# **Product Datasheet**

## ATF6 Antibody (70B1413.1) - BSA Free NBP1-40256SS

Unit Size: 0.025 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

www.novusbio.com



technical@novusbio.com

### Reviews: 3 Publications: 246

Protocols, Publications, Related Products, Reviews, Research Tools and Images at: www.novusbio.com/NBP1-40256

Updated 12/5/2024 v.20.1

# Earn rewards for product reviews and publications.

Submit a publication at www.novusbio.com/publications Submit a review at www.novusbio.com/reviews/destination/NBP1-40256



## NBP1-40256SS

ATF6 Antibody (70B1413.1) - BSA Free

Product Information	
Unit Size	0.025 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	70B1413.1
Preservative	0.05% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	TBS
Product Description	



Description	<ol> <li>(1) The ATF6 (IMG-273) has been cited to recognize both full-length and cleaved forms of ATF6. Please refer to the references in the Product Citation list for more comprehensive information.</li> <li>(2) The ATF6 transfected cell lysate (40210) is recommended as a useful western blot positive control to run in parallel with your experimental samples.</li> <li>(3) Active/cleaved forms of ATF6 are generated through proteolytic cleavage during ER stress. Different molecular weights have been described for cleaved forms. A sample protocol was first described in Luo and Lee (2002) wherein NIH3T3 cells were treated with the amino acid analogue azetidine (AzC), and full-length and cleaved forms ATF6 were detected with IMG-273. The results show that in addition to the major 90 kDa full length ATF6, protein bands over the range of 50-70 kDa were detectable following AzC treatment (Luo and Lee, 2002: Figure 4A, page 791). Many protocols and other cleaved forms have been described since, please consult the literature for additional information.</li> <li>(4) For immunofluorescence microscopy, cells were fixed in methanol at -20 degrees C (Thomas et al, 2005). Thomas et al used immunofluorescence to identify ATF6 with MC 272 in the avelage.</li> </ol>
	<ul> <li>(5) The active/cleaved 50 kDa nuclear form of ATF6 using IMG-273 has been found to be strongly expressed in certain tumor cell lines derived from B cell lymphoma (DEL), primary effusion lymphoma [BC-3 (ATCC CRL-2277), PEL-SY, HBL-6], lymphoblastic leukemia (DS-1) and multiple myeloma (RPMI-8226, NCI-H929), (Jenner et al. 2003).</li> <li>(6) Cleaved 60 and 36 kDa ATF6 forms have also been described in the nucleus (Mao et al. 2007)</li> </ul>
	<ul><li>(7) The ATF6 antibody is reported to be specific for ATF6a, recognizing ATF6a but not ATF6b (Bommiasamy et al, 2009).</li></ul>
	(8) In western blots, the binding pattern of ATF6 may vary. Researchers are encouraged to consult the body of literature citing the ATF6 IMG-273 antibody (see Product citation list) for additional information. General ATF6 literature is also helpful. For example, Yoshida (1998) show a multiple band pattern in HeLa in both untreated and stressed cells (Fig 11B). In this early landmark ATF6 publication, multiple bands were seen between the 66 and 116 kDa markers as well as one or more bands between the 45 and 66 kDa markers.
	(9) We highly recommend the use of a maximum sensitivity ECL substrate (Femto sensitive) for efficient detection of this antibody in Western blot applications.
Host	Mouse
Gene ID	22926
Gene Symbol	ATF6
Species	Human, Mouse, Rat, Porcine, Plant, Rabbit
Reactivity Notes	Plant reactivity reported in scientific literature (PMID: 31531232). Use in Plant reported in scientific literature (PMID:31531232).
Specificity/Sensitivity	This ATF6 antibody detects both the full length and the cleaved/active protein.
Immunogen	This monoclonal antibody was made against a partial protein containing amino acids 1-273 of human ATF6.

## **Product Application Details**

www.novusbio.com



Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), CyTOF-ready, Knockout Validated
Recommended Dilutions	Western Blot 1-5 ug/ml, Flow Cytometry 1 ug per million cells, Immunohistochemistry 1:10-1:500, Immunocytochemistry/ Immunofluorescence 1:10-1:500, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:50, Immunohistochemistry-Frozen 1:10-1:500, Immunoblotting, Flow (Intracellular), Chromatin Immunoprecipitation (ChIP) 1:10-1:500, CyTOF-ready, Knockout Validated
Application Notes	ICC: See Thomas et al (2005) and Kikuchi et al (2006) for details. Immunohistochemistry (Frozen): See Zhu et al (2008) for details. Immunohistochemistry (Paraffin): See van Kollenburg et al (2006) for details. Immunoprecipitation: See Hong et al, 2004 for details. Use in Immunoblotting reported in scientific literature (PMID 28550308). Knockout validation (PMID: 31531232). In Western blot do not use milk in the diluent as it can inhibit the observed signal. This antibody is CyTOF ready.

## Images

Western Blot: ATF6 Antibody (70B1413.1) [NBP1-40256] - Hypoxia leads to EMT and ER-stress in CRC cells. B. C. Confluent growing SW480 (B) and HCT116 (C) cells were cultured under conditions of normoxia or hypoxia-like conditions (serum free; 100 uM CoCl2, 1-9 h. B- actin (B-act) as loading control. HIF1a was detectable after 3 h of CoCl2 incubation, the amount of the 50 kD-ATF6 fragment, was already enhanced after 1 h of addition of CoCl2. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0087386) licensed under a CC-BY license.	B Co H1h H3h H6h H9h CoCl <sub>2</sub> Co H3h H6h C HIF1α ATF6 β-act β-act
Western Blot: ATF6 Antibody (70B1413.1) [NBP1-40256] - Analysis of ATF6 in mouse liver tissue using 3 ug/ml of ATF6 antibody and 0.25 ug/ml of GAPDH antibody. Lane A contains 20 ugs of whole mouse liver lysate, lane B contains 20 ugs of total ER fraction, and lane C contains 20 ugs of rough ER fraction. The ATF6 band may represent under glycosylated or cleaved/active ATF6.	MW (kDa) A B C 200 ATF6 116 ATF6 66 ATF6* 55 A 36 ATF6* 31 A 21 A 14 A 6 AGAPDH



Immunocytochemistry/Immunofluorescence: ATF6 Antibody (70B1413.1) [NBP1-40256] - Untreated HeLa cells were fixed in -20C methanol for 10 min, air dried and rehydrated in PBS at room temperature for 5 minutes. Cells were incubated with anti-ATF6 (1:20) for one hour at room temperature. ATF6 reactivity (green) was detected with anti-mouse Dylight-488 secondary antibody. Nuclei were counterstained with DAPI (blue). Note the ER localization of ATF6.

Immunohistochemistry-Paraffin: ATF6 Antibody (70B1413.1) [NBP1-40256] - Analysis of ATF6 antibody on Human placenta. Fixed paraffinembedded sections. Antibody dilution 1:50. Incubated overnight in 4C. Image from verified customer review.



Flow (Intracellular): ATF6 Antibody (70B1413.1) [NBP1-40256] - An intracellular stain was performed on HeLa cells with ATF6 Antibody (70B1413.1) NBP1-40256F (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 10 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to FITC.. Using the FITC format of this antibody.

Relative Cell Number 300 200 100 Ó. 105 104 106 10 0 ATF6 FITC ATF6 (1-373) ATF6 MW 293 (kDa) 200 116 ATF6 (full-length 66 55 ATF6 (partial 36 -31 -21 14 6

Western Blot: ATF6 Antibody (70B1413.1) [NBP1-40256] - Lane 1: 293 cells transfected with full-length ATF6.

Lane 2: 293 cells transfected with partial length ATF6 (amino acids 1-373).

Lane 3: Untransfected 293 cells.

Western blots were probed with 4 ug/ml of the ATF6 monoclonal antibody and visualized with PicoTect Western Blot Chemiluminescence Substrate (10087K). Film was exposed for 1 min. The top arrow corresponds to the 90 kDa form of ATF6 described as full-length in the literature. The human full-length and partial length ATF6 plasmids are described in Luo and Lee (2002).



2 3

500

400

Immunohistochemistry-Paraffin: ATF6 Antibody (70B1413.1) [NBP1-40256] - Human placenta, followed by biotinylated horse anti-mouse IgG secondary antibody, alkaline phosphatase-streptavidin and chromogen. Dilution 10ug/ml



Flow Cytometry: ATF6 Antibody (70B1413.1) [NBP1-40256] - Intracellular flow cytometric staining of 1 x 10^6 MCF-7 cells using ATF6 antibody (dark blue). Isotype control shown in orange. An antibody concentration of 1 ug/1x10^6 cells was used.	1.5K 1.0K 500 0 1.0K
Western Blot: ATF6 Antibody (70B1413.1) [NBP1-40256] - Lysate of DU145 cells. Image from verified customer review.	MW, KDa
	260
	140
	140
	100
	70
	50
	40
Western Blot: ATF6 Antibody (70B1413.1) [NBP1-40256] - Lysate of PC- 3 cells. Image from verified customer review.	MW, KDa
	260
	140
	100
	70
	<sup>50</sup> 40



Page 6 of 13 v.20.1 Updated 12/5/2024



www.novusbio.com







D Western Blot: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] -Disturbance of ER homeostasis in ADTKD-UMOD.(A) ADTKD-UMOD is characterized by maturation & trafficking defect of mutant UMOD & intracellular accumulation of UMOD in TAL cells. UMOD immunolocalization revealed a diffuse cytoplasmic staining with enforcement of the luminal membrane in TAL cells of a wild-type mouse. In contrast, TAL cells of an UmodC93F mutant mouse displayed a strong paranuclear immunopositivity for UMOD. Wild-type: Umodwt mouse; UmodC93F: homozygous UmodC93F mutant mouse. Age of mice analysed: four months. Chromogen: DAB, nuclear staining: haemalum. (B) Heat map of relative expression values (z scores) showed differential abundance of several proteins localized in the ER. (C) In the outer medulla of Umod mutant mice of both mouse lines, a strong accumulation of immature UMOD was present. (D) Protein abundances of BiP, phospho-eIF2 $\alpha$ , eIF2 $\alpha$ , ATF4, both full-length (§) & cleaved activated (#) ATF6, & CHOP were increased in Umod mutant mice compared to wild-type mice. Signal intensities were corrected for GAPDH signal intensities of the same PVDF-membrane, which was stripped several times to facilitate the detection of multiple proteins. Mean of protein abundance of wild-type mice was set on a value of 1 [mean (wild-type) = 1]. Data are shown as means ± SD. One-way ANOVA with Newman-Keuls's post hoc test: p vs. wild-type. \*p < 0.05: \*\*p < 0.01: \*\*\*p < 0.001. Image collected & cropped by CiteAb from the following publication (https://www.nature.com/articles/srep42970), licensed under a CC-BY license. Not internally tested by Novus Biologicals. Western Blot: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] -The effect of Ezetimibe on unfolded protein response (UPR) gene expression in THP-1 cells exposed to ischemia-reperfusion (IR). (a) The mRNA expression of activating transcription factor 6 (ATF6) & CCAATenhancer-binding protein homologous protein (CHOP). (b) Representative Western blot analyses for the indicated proteins. (c.d) The average quantification of ATF6 & CHOP obtained by the densitometric analysis of three independent experiments. mRNA was analyzed by quantitative real-time PCR; normalized gene expression levels are given as the ratio between the mean value for the target gene & that for β-actin in each sample. Data are expressed as mean  $\pm$  SD. \* p < 0.01 vs. control (up-regulation); \*\* p < 0.01 vs. IR; Sp < 0.01 vs. control (down-regulation). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32340270), licensed under a CC-BY license. Not internally tested by Novus Biologicals.





Western Blot: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] -APPswe induction of OB-senescence via ER stress.a Heat map of differentially expressed ER stress or anti-stress related genes identified by RNA-seg in control (OCN-Cre; Ai9) & TgAPPsweOCN; Ai9 Td+ OBprogenitors (detail analysis was described in Methods). b RT-PCR analysis of ER stress-related genes Grp78, Atf6, Hsp90b1, Eif2ak3, Ern1, Hsp90aa1, & Hspa2 & anti-stress related gene Sirt3 gene expression in purified Td+ BMSCs from 6-MO control (OCN-Cre; Ai9) & TgAPPsweOCN; Ai9 mice, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, mean  $\pm$  SD, n = 3, Mann–Whitney U test. c Western blot analysis of indicated protein expression in BMSCs from mice with indicated genotypes (at 6-MO). GAPDH was used as a loading control. d Quantification of data in c, \*p < 0.05, \*\*p < 0.01. mean ± SD, n = 4, Student's t test. e Western blot analysis of indicated protein expression in BMSCs from 6-MO control & TgAPPsweOCN with or without 0.25 mM 4-PBA (4-Phenylbutyric acid) treatment. f Quantification analyses of the data in e, p < 0.05, n = 3. g SA-B-gal staining of 6-MO control & TgAPPsweOCN BMSCs with vehicle (Veh)(PBS) & 4-PBA treatment, respectively, scale bar, 20 µm. h Quantification of SA- $\beta$ -gal+ cell densities in g (mean ± SD; n = 5, \*\*p < 0.01, \*\*\*p < 0.001). Two-way analysis of variance test was used in f & h. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/34824365), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Immunocytochemistry/ Immunofluorescence: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] - Effect of rapamycin on markers of the UPR pathways(A) Cells were incubated in medium alone (0.05% FBS), with 3 nM IGF-1 or with both 3 nM IGF-1 & 10 nM rapamycin (IGF-1 + rapamycin). Total protein extracts prepared from cells incubated for 24 h in those conditions were subjected to Western Blot analysis. Protein expression levels were assessed for phosphorylated or total forms of PERK, eIF2a & for CHOP, BiP, XBP1-s & XBP1-u proteins. Efficiency of rapamycin to inhibit mTORC1 pathway was also checked by immunoblot with phosphorylated & total forms of p70S6K1 & 4E-BP1. α-tubulin was used as internal control. (B & C) Cells were incubated with 10nM rapamycin for 1 h (B) or 24 h (C). Immunoblots for phosphorylated & total forms of PERK, eIF2α, p70S6K1 & 4E-BP1 or for ATF4, CHOP, BiP, XBP1-s & XBP1-u protein expression were performed. α-tubulin was used as internal control. Blots of P-p70S6K1, p70S6K1, P-PERK, PERK, & α-tubulin of Figure 4B & blots of Figure 4C have been performed on the same electrophoresis gel, but cut & reconstituted. (D) Cells were incubated in medium alone (Ctrl) or with 10 nM rapamycin for 24 h. Nuclear localization of ATF6 was assessed using immunofluorescence with ATF6-antibody (red) & Hoechst dye. Magnification ×1000. (E) Bar graphs were obtained by quantification of ATF6 nuclear staining. Results are representative of at least 3 experiments. Image collected & cropped by CiteAb from the following publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.15469),

licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Page 9 of 13 v.20.1 Updated 12/5/2024





Page 10 of 13 v.20.1 Updated 12/5/2024





	Page 11 of 13 v.20.1 Updated 12/5/2024
Western Blot: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] - Tm induced UPR increases expression of RANKL in cultured osteoblastic & osteocytic cells. (A) Western blotting of cell lysates obtained from neonatal calvaria derived osteoblastic cells (Calvaria Ob) treated with 2.2 $\mu$ g/mL Tm for the indicated times. (B D) Gene expression as determined by qRT PCR in (B) calvaria derived osteoblastic cells (n = 3/group) (C) Osteoblastic UAMS 32 cells (UAMS 32 Ob) (n = 4/group) or (D) osteocytic MLO Y4 cells (MLO Y4 Ot) (n = 4/group),maintained in presence of vehicle (, 0.1% DMSO) or 2.2 $\mu$ g/ml Tm () for 4 hours. (E) Western blot of RANKL protein in cell lysates obtained from calvaria derived osteoblastic cells as described in A in a separate study. Data shown are the mean & SD with individual data points. *P < .05 vs vehicle by Student's t test Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32259048), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	<ul> <li>(A) Calvaria Ob</li> <li>Tm (min) 0 30 60 240</li> <li>p-elF2α</li> <li>t-elF2α</li> <li>ATF6</li> <li>tubulin</li> </ul>
Immunocytochemistry/Immunofluorescence: Mouse Monoclonal ATF6 Antibody (70B1413.1) [IMGENEX: IMG-273] [NBP1-40256] - Mice hepatocytes were stained for ATF6. Image from a verified customer review.	
Western Blot: Mouse Monoclonal ATF6 Antibody (70B1413.1) [IMGENEX: IMG-273] [NBP1-40256] - Western blot of the mouse liver homogenates from control (1) and alcohol-fed mice (2). Image from a verified customer review.	MW, KDa 1 2 260 140 100 70 50 40

www.novusbio.com



#### **Publications**

Elahe Zarini-Gakiye, Gholamhassan Vaezi, Kazem Parivar, Nima Sanadgol Age and Dose-Dependent Effects of Alpha-Lipoic Acid on Human Microtubule- Associated Protein Tau-Induced Endoplasmic Reticulum Unfolded Protein Response: Implications for Alzheimer's Disease. CNS & neurological disorders drug targets 2022-01-12 [PMID: 33573583]

Yuchang Li, Liting Chen, Lu Li, Chantal Sottas, Stephanie K. Petrillo, Anthoula Lazaris, Peter Metrakos, Hangyu Wu, Yuji Ishida, Takeshi Saito, Lucy Golden-Mason, Hugo R. Rosen, Jeremy J. Wolff, Cristina I. Silvescu, Samuel Garza, Garett Cheung, Tiffany Huang, Jinjiang Fan, Martine Culty, Bangyan Stiles, Kinji Asahina, Vassilios Papadopoulos Cholesterol-binding translocator protein TSPO regulates steatosis and bile acid synthesis in nonalcoholic fatty liver disease iScience 2021-05-01 [PMID: 34013171]

Liu D, Zhang P, Zhou J et al. TNFAIP3 Interacting Protein 3 Overexpression Suppresses Nonalcoholic Steatohepatitis by Blocking TAK1 Activation Cell Metab. 2020-04-07 [PMID: 32268115]

Rudalska R, Harbig J, Snaebjornsson M et al. LXR alpha activation and Raf inhibition trigger lethal lipotoxicity in liver cancer Nature Cancer 2021-02-01 [PMID: 35122079]

Amit U Joshi, Nay L Saw, Hannes Vogel, Anna D Cunnigham, Mehrdad Shamloo, Daria Mochly Rosen Inhibition of Drp1/Fis1 interaction slows progression of amyotrophic lateral sclerosis EMBO Molecular Medicine 2018-01-15 [PMID: 29335339]

M Navas-Madr, E Castelblan, M Camacho, M Consegal, A Ramirez-Mo, MR Sarrias, P Perez, N Alonso, M Galán, D Mauricio Role of the Scavenger Receptor CD36 in Accelerated Diabetic Atherosclerosis Int J Mol Sci, 2020-10-05;21 (19):. 2020-10-05 [PMID: 33028031]

Nancy Ahuja, Shalini Gupta, Rashmi Arora, Ella Bhagyaraj, Drishti Tiwari, Sumit Kumar, Pawan Gupta Nr1h4 and Thrb ameliorate ER stress and provide protection in the MPTP mouse model of Parkinson's Life Science Alliance 2024-04-12 [PMID: 38609183]

Ming-Hong Sun, Wen-Jie Jiang, Xiao-Han Li, Song-Hee Lee, Geun Heo, Dongjie Zhou, Zhi Chen, Xiang-Shun Cui ATF6 aggravates apoptosis in early porcine embryonic development by regulating organelle homeostasis under high-temperature conditions Zoological Research 2023-09-18 [PMID: 37501400]

Ryan D.R. Brown, Christopher D. Green, Cynthia Weigel, Bin Ni, Francesco S. Celi, Richard L. Proia, Sarah Spiegel Overexpression of ORMDL3 confers sexual dimorphism in diet-induced non-alcoholic steatohepatitis Molecular Metabolism 2023-12-09 [PMID: 38081412]

Shimizu Y, Nakamura K, Yoshii A et al. Paneth cell alpha-defensin misfolding correlates with dysbiosis and ileitis in Crohn\'s disease model mice Life Sci Alliance 2020-06-01 [PMID: 32345659]

Suravi Majumder, Abhijnan Chattopadhyay, Jamie M Wright, Pujun Guan, L Maximilian Buja, Callie S Kwartler, Dianna M Milewicz Pericentrin deficiency in smooth muscle cells augments atherosclerosis through HSF1-driven cholesterol biosynthesis and PERK activation. JCI insight 2023-11-14 [PMID: 37937642]

Alexandru P, Chiritoiu G, Lixandru D et al. EDEM1 regulates the insulin mRNA level by inhibiting the endoplasmic reticulum stress-induced IRE1/JNK/c-Jun pathway iScience 2023-09-01 [PMID: 37822496] (WB, Rat)

More publications at http://www.novusbio.com/NBP1-40256





## Novus Biologicals USA

10730 E. Briarwood Avenue Centennial, CO 80112 USA Phone: 303.730.1950 Toll Free: 1.888.506.6887 Fax: 303.730.1966 nb-customerservice@bio-techne.com

## **Bio-Techne Canada**

21 Canmotor Ave Toronto, ON M8Z 4E6 Canada Phone: 905.827.6400 Toll Free: 855.668.8722 Fax: 905.827.6402 canada.inquires@bio-techne.com

## **Bio-Techne Ltd**

19 Barton Lane Abingdon Science Park Abingdon, OX14 3NB, United Kingdom Phone: (44) (0) 1235 529449 Free Phone: 0800 37 34 15 Fax: (44) (0) 1235 533420 info.EMEA@bio-techne.com

## **General Contact Information**

www.novusbio.com Technical Support: nb-technical@biotechne.com Orders: nb-customerservice@bio-techne.com General: novus@novusbio.com

#### 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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NBP1-40256

Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications

