Product Datasheet

NFAT5 Antibody
NB120-3446

Unit Size: 50 ug

Store at -20C. Avoid freeze-thaw cycles.

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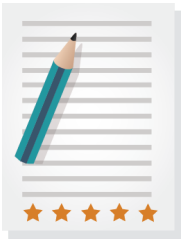
Updated 12/20/2023 v.20.1



NB120-3446

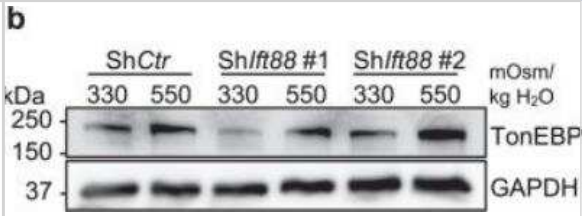
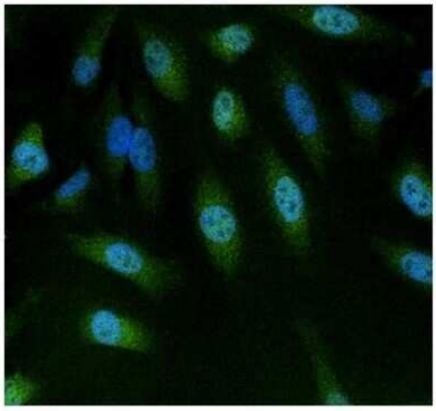
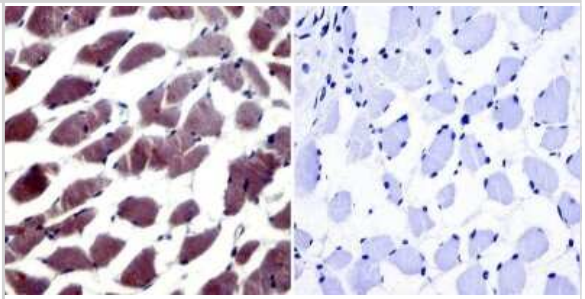
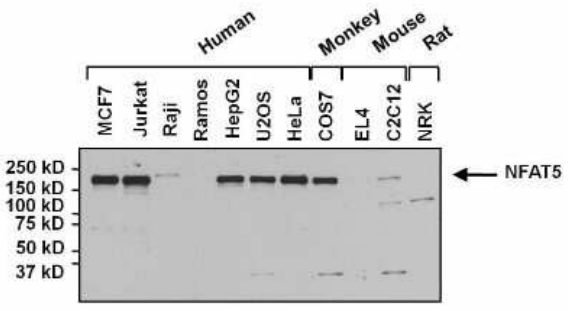
NFAT5 Antibody

Product Information	
Unit Size	50 ug
Concentration	1 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS with 1 mg/ml BSA

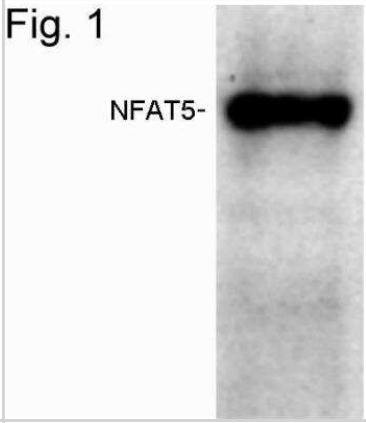
Product Description	
Host	Rabbit
Gene ID	10725
Gene Symbol	NFAT5
Species	Human, Mouse, Rat, Canine, Hamster, Rabbit
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID: 19412173). Rat reactivity reported in scientific literature (PMID: 12824075). Rabbit reactivity reported in scientific literature (PMID: 22944138). Canine reactivity reported in scientific literature (PMID: 31066233).
Immunogen	Synthetic peptide corresponding to residues C D(1439) L L V S L Q N Q G N N L T G S F(1455) of human NFAT5.

Product Application Details	
Applications	Western Blot, Simple Western, Gel Super Shift Assays, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Knockdown Validated
Recommended Dilutions	Western Blot 1:1000, Simple Western, Immunohistochemistry 1:20, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation 3 ug, Immunohistochemistry-Paraffin 1:20, Gel Super Shift Assays 1:1 - 1:100, Chromatin Immunoprecipitation (ChIP) 1:10-1:500, Knockdown Validated
Application Notes	ChIP usage was reported in scientific literature (PMID: 22266867). WB: Detects an approx. 170 kDa protein representing NFAT 5 in HEK293 cells transfected with the human NFAT 5 gene. ICC: Staining of NFAT 5 in HEK293 cells transfected with the human NFAT5 gene results in primarily cytoplasmic staining.Use in Immunocytochemistry/immunofluorescence reported in scientific literature (PMID 28356704). Knockdown Validated reported in scientific literature (PMID: 31066233). Simple Western reported by an internal validation. Separated by Size-All, antibody dilution of 1:50; matrix was 12-230 kDa.

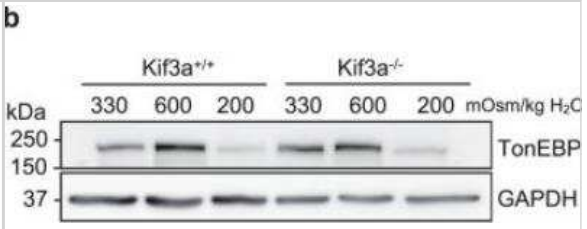
Images

Western Blot: NFAT5 Antibody [NB120-3446] - Western blot image showing increased TonEBP/NFAT5 expression in response to hyperosmolarity (550mOsm/kg H2O) independently of Ift88 knockdown. Image collected and cropped by CiteAb from the following publication (nature.com/articles/s41598-019-51939-7), licensed under a CC-BY license.	
Immunocytochemistry/Immunofluorescence: NFAT5 Antibody [NB120-3446] - Analysis of NFAT5 using anti-NFAT5 polyclonal antibody (shown in green) in HeLa cells.	
Immunohistochemistry-Paraffin: NFAT5 Antibody [NB120-3446] - Normal biopsies of deparaffinized human skeletal muscle tissue.	
Western Blot: NFAT5 Antibody [NB120-3446] - Analysis of NFAT5 was performed by loading 25ug of various whole cell lysates 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 probed with a rabbit polyclonal antibody recognizing NFAT5 at a dilution of 1:1000 overnight at 4C on a rocking platform. 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 using Super Signal West Dura.	

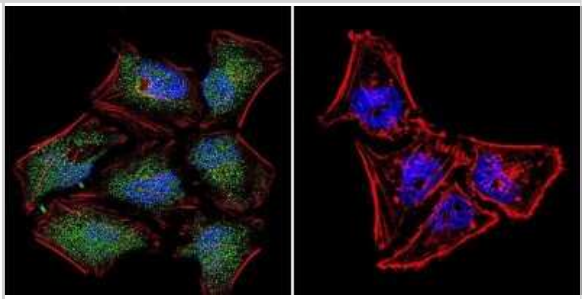
Western Blot: NFAT5 Antibody [NB120-3446] - Analysis of human NFAT5 from transfected BHK cell lysate.



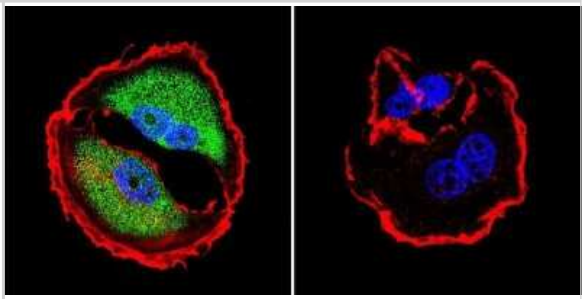
Western Blot: NFAT5 Antibody [NB120-3446] - Western blot image and corresponding densitometry analyses showing TonEBP/NFAT5 levels under different osmotic conditions in wild-type and Kif3a null MEFs. Kif3a null MEFs show slightly attenuated hyperosmotic increase but unaffected hypoosmotic decrease in TonEBP expression. Image collected and cropped by CiteAb from the following publication (nature.com/articles/s41598-019-51939-7), licensed under a CC-BY license.



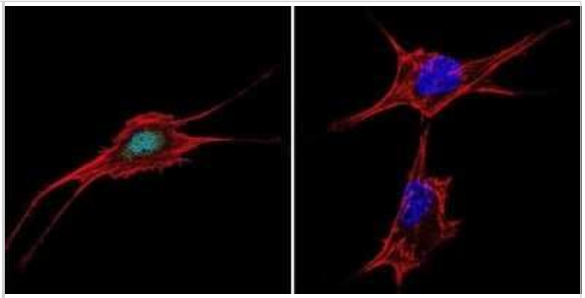
Immunocytochemistry/Immunofluorescence: NFAT5 Antibody [NB120-3446] - Analysis of NFAT5 in HeLa Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a NFAT5 polyclonal antibody at a dilution of 1:20 overnight at 4C, washed 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.



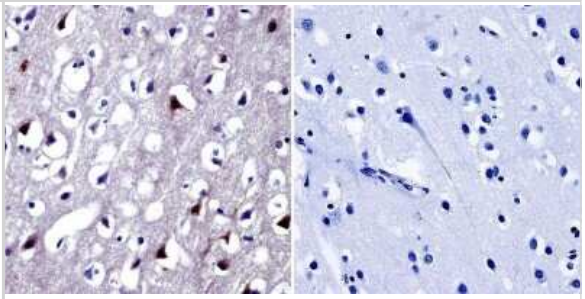
Immunocytochemistry/Immunofluorescence: NFAT5 Antibody [NB120-3446] - Analysis of NFAT5 in MCF-7 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a NFAT5 polyclonal antibody at a dilution of 1:200 overnight at 4C, washed 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.



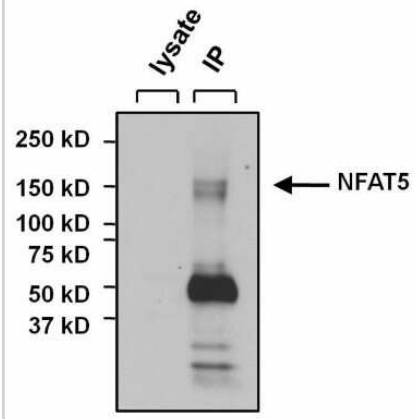
Immunocytochemistry/Immunofluorescence: NFAT5 Antibody [NB120-3446] - Analysis of NFAT5 in NIH-3T3 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a NFAT5 polyclonal antibody at a dilution of 1:20 overnight at 4C, washed 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.



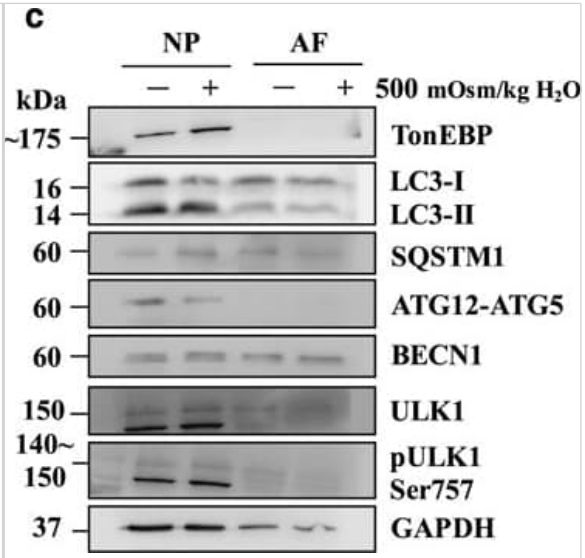
Immunohistochemistry-Paraffin: NFAT5 Antibody [NB120-3446] - Normal biopsies of deparaffinized human brain tissue.



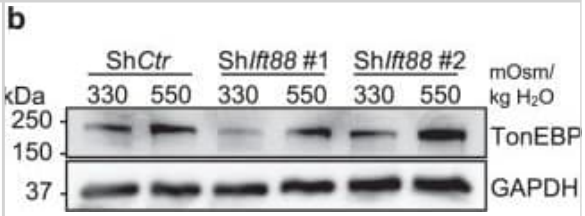
Immunoprecipitation: NFAT5 Antibody [NB120-3446] - Analysis of NFAT5 was performed on U2OS cells. The antigen:antibody complex was formed by incubating 500ug whole cell lysate with 3ug of rabbit polyclonal antibody recognizing NFAT5 overnight on a rocking platform at 4C. The immune-complex was captured on 50ul 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 using Super Signal West Dura.



Analyses of ECM components in murine ON astrocytes following treatment with TGF-β2 and CCN2/CTGF. (A) Immunocytochemical staining against fibronectin (green, upper panel) showed a markedly increase following the treatment with 1 ng/ml TGF-β2 or 50 ng/ml CCN2/CTGF. Immunocytochemical staining against tropoelastin (green, lower panel) showed a pronounced increase after the treatment with 1 ng/ml TGF-β2 or 50 ng/ml CCN2/CTGF. Nuclei were stained with Dapi (blue). (B) Real-time RT-PCR analyses shows an intense upregulation of collagen 3α1, tropoelastin and fibronectin mRNA in murine ON astrocytes after treatment with 1 ng/ml TGF-β2, 50 ng/ml CCN2/CTGF or 100 ng/ml CCN2/CTGF for 24 h compared to untreated control cells (collagen 3α1: n = 3; TGF-β2 ***p = 0.0000004, 50 ngCTGF **p = 0.005, 100ngCTGF **p = 0.008; fibronectin: n = 6, TGF-β2 *p = 0.00004, 50 ng CTGF *p = 0.016, 100 ngCTGF *p = 0.05; elastin: n = 5, TGF-β2 *p = 0.026, 50 ng CTGF *0.04, 100 ng CTGF *p = 0.03). mRNA expression was normalized to Gnb2l and mean value of control cells was set at 1. (C) Western Blot analysis show an increase in protein synthesis of collagen 3α1, elastin and fibronectin in murine ON astrocytes after treatment with 1 ng/ml TGF-β2, 50 ng/ml CCN2/CTGF or 100 ng/ml CCN2/CTGF for 24 h compared to untreated control cells (collagen 3α1: n = 3, TGF-β2 *p = 0.03, 100 ngCTGF **p = 0.009; fibronectin: n = 5, **p = 0.005, 50 ngCTGF **p = 0.006, 100 ng CTGF *p = 0.02; tropoelastin: n = 5, TGF-β2 ***p = 0.0004, 50 ngCTGF **p = 0.002, 100 ngCTGF ***p = 0.00001). GAPDH and α-tubulin were used to normalize protein synthesis and mean value of control cells was set at 1. Right panel shows representative Western Blots for all three proteins. Data represented as mean ± SD. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/35493079), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Small interfering RNA against Nox4 (siNox4) alleviated oxidative stress and DNA damage induced by high oxygen tension in NP cells. (a, b) RT-qPCR analysis (N = 3) and representative immunoblot analysis of Nox4 in NP cells. The knockdown of Nox4 in NP cells was confirmed. (c) ROS production in NP cells (N = 3). (d) RT-qPCR analysis of MsrbA, MsrB1, and MsrB2 in NP cells (N = 3). (e, f) Immunofluorescence staining of γ-H2A.X and percentage of γ-H2A.X-positive cells in NP cells (N = 6). NP cells were transfected with siNox4 or scrambled siRNA control (siCtrl) before high oxygen tension treatment. □, P value < 0.05, error bars represent standard error. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/29147462), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Laban H, Siegmund S, Zappe M et al. NFAT5/TonEBP Limits Pulmonary Vascular Resistance in the Hypoxic Lung by Controlling Mitochondrial Reactive Oxygen Species Generation in Arterial Smooth Muscle Cells Cells 2021-11-24 [PMID: 34943801]

Kappert L, Ruzicka P, Kutikhin A Et al. Loss of Nfat5 promotes lipid accumulation in vascular smooth muscle cells FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2021-09-01 [PMID: 34383982] (IF/IHC, ICC/IF, WB, Human, Mouse)

Pelzl L, Sahu I, Ma K et al. Beta-Glycerophosphate-Induced ORAI1 Expression and Store Operated Ca2+ Entry in Megakaryocytes Sci Rep 2020-02-03 [PMID: 32015442] (WB, Human)

Choi H, Madhu V, Shapiro IM, Risbud MV Nucleus pulposus primary cilia alter their length in response to changes in extracellular osmolarity but do not control TonEBP-mediated osmoregulation Sci Rep 2019-10-29 [PMID: 31664118] (WB, Human)

Chen L, Cao J, Cao D et al. Protective effect of dexmedetomidine against diabetic hyperglycemia-exacerbated cerebral ischemia/reperfusion injury: An in vivo and in vitro study Life Sci. 2019-06-08 [PMID: 31185237] (WB, Mouse)

Rasmussen RN, Christensen KV, Holm R, Nielsen CU Nfat5 is involved in the hyperosmotic regulation of Tmem184b: a putative modulator of ibuprofen transport in renal MDCK I cells FEBS Open Bio 2019-06-01 [PMID: 31066233] (WB, Knockdown Validated, Canine)

Choi H, Chaiyamongkol W, Doolittle AC et al. COX-2 expression mediated by calcium-TonEBP signaling axis under hyperosmotic conditions serves osmoprotective function in nucleus pulposus cells. J Biol Chem 2018-06-08 [PMID: 29700115] (WB, Mouse)

Hollborn M, Fischer S, Kuhrt H et al. Osmotic regulation of NFAT5 expression in RPE cells: The involvement of purinergic receptor signaling. Mol. Vis. 2017-03-30 [PMID: 28356704] (ICC/IF, Human)

Johnson ZI, Shapiro IM, Risbud MV. RNA sequencing reveals a role of TonEBP in regulation of pro-inflammatory genes in response to hyperosmolarity in healthy nucleus pulposus cells: A homeostatic response?. J. Biol. Chem. 2016-11-08 [PMID: 27875309] (WB, Rat)

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Products Related to NB120-3446

HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
H00010725-Q01-10ug	Recombinant Human NFAT5 GST (N-Term) Protein

Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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