Anti-ATF6 α antibody, mouse monoclonal (1-7)

Product code	73-500
Size	50 µg
Storage	-20℃
Concentration	1.0 mg/ml
Buffer	PBS- with 50% glycerol
Purity	Purified IgG fraction with protein A from hybridoma cell culture medium
Immunogen	Recombinant ATF6α (His-tagged amino-terminal fragment of ATF6α)
Isotype	Mouse IgG2aκ
Reactivity	Specific to human ATF6α. No cross reactivity with mouse ATF6α (for mouse, BioAcademia 73-005 clone 37-1 is recommended) This antibody is one of the two antibodies currently on market that can detect both precursor and activated forms of ATF6α at endogenous level.
Special notes	N/A
Application	1. Western blotting (1/500-1/1,000) 2. Immunoprecipitation (1/100-1/500) 3. Immunofluorescence staining (1/100-1/500)
Background	**ATF6** (**a**ctivating **t**ranscription **f**actor **6**) is an endoplasmic reticulum (ER) membrane-bound transcription factor activated in response to ER stress. When unfolded proteins accumulate in the ER, **ATF6** is cleaved by regulated intramembrane proteolysis. The resulting amino-terminal fragment translocates to the nucleus and activates transcription by binding to ER stress-response elements present in the promoter regions of ER stress-inducible genes including those encoding ER chaperones and components of ER-associated degradation. The mammalian **ATF6** family consists of two closely related homologs, **ATF6α** and ATF6β. **ATF6α** but not ATF6β plays a pivotal role in transcriptional control. The monoclonal antibody was characterized in the laboratory of Professor Kazutoshi Mori of Kyoto University. The antibody was produced from hybridoma cultured in serum-free medium and purified under mild conditions by propriety chromatography processes.
Data Link	UniProtKB P18850 (ATF6A_HUMAN)

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Data Images: 73-500 Anti-ATF6α antibody, mouse monoclonal (1-7)

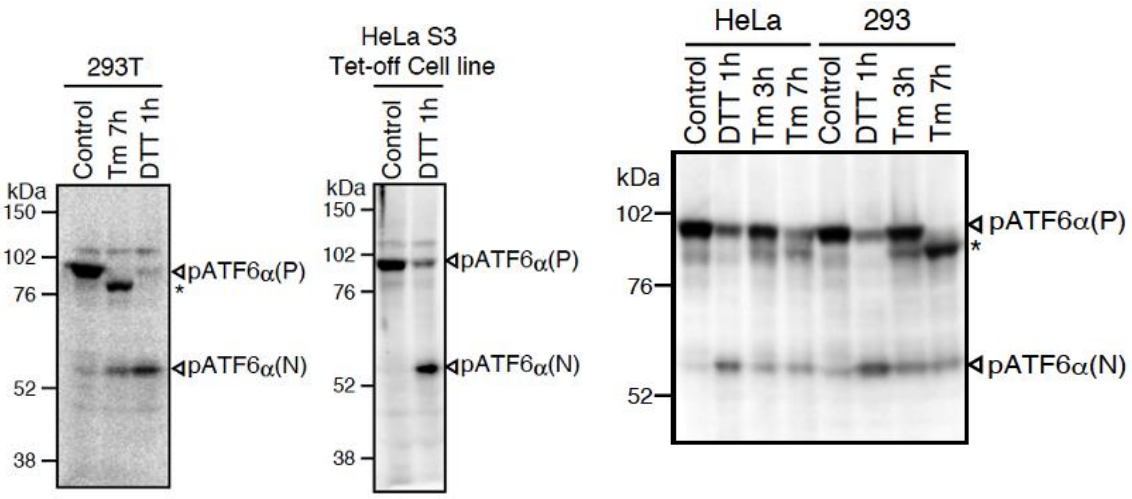

Fig.1 Western blot analysis of human cell extracts using this antibody: Conversion of pATF6 α (P) to pATF6 α (N) in DTT- or tunicamycin-treated cells.

Tm: 2 μg/ml tunicamycin (inhibitor of N-glycosylation). DTT: 1mM dithiothreitol (reducing reagent). The asterisk denotes an unglycosylated form of pATF6α(P).

ATF6 α is constitutively expressed as pATF6□(P) (~90-kDa protein), and converted to pATF6α(N) (>50-kDa protein) in ER-stressed cells.

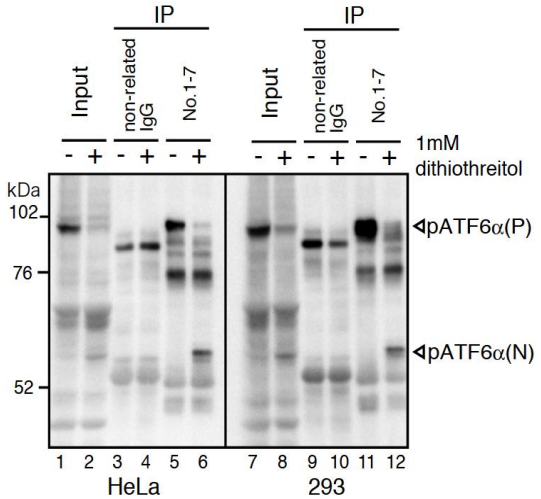

Fig.2 IP-Western blot analysis of human cell extracts using this antibody.

ATF6 α was detected by Western blot (Input; lanes 1, 2, 7, and 8) using this antibody (No.1-7). After immunoprecipitation (IP) with non-related IgG (IP; lanes 3, 4, 9, and 10) or this antibody (No. 1-7) (IP; lanes 5, 6, 11, and 12), samples were subjected to SDS-PAGE and analyzed by Western blot using this antibody (# 1-7) and anti-mouse IgG antibody (light chain specific).

Detection of pATF6 α (P) and pATF6 α (N) is better in IP-Western blotting than Western blotting.

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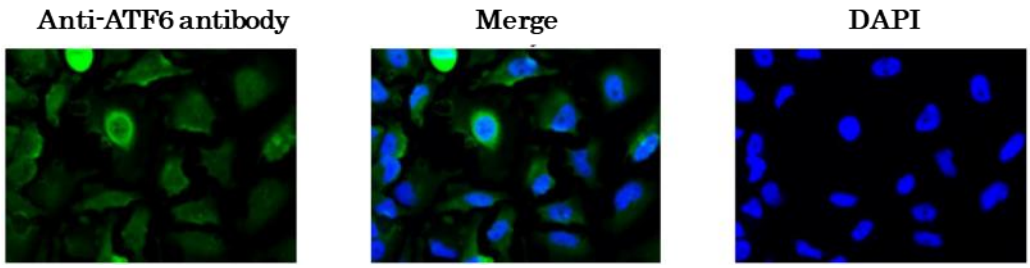

Fig.3 Immunofluorescence staining of ATF6 in HeLa cells by using anti-ATF6 antibody.

The cells were fixed with paraformaldehyde. The antibody was used at 1/100 dilution.

As the second antibody, Alexa Fluor 488 conjugated goat anti-mouse IgG antibody was used at 1/1,000 dilution.

In unstressed cell ATF6 α is localized in endoplasmic reticulum and upon receiving stress signal it is proteolytically processed and translocated into nucleus where it functions as transcriptional activator.

References: This antibody was described in Ref. 1 and has been used in the following publications.

1. Mori K .Divest yourself of a preconceived idea: transcription factor ATF6 is not a soluble protein! Mol Biol Cell 21: 11435-8 (2010) PMID: 20219975 Review article
2. Maiuolo J *et al.* Selective activation of the transcription factor ATF6 mediates endoplasmic reticulum proliferation triggered by a membrane protein. PNAS **108**: 7832-7 (2011) PMID: 21521793 **WB (human)**
3. Amyot J et al. Binding of activating transcription factor 6 to the A5/Core of the rat insulin II gene promoter does not mediate its transcriptional repression. J Mol Endocrinol. 2011 Sep30;47(3):273-83. PMID:21821716 WB (human)
4. Higa A. et al. Role of pro-oncogenic protein disulfide isomerase (PDI) family member anterior gradient 2 (AGR2) in the control of endoplasmic reticulum homeostasis. J Biol Chem. 2011 Dec 30;286(52):44855-68 PMID:22025610 **WB (human)**
5. Bouchecareilh M et al. Small GTPase Signaling and the Unfolded Protein Response. Methods in Enzymology Vol. 491; p. 343-360 (2011). PMID:21329809 **WB (human)**
6. Kondo S. et al. Activation of OASIS family, ER stress transducers, is dependent on its stabilization Cell Death and Differentiation (2012) **19,** 1939–1949. PMID: 22705851 **WB (human)**
7. Chiang WC et al. Selective Activation of ATF6 and PERK Endoplasmic Reticulum Stress Signaling Pathways Prevent Mutant Rhodopsin Accumulation. Invest Ophthalmol Vis Sci. 2012 Oct; 53(11): 7159–7166. PMID:22956602 **WB (human)**
8. Yi P et al. Sorafenib-mediated targeting of the AAA$^+$ ATPase p97/VCP leads to disruption of the secretorypathway, endoplasmic reticulum stress, and hepatocellular cancer cell death. Mol Cancer Ther. 2012 Dec;11(12):2610-20. PMID: 23041544 **WB (human)**
9. Shoulders MD et al. Stress-independent activation of XBP1s and/or ATF6 reveals three functionally diverse ERproteostasis environments. Cell Rep. 2013 Apr 25;3(4):1279-92. PMID:23583182 **WB**

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(human)

10.Kitai Y et al. Membrane lipid saturation activates IRE1α without inducing clustering. Genes Cells. 2013 Sep;18(9):798-809. PMID:23803178 **WB (human)**

11. Higa A. et al. Endoplasmic reticulum stress-activated transcription factor ATF6α requires the disulfide isomerase PDIA5 to modulate chemoresistance. Mol Cell Biol. 2014 May;34(10):1839-49. PMID: 24636989 **WB (human)**

12. Miyata S. et al. **X**anthohumol Improves Diet-induced Obesity and Fatty Liver by Suppressing Sterol Regulatory Element-binding Protein (SREBP) Activation. J Biol Chem. 2015 Aug 14;290(33):20565-79. PMID: 26140926 **WB (human)**

13. Plate L et al. Small molecule proteostasis regulators that reprogram the ER to reduce extracellular protein aggregation. Elife. 2016 Jul 20;5. pii: e15550. PMID:27435961 **WB (human)**

Protocol for ATF6 α analysis using anti-human ATF6 α monoclonal antibody

Both endogenous precursor ATF6□, pATF6□(P), and its cleaved product, pATF6 α (N), can be detected in human cells such as HEK293T, HEK293 and HeLa cells by western blot analysis using anti-human ATF6α monoclonal antibody clone 1-7 (Fig. 1), according to the procedures described below. Clarity of the results may depend on cell types and culture conditions. If clear results could not be obtained by western blot analysis as seen with HeLa and HEK293 cells (Fig.1 right panel), it is worth while trying immunoprecipitation followed by western blot analysis according to the procedures described below.

Western blotting

SDS-sample buffer: 50 mM Tris/HCl, pH6.8, containing 2% SDS, (100 mM DTT), 10% glycerol and BPB

PBST: PBS containing 0.1% Tween 20 Blocking buffer: PBS containing 0.1% Tween 20 and 5% skim milk

Sample Preparation (for HeLa or HEK293 cells cultured in 6cm dish)

(1) Wash cells with ice-cold PBS.
(2) Scrape cells in 500 µl of ice-cold PBS (+ protease inhibitor cocktail and 10 µM MG132) 2 times and collect cells by centrifugation at 5,000 rpm for 2 min.
(3) Lyse cells directly in 100 µl of SDS-sample buffer without reducing reagent (+ protease inhibitor cocktail and 10 µM MG132).
(4) Voltex mix vigorously.
(5) Boil the lysate for 5 min and voltex well.
(6) If the lysate is still viscous, boil again and voltex mix vigorously.
(7) Centrifuge at 14,000 rpm for 2 min.
(8) Determine protein concentration using BCA protein assay kit.

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SDS-PAGE and incubation with antibody

(9) Add one-tenth volume of 1 M DTT and boil for 5 min.

(10) Subject 50 μg of the lysate to 8% SDS-PAGE.

(11) Transfer to nitrocellulose membrane (such as Hybond-ECL, GE Healthcare).

(12) Incubate the membrane in Blocking buffer overnight at 4˚C.

(13) Incubate the membrane with primary antibody diluted in Blocking buffer (1:500-1:1000) for 1 h at room temperature or overnight at 4˚C. Wash the membrane 3 times each for 5 min with PBST.

(14) Incubate the membrane with HRP-conjugated secondary antibody for 1 h at room temperature. We recommend "ECL anti-mouse IgG, Horseradish Peroxidase linked F(ab')2 fragment" (GE Healthcare NA9310V-1ML) or "Peroxidase-conjugated AffniPure Goat Anti-Mouse IgG, Light Chain Specific" (Jackson ImmunoReseach 115-035-174).

(15) Wash the membrane 3 times each for 5 min with PBST.
Detect signals using an appropriate luminescent reagent.

Immunoprecipitation

(For HeLa or HEK293 cells cultured in 6 cm dish)

Lysis buffer: 50 mM Tris-HCl pH7.5, containing 150 mM NaCl, 1% NP-40, protease inhibitor cocktail and 10 μM MG132)

(1) Wash cells with ice-cold PBS, suspend them in 400 μl of Lysis buffer and stand on ice for 10 min.

(2) Clear the lysate by centrifugation at 14,000rpm for 10 min at 4˚C and transfer 300 μl of the supernatant to a new tube.

(3) Add anti-ATF6α antibody (1-3 μg) into the supernatant and incubate with gentle rotation for 2 h～overnight at 4˚C.

(4) Add 30 μl of the 50% slurry of ProteinG-Sepharose suspended in Lysis buffer into the tube and incubate with gentle rotation for 1 h at 4˚C.

(5) Wash the Sepharose beads 2 times with Lysis buffer.

(6) Wash the Sepharose beads with PBS.

(7) Resuspend the Sepharose beads in 30 μl of SDS-sample buffer containing 100 mM DTT. Use 10 μl aliquot of the sample for Western blot analysis.

***Critical point■**

In IP-western blot analysis, use a light chain specific anti-mouse IgG antibody as a secondary antibody. "Peroxidase-conjugated AffniPure Goat Anti-Mouse IgG, Light Chain Specific" (Jackson ImmunoReseach 115-035-174) is recommended.

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