



## **Anti-ATF6 $\alpha$ antibody, mouse monoclonal (1-7)**

### **BACKGROUND**

**ATF6** (activating transcription factor **6**) is an endoplasmic reticulum (ER) membrane-bound transcription factor activated in response to ER stress. When unfolded proteins accumulate in the ER, **ATF6  $\alpha$**  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, **ATF6  $\alpha$**  and **ATF6  $\beta$** . **ATF6  $\alpha$**  but not **ATF6  $\beta$**  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.

### **Applications: (Detailed Protocol is given below)**

1. Western blotting
2. Immunoprecipitation (IP)

This antibody does not work for immunofluorescence analyses.

**Immunogen:** Recombinant ATF6  $\alpha$  (His-tagged amino-terminal fragment of ATF6  $\alpha$ )

**Epitope:** Not determined

**Isotype:** Mouse IgG2a  $\kappa$

**Form:** Purified monoclonal antibody (IgG) 1mg/ml in PBS, 50% glycerol, filter-sterilized

**Specificity:** Specific to human ATF6  $\alpha$ , no cross reactivity with mouse ATF6  $\alpha$

**Size:** 50 ug

**Storage:** Shipped at 4°C or -20°C and stored at -20°C.

**Data Link:** UniProtKB/Swiss-Prot [P18850](#) (ATF6A\_HUMAN)

**References:** This antibody was used in Ref. 4 and described in Ref. 5..

1. Hai T *et al* "Transcription factor ATF cDNA clones: an extensive family of leucine zipper proteins able to selectively form DNA-binding heterodimers" *Genes Dev* **3**: 2083-2090 (1989) PMID: [2516827](#)
2. Haze K *et al* "Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress" *Mol Biol Cell* **10**: 3787-3799 (1999) PMID: [10564271](#)
3. Yamamoto K *et al* "Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6 and XBP1" *Dev Cell* **13**: 365-376 (2007) PMID: [17765680](#)
4. 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](#)
5. 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](#)

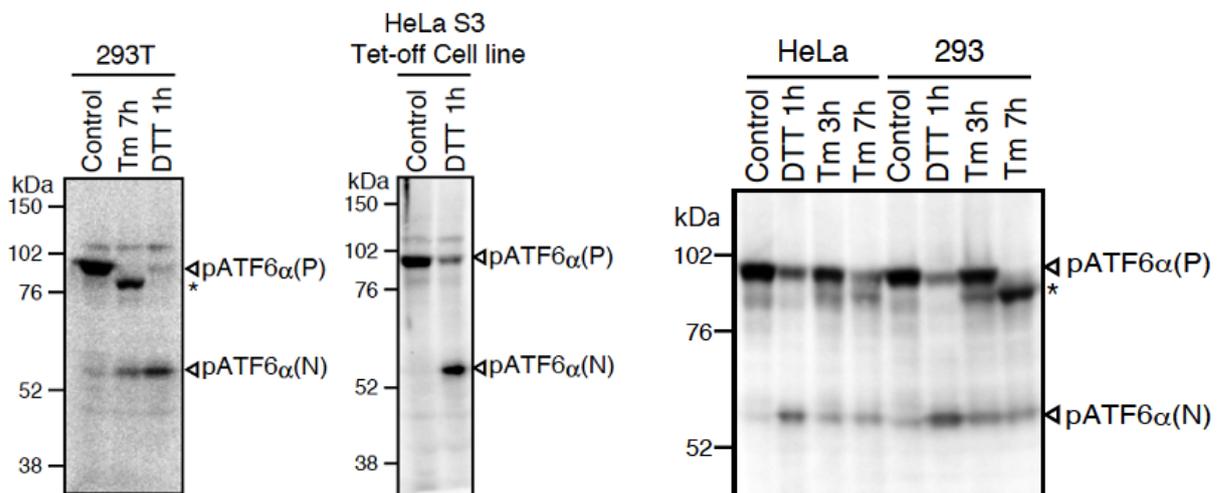


**Protocol for ATF6 $\alpha$  analysis using anti-human ATF6 $\alpha$  monoclonal antibody**

Both endogenous precursor ATF6 $\alpha$ , pATF6 $\alpha$  (P), and its cleaved product, pATF6 $\alpha$  (N), can be detected in human cells such as HEK293T, HEK293 and HeLa cells by western blot analysis using anti-human ATF6 $\alpha$  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.

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

Tm: 2  $\mu$ g/ml tunicamycin (inhibitor of N-glycosylation). DTT: 1mM dithiothreitol (reducing reagent). The asterisk denotes an unglycosylated form of pATF6 $\alpha$  (P). ATF6 $\alpha$  is constitutively expressed as pATF6 $\alpha$  (P) (~90-kDa protein), and converted to pATF6 $\alpha$  (N) (>50-kDa protein) in ER-stressed cells.



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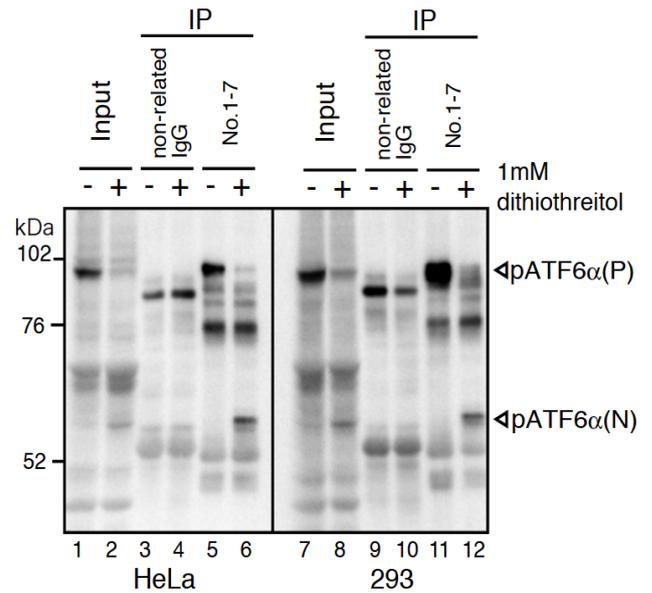


Anti-ATF6 $\alpha$  antibody, mouse monoclonal (1-7)

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

ATF6 $\alpha$  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 $\alpha$  (P) and pATF6 $\alpha$  (N) is better in IP-Western blotting than Western blotting.



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**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  $\mu$ l of ice-cold PBS (+ protease inhibitor cocktail and 10  $\mu$ M MG132) 2 times and collect cells by centrifugation at 5,000 rpm for 2 min.
- (3) Lyse cells directly in 100  $\mu$ l of SDS-sample buffer without reducing reagent (+ protease inhibitor cocktail and 10  $\mu$ M MG132).
- (4) Vortex mix vigorously.
- (5) Boil the lysate for 5 min and vortex well.
- (6) If the lysate is still viscous, boil again and vortex mix vigorously.
- (7) Centrifuge at 14,000 rpm for 2 min.
- (8) Determine protein concentration using BCA protein assay kit.

• SDS-PAGE and incubation with antibody

- (9) Add one-tenth volume of 1 M DTT and boil for 5 min.
- (10) Subject 50  $\mu$ g of the lysate to 8% SDS-PAGE.
- (11) Transfer to nitrocellulose membrane.
- (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.
- (15) Wash the membrane 3 times each for 5 min with PBST.
- (16) Detect signals using an appropriate luminescent reagent.

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**Immunoprecipitation**

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

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

- (1) Wash cells with ice-cold PBS, suspend them in 400  $\mu$ l 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  $\mu$ l of the supernatant to a new tube.
- (3) Add anti-ATF6 $\alpha$  antibody (1-3  $\mu$ g) into the supernatant and incubate with gentle rotation for 2 h~overnight at 4°C.
- (4) Add 30  $\mu$ l 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  $\mu$ l of SDS-sample buffer containing 100 mM DTT. Use 10  $\mu$ l aliquot of the sample for Western blot analysis.

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