Product Datasheet

NFAT5 Antibody
NB120-3446

Unit Size: 50 ug

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

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Reviews: 1 Publications: 11

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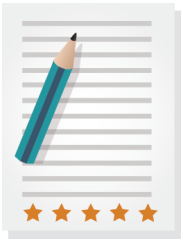
Updated 10/23/2024 v.20.1



NB120-3446

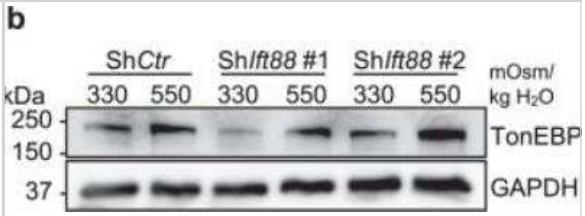
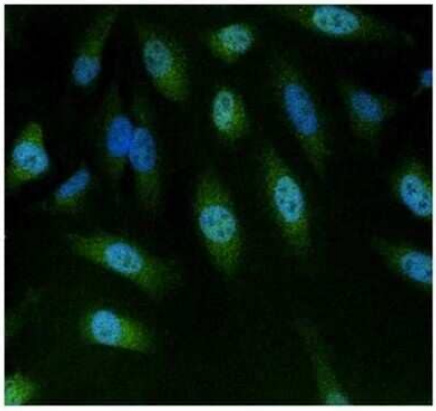
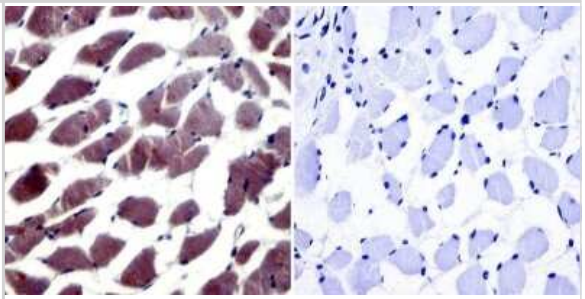
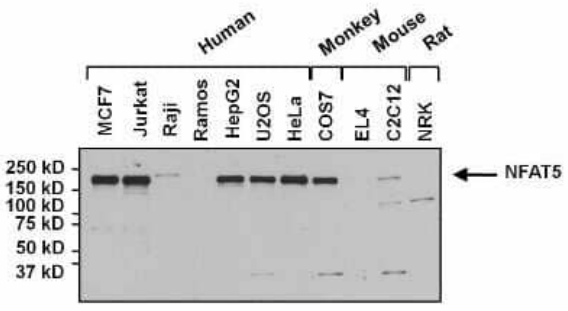
NFAT5 Antibody

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

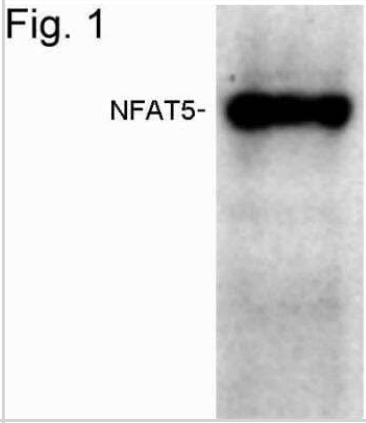
Product Description	
Host	Rabbit
Gene ID	10725
Gene Symbol	NFAT5
Species	Human, Mouse, Rat, Canine, Hamster, Rabbit
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID: 19412173). Rat reactivity reported in scientific literature (PMID: 12824075). Rabbit reactivity reported in scientific 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). See Simple Western Antibody Database for Simple Western validation: tested in HeLa lysate; separated by size, antibody dilution of 1:50; matrix was 12-230 kDa.

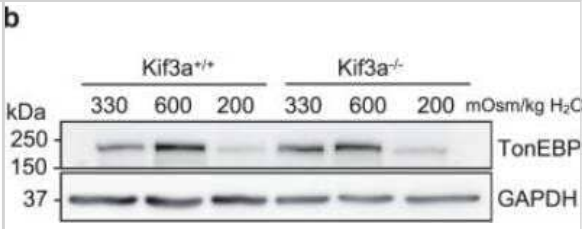
Images

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.	

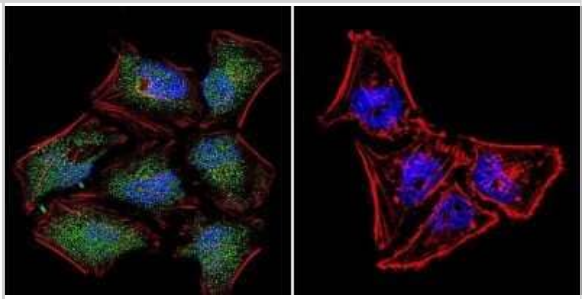
Western Blot: NFAT5 Antibody [NB120-3446] - Analysis of human NFAT5 from transfected BHK cell lysate.



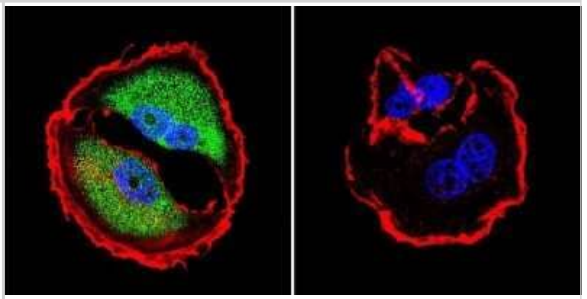
Western Blot: NFAT5 Antibody [NB120-3446] - Western blot image and corresponding densitometry analyses showing TonEBP/NFAT5 levels under different osmotic conditions in wild-type and Kif3a null MEFs. Kif3a null MEFs show slightly attenuated hyperosmotic increase but unaffected hypoosmotic decrease in 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.



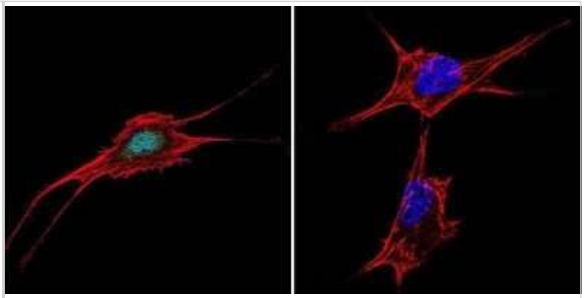
Immunocytochemistry/Immunofluorescence: NFAT5 Antibody [NB120-3446] - Analysis of NFAT5 in HeLa Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a NFAT5 polyclonal antibody at a dilution of 1:20 overnight at 4C, washed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



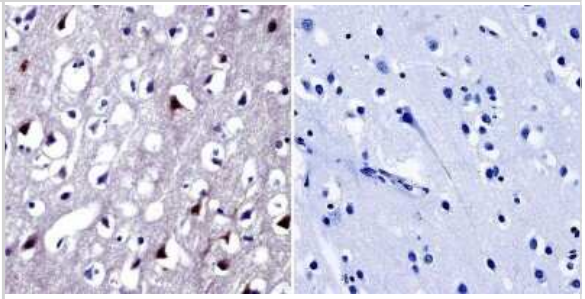
Immunocytochemistry/Immunofluorescence: NFAT5 Antibody [NB120-3446] - Analysis of NFAT5 in MCF-7 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a NFAT5 polyclonal antibody at a dilution of 1:200 overnight at 4C, washed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



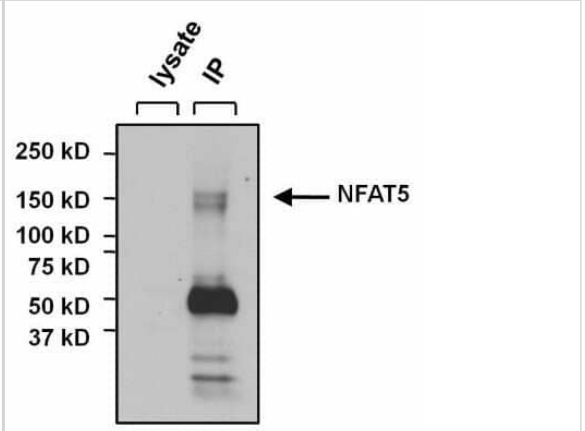
Immunocytochemistry/Immunofluorescence: NFAT5 Antibody [NB120-3446] - Analysis of NFAT5 in NIH-3T3 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a NFAT5 polyclonal antibody at a dilution of 1:20 overnight at 4C, washed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



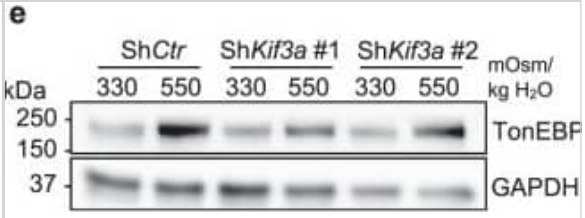
Immunohistochemistry-Paraffin: NFAT5 Antibody [NB120-3446] - Normal biopsies of deparaffinized human brain tissue.

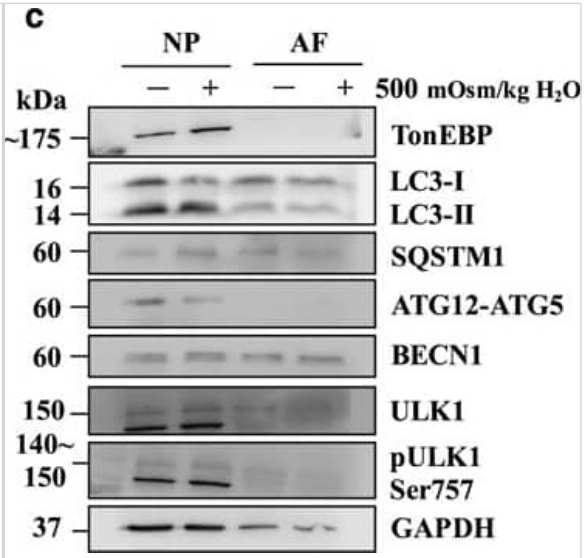
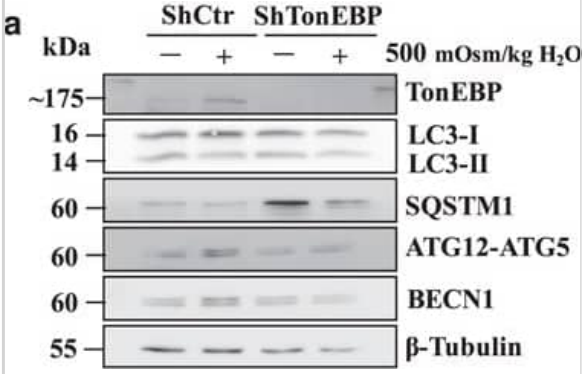


Immunoprecipitation: NFAT5 Antibody [NB120-3446] - Analysis of NFAT5 was performed on U2OS cells. The antigen:antibody complex was formed by incubating 500ug whole cell lysate with 3ug of rabbit polyclonal antibody recognizing NFAT5 overnight on a rocking platform at 4C. The immune-complex was captured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

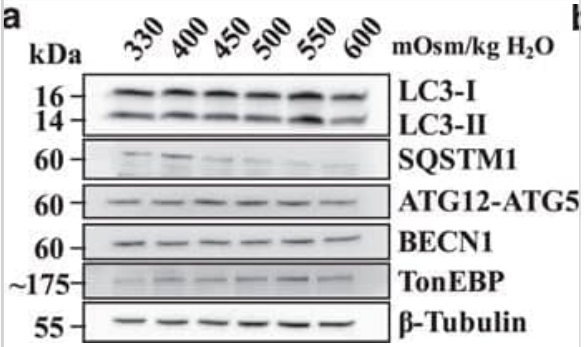


Western Blot: NFAT5 Antibody [NB120-3446] - Knockdown of Ift88 or Kif3a in NP cells does not affect hyperosmotic upregulation of TonEBP. (a) TonEBP/Nfat5 mRNA levels in NP cells with Ift88 knockdown (n ≥ 5). (b) Western blot image showing increased TonEBP expression in response to hyperosmolarity (550 mOsm/kg H2O) independently of Ift88 knockdown. (c) Densitometry analyses of TonEBP with Ift88 knockdown (n ≥ 4). (d) TonEBP/Nfat5 mRNA levels in NP cells with Kif3a knockdown (n ≥ 3). (e) Western blot image showing that hyperosmotic induction of TonEBP is maintained after Kif3a knockdown. (f) Densitometry analyses of TonEBP after Kif3a knockdown (n ≥ 4). Data are represented as scatter plots (mean ± SEM). ns = not significant. One-way ANOVA or Kruskal-Wallis test with Sidak's or Dunn's multiple comparison test was used based on the distribution of the data to determine statistical significance. Western blot images were cropped & acquired under same experimental conditions. See Supplementary Fig. S1-1 for un-cropped Western blot images. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31664118), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: NFAT5 Antibody [NB120-3446] - NP cells do not induce autophagy in response to hyperosmotic stimulus in an ex vivo disc organ culture model. (a) A schematic depicting ex vivo rat intervertebral disc organ culture model. (b) H&E & alcian blue staining of discs cultured under iso- (330 mOsm/kg H2O) or hyperosmotic (500 mOsm/kg H2O) conditions showing that NP maintained its structure & cellular morphology. Scale bar: 100 µm. (c) Western blot analysis of tissue proteins from NP or AF (annulus fibrosus) compartments of the organ culture discs. The level of TonEBP increased with hyperosmotic stimulus only in the NP. However, the levels of LC3-II, SQSTM1, ATG12-ATG5, BECN1, as well as pULK1 Ser757 did not change with hyperosmolarity in both NP & AF. (d–f) Densitometric analyses of multiple Western blots represented in (c). Bars represent mean ± SEM (n = 3; For each independent experiment, one motion segment per group was used for histology & 6 motion segments per group were used for tissue protein Western blot). Student t test was used to determine statistical significance. NS, non-significant. Western blot images were cropped & acquired under same experimental conditions. See Supplementary Fig. S1 for examples of uncropped images. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28674405), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: NFAT5 Antibody [NB120-3446] - TonEBP does not control autophagy in NP cells. (a) Western blot analysis of NP cells transduced with a lentivirus expressing either control shRNA or shTonEBP plasmid showed that TonEBP silencing did not affect the levels of LC3-II, ATG12-ATG5, & BECN1. The levels of SQSTM1 increased with TonEBP silencing. (b–f) Densitometric analyses of multiple Western blots shown in (a). (g) Western blot analysis of ULK1 activation status showed that the levels of pULK1 Ser757 was slightly lower, while that of pULK1 Ser777 remained unaffected after TonEBP knockdown under hyperosmotic conditions. (h,i) Densitometric analyses of multiple western blots shown in (g). Bars represent mean ± SEM (n = 4). Two-way ANOVA with Tukey's multiple comparisons test was used to determine statistical significance. NS, non-significant. Western blot images were cropped & acquired under same experimental conditions. See Supplementary Fig. S1 for examples of uncropped images. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28674405), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Western Blot: NFAT5 Antibody [NB120-3446] - Hyperosmolarity does not upregulate the levels of canonical autophagic markers. (a) Western blot analysis of NP cells cultured under increasing osmolarity (330–600 mOsm/kg H2O) showed that the levels of LC3-II, SQSTM1, ATG12-ATG5, & BECN1 did not change by hyperosmolarity. However, TonEBP expression increased under hyperosmotic condition. (b–d) Densitometric analyses of multiple Western blots represented by (a) confirmed significant induction of TonEBP, while LC3-II & SQSTM1 levels remained unaltered (n = 5). (e) Western blot analysis of NP cells cultured under hyperosmotic condition for increasing lengths of time demonstrated that LC3-II, SQSTM1, ATG12-ATG5, & BECN1 levels were unaffected by hyperosmolarity up till 72 h. (f–i) Densitometric analyses of multiple Western blots shown in (e) (n = 3). Bars represent mean ± SEM. One-way ANOVA with Sidak's multiple comparisons test was used to determine statistical significance. NS, non-significant. Western blot images were cropped & acquired under same experimental conditions. See Supplementary Fig. S1 for examples of uncropped images. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28674405), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Liqiong He, Shengyun Ma, Zijin Ding, Zhifeng Huang, Yu Zhang, Caiyun Xi, Kailu Zou, Qingwei Deng, Wendy Jia Men Huang, Qulian Guo, Changsheng Huang Inhibition of NFAT5□Dependent Astrocyte Swelling Alleviates Neuropathic Pain Advanced Science 2024-01-09 [PMID: 38195869]

Liu C, Choi H, Johnson ZI et al. Lack of evidence for involvement of TonEBP and hyperosmotic stimulus in induction of autophagy in the nucleus pulposus Sci Rep 2017-07-03 [PMID: 28674405]

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

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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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