Anti-ATF6 α antibody, mouse monoclonal (37-1)

73-505, 100 μg

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. **ATF6** consists of two closely related factors, **ATF6α** and ATF6β, in mammals. **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.

Applications: (Detailed Protocol is given below)

1. Western blotting
2. Immunoprecipitation (IP) (less efficient than clone1-7)

This antibody does not work for immunofluorescence analyses.

Immunogen: Recombinant ATF6α (amino-terminal fragment of ATF6α fused to GST)

Isotype: mouse IgG1 κ **Epitope:** not determined

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

Specificity: Reactive to human and mouse ATF6α. However, clone 1-7 antibody (#73-500) is recommended for human cells.

Storage: -20℃ (long period, -70℃)

Data Link Swiss-Prot P18850 (human ATF6 alpha)

References: This antibody is described in Ref 4.

1. Hai T *et al* (1989) "Transcription factor ATF cDNA clones: an extensive family of leucine zipper proteins able to selectively form DNA-binding heterodimers." *Genes Dev* **3**: 2083-2090 PMID 2516827
2. Haze K *et al* (1999) "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 PMID: 10564271
3. Yamamoto K *et al* (2007) "Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6α and XBP1". *Dev. Cell* **13**: 365-376 PMID: 17765680
4. 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

Related Product: #73-500 anti-ATF6 alpha (clone 1-7)

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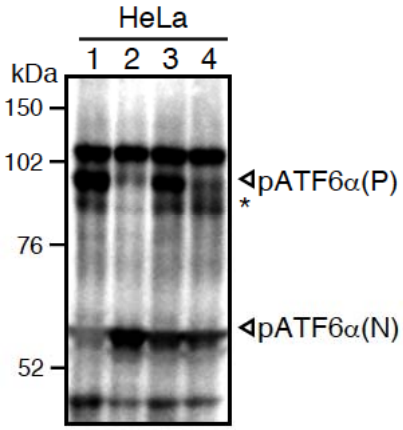
Protocol for ATF6α analysis using anti-human ATF6α monoclonal antibody (37-1)

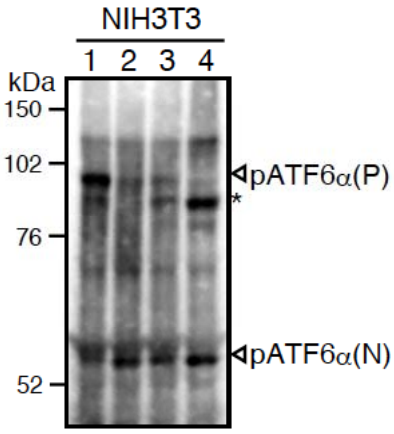
Both endogenous precursor ATF6α, pATF6α(P), and its cleaved product, pATF6α(N), can be detected in human cells such as HeLa cells by western blot analysis using anti-human ATF6α monoclonal antibody clone 37-1 (Fig. 1), according to the procedures described below.

As clone 37-1 cross reacts with mouse ATF6α, both endogenous precursor ATF6α, pATF6α(P), and its cleaved product, pATF6α(N), can be detected in mouse cells such as NIH3T3 cells by western blot analysis (Fig. 2), according to the procedures described below.

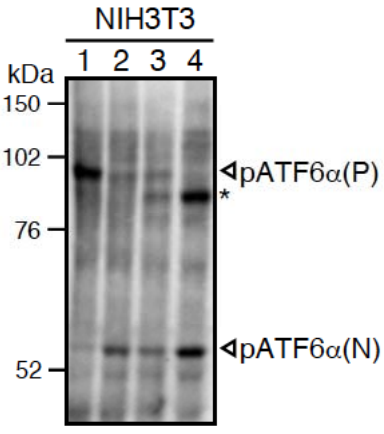
Fig.1 Western blot analysis of human cell extracts using this antibody: Conversion of pATF6α(P) to pATF6α(N) in DTT- or tunicamycin-treated cells.

1) untreated
2) DTT: 1mM dithiothreitol (reducing reagent) for 1 h.
3) Tm: 2 μg/ml tunicamycin (inhibitor of N-glycosylation) for 3 h.
4) Tm: 2 μg/ml tunicamycin (inhibitor of N-glycosylation) for 7 h.
The asterisk denotes an unglycosylated form of pATF6α (P).
ATF6α is constitutively expressed as pATF6α(P) (~90-kDa protein), and converted to pATF6α(N) (>50-kDa protein) in ER-stressed cells.





10 min exposure



1 min exposure with Can Get Signal

Fig.2 Western blot analysis of mouse cell extracts using this antibody: Conversion of pATF6α(P) to pATF6α(N) in DTT- or tunicamycin-treated cells.

1) untreated.
2) DTT: 1mM dithiothreitol for 1 h.
3) Tm: 2 μg/ml tunicamycin for 3 h.
4) Tm: 2 μg/ml tunicamycin for 7 h.
The asterisk denotes an unglycosylated form of pATF6α (P).
ATF6α is constitutively expressed as pATF6α(P) (~90-kDa protein), and converted to pATF6α(N) (>50-kDa protein) in ER-stressed cells.

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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 NIH3T3 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.

・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 (**overnight incubation is esssntial**).

(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).

(15) Wash the membrane 3 times each for 5 min with PBST.

(16) Detect signals using an appropriate luminescent reagent.

*Clearer results can be obtained by using ‘Can Get Signal (TOYOBO NKB-101T)’ during incubation with primary and secondary antibodies, according to the manufacture’s instructions.

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