

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 8/21/2025 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	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS

Product Description

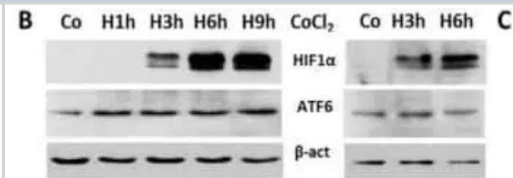
Description	<p>(1) The ATF6 (IMG-273) has been cited to recognize both full-length and cleaved forms of ATF6. Please refer to the references in the Product Citation list for more comprehensive information.</p> <p>(2) The ATF6 transfected cell lysate (40210) is recommended as a useful western blot positive control to run in parallel with your experimental samples.</p> <p>(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.</p> <p>(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 IMG-273 in the nucleus.</p> <p>(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).</p> <p>(6) Cleaved 60 and 36 kDa ATF6 forms have also been described in the nucleus (Mao et al, 2007).</p> <p>(7) The ATF6 antibody is reported to be specific for ATF6a, recognizing ATF6a but not ATF6b (Bommiasamy et al, 2009).</p> <p>(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.</p> <p>(9) We highly recommend the use of a maximum sensitivity ECL substrate (Femto sensitive) for efficient detection of this antibody in Western blot applications.</p>
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

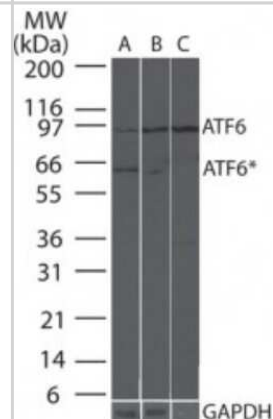
Applications	Western Blot, Simple Western, Immunohistochemistry-Paraffin, Flow Cytometry, Flow (Intracellular), Immunoblotting, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), CyTOF-ready, Knockout Validated
Recommended Dilutions	Western Blot 1-5 ug/ml, Simple Western, 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

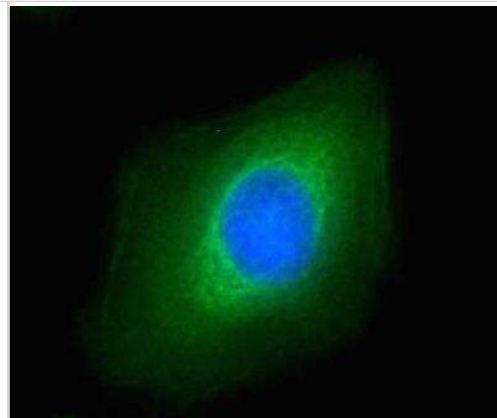
Western Blot: ATF6 Antibody (70B1413.1) [NBP1-40256] - Hypoxia leads to EMT and ER-stress in CRC cells. B. C. Confluent growing SW480 (B) and HCT116 (C) cells were cultured under conditions of normoxia or hypoxia-like conditions (serum free; 100 uM CoCl₂, 1-9 h. B-actin (B-act) as loading control. HIF1a 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](https://doi.org/10.1371/journal.pone.0087386)) licensed under a CC-BY license.



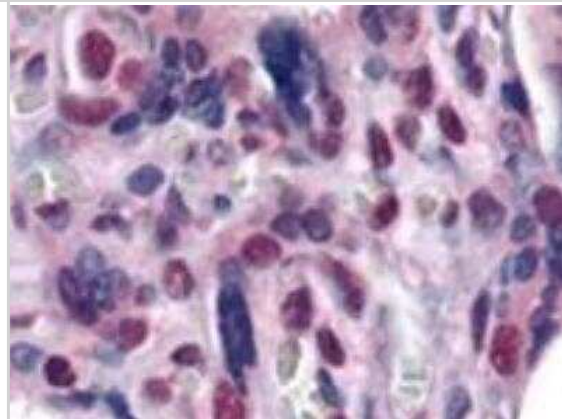
Western Blot: ATF6 Antibody (70B1413.1) [NBP1-40256] - Analysis of ATF6 in mouse liver tissue using 3 ug/ml of ATF6 antibody and 0.25 ug/ml of GAPDH antibody. Lane A contains 20 ugs of whole mouse liver lysate, lane B contains 20 ugs of total ER fraction, and lane C contains 20 ugs of rough ER fraction. The ATF6 band may represent under glycosylated or cleaved/active ATF6.



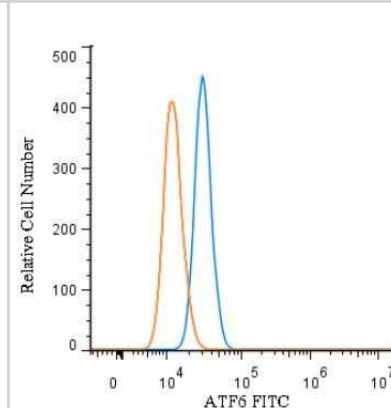
Immunocytochemistry/Immunofluorescence: ATF6 Antibody (70B1413.1) [NBP1-40256] - Untreated HeLa cells were fixed in -20C methanol for 10 min, air dried and rehydrated in PBS at room temperature for 5 minutes. Cells were incubated with anti-ATF6 (1:20) for one hour at room temperature. ATF6 reactivity (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 paraffin-embedded 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.

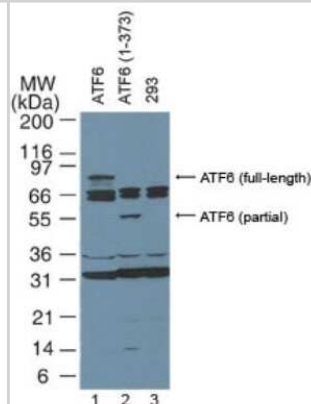


Western Blot: ATF6 Antibody (70B1413.1) [NBP1-40256] - Lane 1: 293 cells transfected with full-length ATF6.

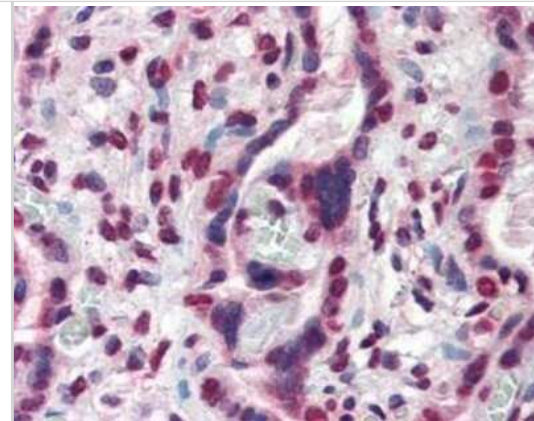
Lane 2: 293 cells transfected with partial length ATF6 (amino acids 1-373).

Lane 3: Untransfected 293 cells.

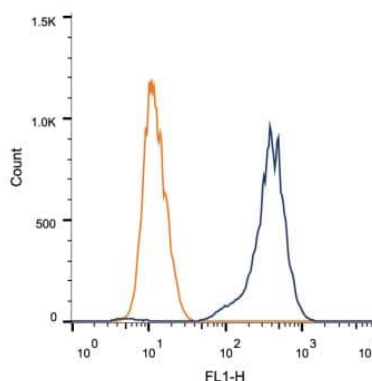
Western blots were probed with 4 ug/ml of the ATF6 monoclonal antibody and visualized with PicoTect Western Blot Chemiluminescence Substrate (10087K). Film was exposed for 1 min. The top arrow corresponds to the 90 kDa form of ATF6 described as full-length in the literature. The human full-length and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



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

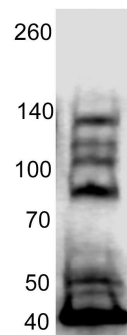


Flow Cytometry: ATF6 Antibody (70B1413.1) [NBP1-40256] - Intracellular flow cytometric staining of 1×10^6 MCF-7 cells using ATF6 antibody (dark blue). Isotype control shown in orange. An antibody concentration of 1 ug/1x10⁶ cells was used.



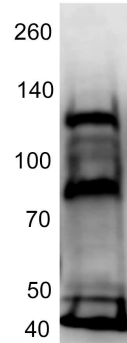
Western Blot: ATF6 Antibody (70B1413.1) [NBP1-40256] - Lysate of DU145 cells. Image from verified customer review.

MW, KDa



Western Blot: ATF6 Antibody (70B1413.1) [NBP1-40256] - Lysate of PC-3 cells. Image from verified customer review.

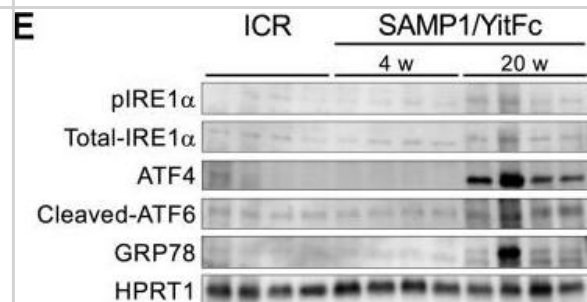
MW, KDa



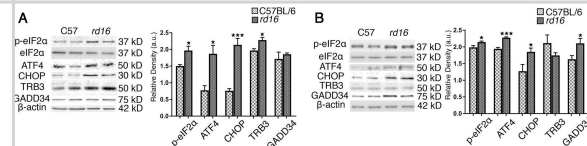
Expression of ER stress-associated transcription factors in NASH, NAFL and normal liver tissues. β -Actin was used as an internal control. Horizontal lines represent means of densitometry signals from the western blot analyses for all tissue groups. #, significant differences in signals between NASH and normal liver tissues ($P < 0.05$). Data for NAFL tissues were not used for statistical comparisons because of limited sample number ($n = 2$).



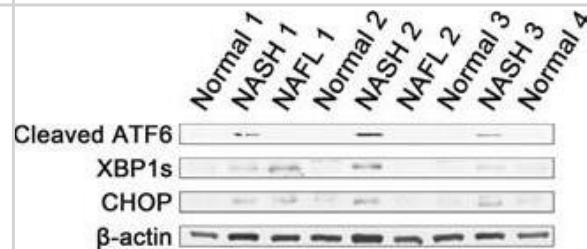
Abnormal Paneth cells show ER stress. (A, B) Representative transmission electron microscopy images of Paneth cells at the base of ileal crypts in (A) ICR & (B) SAMP1/YitFc mice. Scale bars indicate 2 μ m. (C, D) Quantitative analysis of (C) granule number & (D) ER lumen diameter in Paneth cells ($n = 3$ /each week for SAMP1/YitFc mice). For the measurements, three Paneth cells were randomly selected from each mouse. (E) SDS-PAGE Western blot analysis of ER stress markers, pIRE1 α , ATF4, cleaved-ATF6, & GRP78 in ileal crypts ($n = 4$ /each group). Total-IRE1 α & HPRT1 was used as loading control. (F) Relative expression level of ER stress markers calculated from the band intensity. Error bars represent mean \pm SEM. (C, D, F) Statistical significance was evaluated by t test in (C, D), & one-way ANOVA followed by Tukey's post hoc test in (F). $P < 0.05$ was considered statistically significant. * $P < 0.05$, † $P < 0.01$, § $P < 0.001$. E, ER; G, granules; N, nucleus; n.s., not significant. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32345659>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



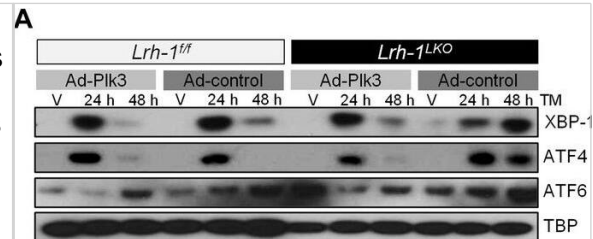
Western Blot: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] - The ISR is active in the retinas of rd16 mice. Western blots & corresponding graphs of ISR markers in the retinas of rd16 mice at P15 (a) & P20 (b). At P15, p-eIF2 α , ATF4, CHOP, & TRB3 are significantly elevated. At P20, levels of p-eIF2 α , ATF4, CHOP, & GADD34 are increased. Markers of the UPR are elevated in rd16 retinas. BiP & cleaved (~50 kD) ATF6 are upregulated in the retinas of rd16 mice at P15 (c) & P20 (d). Relative density measurements correspond to the intensities of the immunoblotting bands or lanes normalized to an internal control. Data are shown as mean \pm SEM. a.u. arbitrary units. P15 $n = 3$. P20 $n = 4$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/29706649>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



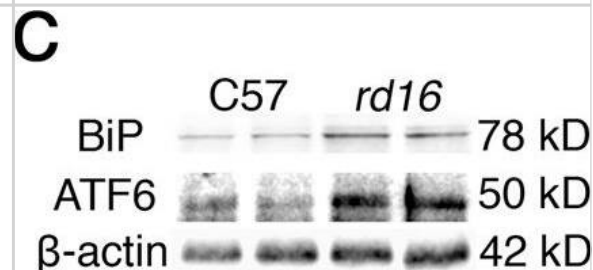
Western Blot: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] - Expression of ER stress-associated transcription factors in NASH, NAFL & normal liver tissues. β -Actin was used as an internal control. Horizontal lines represent means of densitometry signals from the western blot analyses for all tissue groups. #, significant differences in signals between NASH & normal liver tissues ($P < 0.05$). Data for NAFL tissues were not used for statistical comparisons because of limited sample number ($n = 2$). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/28968997>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



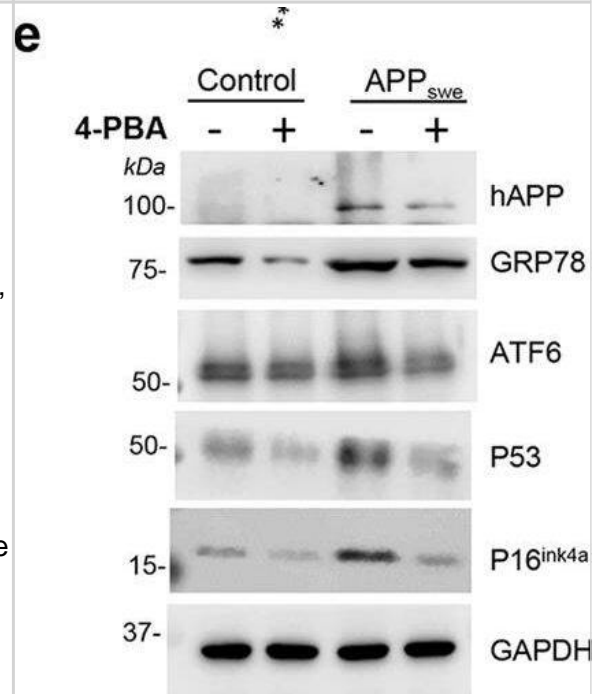
Western Blot: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] - Restoration of Plk3 induction rescues ATF2 phosphorylation & ER stress resolution in Lrh-1LKO mice, & loss or gain of ATF2 transcriptional activity also alters ER stress resolution capacity. (A) Primary hepatocytes prepared from Lrh-1^{fl/fl} and Lrh-1LKO mice & transduced with Ad-Plk3 or Ad-control at a MOI of 100. Cells treated 36 hr later with vehicle or tunicamycin (TM) (0.01 μ g/ml) & doxycycline (1 μ g/ml) to induce Plk3 or LacZ control. Nuclear protein was obtained at timepoints indicated & immunoblotted for UPR transcription factors, with TBP as a loading control. Results representative of 3 independent experiments. Image collected & cropped by CiteAb from the following publication (<https://elifesciences.org/articles/01694>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



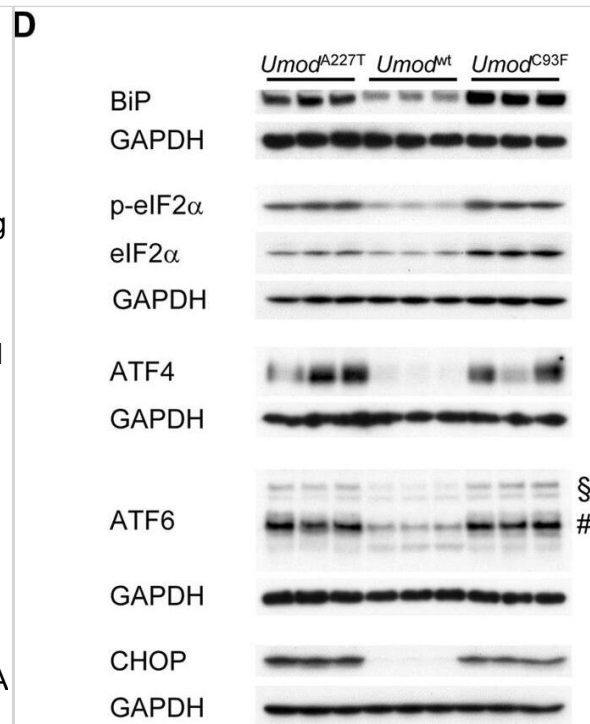
Western Blot: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] - The ISR is active in the retinas of rd16 mice. Western blots & corresponding graphs of ISR markers in the retinas of rd16 mice at P15 (a) & P20 (b). At P15, p-eIF2 α , ATF4, CHOP, & TRB3 are significantly elevated. At P20, levels of p-eIF2 α , ATF4, CHOP, & GADD34 are increased. Markers of the UPR are elevated in rd16 retinas. BiP & cleaved (~50 kD) ATF6 are upregulated in the retinas of rd16 mice at P15 (c) & P20 (d). Relative density measurements correspond to the intensities of the immunoblotting bands or lanes normalized to an internal control. Data are shown as mean \pm SEM. a.u. arbitrary units. P15 n = 3. P20 n = 4. *p < 0.05, **p < 0.01, ***p < 0.001 Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/29706649>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



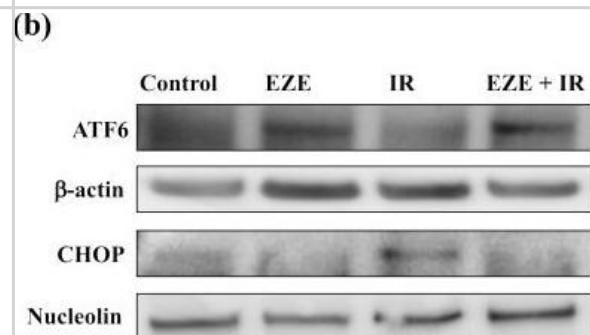
Western Blot: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] - APP^{swe} induction of OB-senescence via ER stress. a Heat map of differentially expressed ER stress or anti-stress related genes identified by RNA-seq in control (OCN-Cre; Ai9) & TgAPP^{swe}OCN; Ai9 Td+ OB-progenitors (detail analysis 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) & TgAPP^{swe}OCN; 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 \pm SD, n = 4, Student's t test. e Western blot analysis of indicated protein expression in BMSCs from 6-MO control & TgAPP^{swe}OCN 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- β -gal staining of 6-MO control & TgAPP^{swe}OCN BMSCs with vehicle (Veh)(PBS) & 4-PBA treatment, respectively, scale bar, 20 μ m. h Quantification of SA- β -gal+ cell densities in g (mean \pm 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.



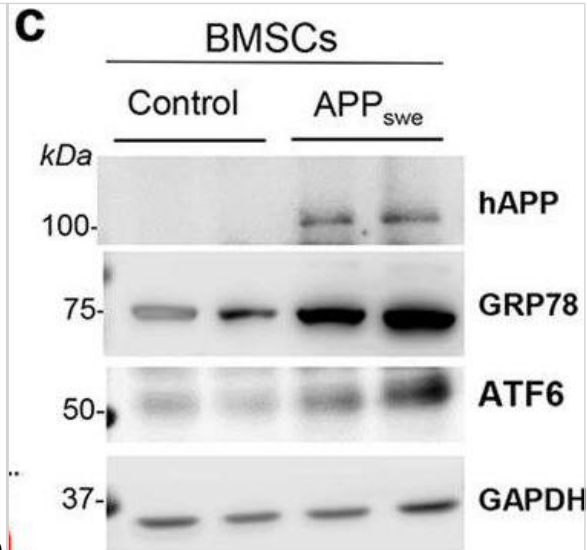
Western Blot: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] - Disturbance of ER homeostasis in ADTKD-UMOD. (A) ADTKD-UMOD is characterized by maturation & trafficking defect of mutant UMOD & intracellular accumulation of UMOD in TAL cells. UMOD immunolocalization revealed a diffuse cytoplasmic staining 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 α , eIF2 α , 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 \pm 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.



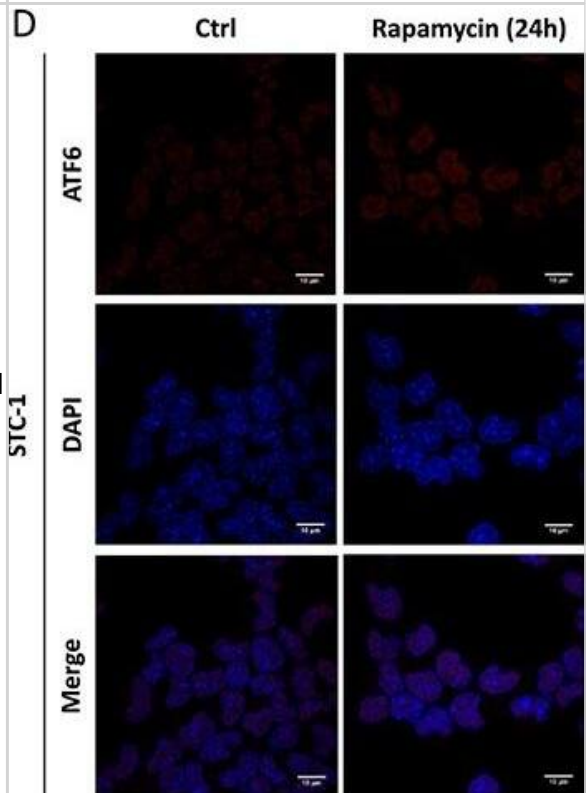
Western Blot: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] - The effect of Ezetimibe on unfolded protein response (UPR) gene expression in THP-1 cells exposed to ischemia-reperfusion (IR). (a) The mRNA expression of activating transcription factor 6 (ATF6) & CCAAT-enhancer-binding protein homologous 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; §p < 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.



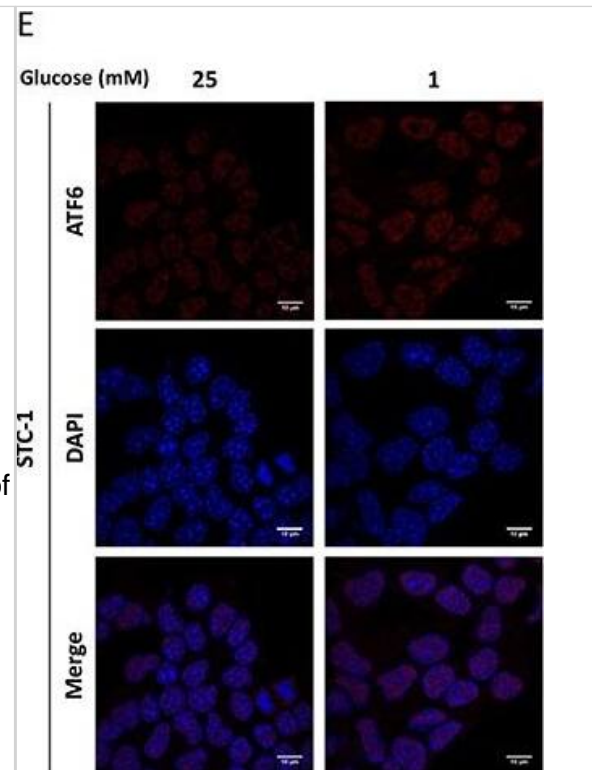
Western Blot: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] - APP^{swe} induction of OB-senescence via ER stress. a Heat map of differentially expressed ER stress or anti-stress related genes identified by RNA-seq in control (OCN-Cre; Ai9) & TgAPP^{swe}OCN; Ai9 Td+ OB-progenitors (detail analysis 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) & TgAPP^{swe}OCN; Ai9 mice, **p* < 0.05, ***p* < 0.01, ****p* < 0.001, mean ± 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 & TgAPP^{swe}OCN 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-β-gal staining of 6-MO control & TgAPP^{swe}OCN BMSCs with vehicle (Veh)(PBS) & 4-PBA treatment, respectively, scale bar, 20 μm. h Quantification of SA-β-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.



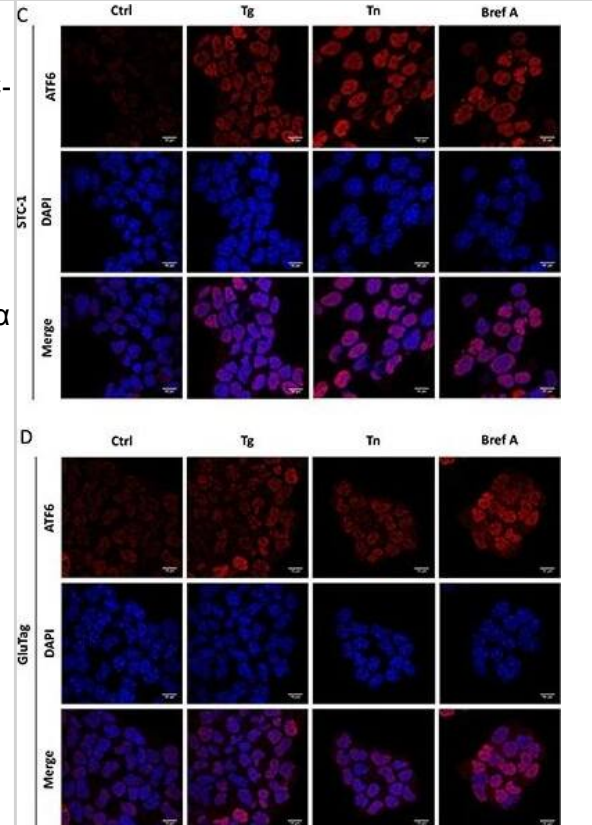
Immunocytochemistry/ Immunofluorescence: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] - Effect of rapamycin on markers of the UPR pathways(A) Cells were incubated in medium alone (0.05% FBS), with 3 nM IGF-1 or with both 3 nM IGF-1 & 10 nM rapamycin (IGF-1 + rapamycin). Total protein extracts prepared 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, eIF2α & 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.



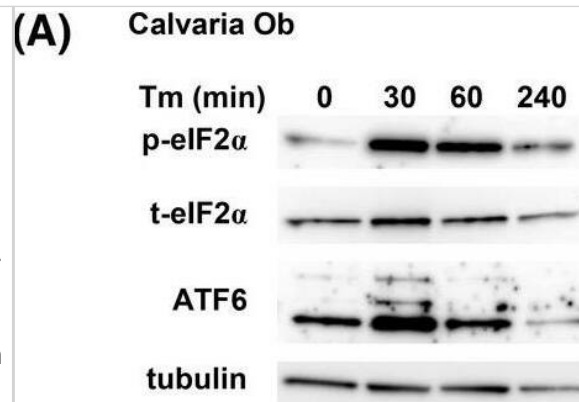
Immunocytochemistry/ Immunofluorescence: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] - Activation of the UPR during hypoxia or glucose depletion. STC-1 & GluTag cells were subjected to hypoxia (1%) or cultivated with decreasing concentration of glucose i.e. 25, 5 or 1 mM, for 24 h. (A) & (C) Protein expression level of phosphorylated & total forms of PERK, eIF2 α & ATF4, CHOP, BiP & C-Caspase 3 protein expression was examined using immunoblots, during hypoxia (A) & glucose depletion (C). α -tubulin was used as internal control. Blots of Figure 2A have been performed on the same electrophoresis gel, but cut & reconstituted. (B) & (D) XBP1 mRNA splicing was analyzed by RT-PCR after Pst1 digestion: XBP1-u, unspliced; XBP1-h, hybrid; XBP1-s, spliced variant of XBP1; *, XBP1-u mRNA fragments after Pst1 digestion. (E) ATF6 nuclear localization was assessed in STC-1 cells using immunofluorescence with anti-ATF6 antibody & hoechst dye. Magnification x1000. Results are representative of at least 3 independent experiments (A–E). (F) Bar graphs were obtained by quantification of ATF6 nuclear staining (*P < 0.05 versus control). Results are representative of 3 independent experiments (A–E) or the mean \pm S.E.M. of an experimental n = 3 (F). 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.



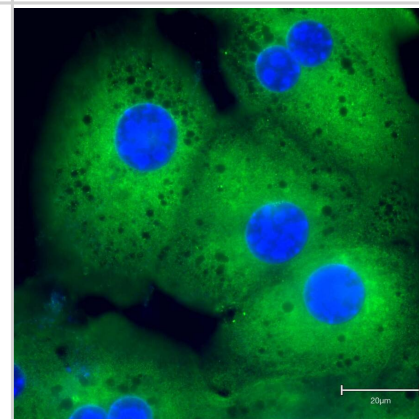
Immunocytochemistry/ Immunofluorescence: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] - UPR status in STC-1 & GluTag cell lines & effect of UPR inducers on markers of the UPR pathways. STC-1 & GluTag cells were incubated in medium (Ctrl) or ER stress-inducing agents thapsigargin (Tg, 300 nM), tunicamycin (Tn, 0.05 μ g/mL) & brefeldin A (Bref A, 3 μ M) for 4 h, 16 h & 8 h respectively. (A) Protein expression level of phosphorylated or total forms of PERK, eIF2 α & CHOP, BiP & cleaved-caspase 3 (C-Caspase 3) protein expression was examined using Western Blot analysis. α -tubulin was used as internal control (B) Densitometric quantification of P-PERK/PERK, P-eIF2 α /eIF2 α & BiP/ α -tubulin ratios analysis in STC-1 or GluTag cell lines (*P < 0.05 versus control). (C–D) The effect of Tg, Tn & Bref A on ATF6 nuclear localization was assessed by immunofluorescence in STC-1 cells (C) & GluTag cells (D) using anti-ATF6 antibody & Hoechst dye. Magnification \times 1000. (E) Bar graphs obtained by quantification of ATF6 nuclear staining (*P < 0.05 versus control). (F) XBP1 mRNA splicing was analyzed by RT-PCR after Pst1 digestion: XBP1-h, hybrid; XBP1-u, unspliced; XBP1-s, spliced variant of XBP1; *, XBP1-u mRNA fragments after Pst1 digestion. Results are representative of 3 independent experiments (A, C, D, F) or the mean \pm S.E.M. of an experimental n = 3 (B, E). 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.



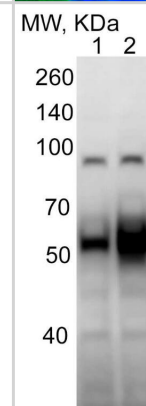
Western Blot: ATF6 Antibody (70B1413.1) - BSA Free [NBP1-40256] - Tm-induced UPR increases expression of RANKL in cultured osteoblastic & osteocytic cells. (A) Western blotting of cell lysates obtained from neonatal calvaria-derived osteoblastic cells (Calvaria Ob) treated with 2.2 µg/mL Tm for the indicated 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 µg/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.



Immunocytochemistry/Immunofluorescence: Mouse Monoclonal ATF6 Antibody (70B1413.1) [IMGEX: IMG-273] [NBP1-40256] - Mice hepatocytes were stained for ATF6. Image from a verified customer review.



Western Blot: Mouse Monoclonal ATF6 Antibody (70B1413.1) [IMGEX: 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.



Publications

Cervia L, Shibue T, Borah A et al. Data from A Ubiquitination Cascade Regulating the Integrated Stress Response and Survival in Carcinomas American Association for Cancer Research 2023-04-04 [PMID: 36576405] (Western Blot)

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] (Western Blot)

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] (Western Blot)

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, Garrett 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 Cunningham, Mehrdad Shamloo, Daria Mochly-Rosen Inhibition of Drp1/Fis1 interaction slows progression of amyotrophic lateral sclerosis EMBO Molecular Medicine 2018-01-15 [PMID: 29335339]

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]

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]

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

Procedures

Flow (Intracellular) Protocol for ATF6 Antibody (NBP1-40256)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2×10^5 and 1×10^6 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 μ L for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.
 - a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of 1×10^6 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 1 mL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

Optional: Perform cell surface staining as described in the previous section.

1. Fix the cells by adding 100 μ L fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 μ L of a permeabilization buffer to every 1×10^6 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
 - a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
 - b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 5 minutes at 400 RCF.
5. Discard supernatant and re-suspend in 1 mL of staining buffer + 0.1% permeabilizer.
6. Stain each sample at 1 μ L/ 1×10^6 cells of primary antibody or 1-3 μ L/ 1×10^6 cells for directly conjugated antibodies. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
7. Following the primary/conjugate incubation, add 2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 5 minutes at 400 RCF.
8. Remove supernatant and re-suspend each sample in 2 mL staining buffer + 0.1% permeabilizer, repeat wash for 5 minutes at 400 RCF.
9. If using a directly conjugated antibody, after the second wash, re-suspend cell pellet to a final volume of 500 μ L per sample and proceed with flow analysis.

Immunocytochemistry/ Immunofluorescence Protocol for ATF6 Antibody (NBP1-40256)**Immunocytochemistry Protocol**

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.

Immunohistochemistry-Paraffin Protocol for ATF6 Antibody (NBP1-40256)**Immunohistochemistry-Paraffin Embedded Sections****Antigen Unmasking:**

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.



Western Blot Protocol for ATF6 Antibody (NBP1-40256)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturer's instructions.





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@bio-techne.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

