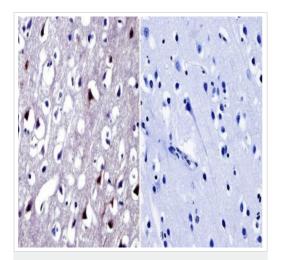
Immunoprecipitation of NFAT5 was performed on U-2 OS (Human bone osteosarcoma epithelial cell line) whole cell lysate (lane 2). The antigen:antibody complex was formed by incubating 500 µg whole cell lysate with 3 µg of ab3446 overnight on a rocking platform at 4°C. The immune-complex was captured on 50 µL Protein A/G Plus Agarose. Captured immune-complexes were washed and proteins eluted with 5X Reducing Sample Loading Dye. Samples were resolved on a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to PVDF membrane and blocked with 5% Milk/TBS-0.1%Tween for at least 1 hour. Membranes were washed in TBS-0.1%Tween 20 and probed with a goat anti-rabbit-HRP secondary antibody at a dilution of 1/20,000 for at least one hour. Membranes were washed and chemiluminescent detection performed.

Lane 1: Only cell lysate.



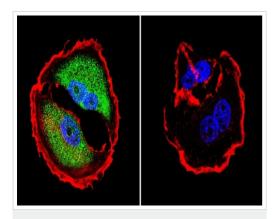
Immunocytochemistry/ Immunofluorescence - Anti-NFAT5 antibody (ab3446)

ICC analysis of NFAT5 in NIH/3T3 (Mouse embryo fibroblast cell line) cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control-right) or with ab3446 at a dilution of 1:20 overnight at 4 C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. NFAT5 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.



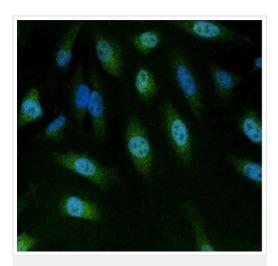
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NFAT5 antibody (ab3446)

Immunohistochemistry was performed on normal biopsies of deparaffinized human brain tissue. 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 1:20 with ab3446 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-NFAT5 antibody (ab3446)

ICC analysis of NFAT5 in MCF7 (Human breast adenocarcinoma cell line) cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control-right) or with ab3446 at a dilution of 1:200 overnight at 4 C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. NFAT5 staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.



Immunocytochemistry/ Immunofluorescence - Anti-NFAT5 antibody (ab3446)

ICC analysis of NFAT5 usingab3446 (shown in green) in HeLa (Human epithelial adenocarcinoma cell line) whole cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature. Cells were then blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with a rabbit polyclonal antibody recognizing NFAT5, at a dilution of 1/100 for at least 1 hour at room temperature. Cells were washed with PBS and incubated with DyLight 488 goat-anti-rabbit secondary antibody at a dilution of 1/400 for 30 minutes at room temperature. Nuclei (blue) were stained with Hoechst 33342 dye. Images were taken on a Thermo Scientific ArrayScan at 20X magnification.

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

#### Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <a href="https://www.abcam.co.jp/abpromise">https://www.abcam.co.jp/abpromise</a> or contact our technical team.

#### Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors