Anti-Phospho-ATF2 (T71) Antibody [SC05-90]

ET1610-30

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IP, IHC-P
Molecular Wt:	Predicted band size: 55 kDa
Clone number:	SC05-90

Description: Eukaryotic gene transcription is regulated by sequence-specific transcription factors which bind modular cis-acting promotor and enhancer elements. The ATF/CREB transcription factor family binds the palindromic cAMP response element (CRE) octanucleotide TGACGTCA. The ATF/CREB family includes CREB-1, CREB-2 (also designated ATF-4), ATF-1, ATF-2 and ATF-3. This family of proteins contain highly divergent N-terminal domains, but share a C-terminal leucine zipper for dimerization and DNA binding. ATF-2 forms homodimers and heterodimers with c-Jun to initiate CRE-dependent transcription. Phosphorylation of ATF-2 at Thr 69 and Thr 71 by stress-activated kinases is necessary for transcriptional activation. Myc also induces phosphorylation of ATF-2 at Thr 69 and Thr 71 to prolong the half-life of ATF-2. ATF-2 also functions as a histone acetyltransferase (HAT) by specifically acetylating histones H2B and H4 in vitro. The gene encoding human ATF-2 maps to chromosome 2q31.1.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Thr71 of Human ATF2 aa 58-94 / 505.

Positive control: Hela, NIH/3T3, SH-SY5Y, rat brain tissue, human prostate tissue, human uterus tissue, mouse colon tissue, human cervix tissue, human skin tissue, human breast tissue, human esophagus tissue, human placenta tissue, mouse large intestine tissue, Hela cells treated with 250ng/mL anisomycin for 30 minutes.

Subcellular location: Mitochondrion outer membrane, Nucleus, Cytoplasm.

Database links: SwissProt: P15336 Human | P16951 Mouse | Q00969 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:100-1:800

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

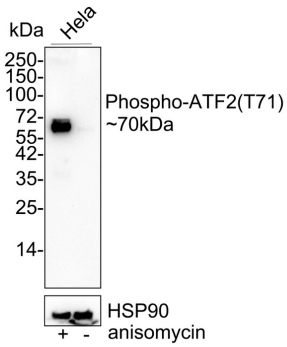

Fig1: Western blot analysis of Phospho-ATF2 (T71) on HeLa cell lysates treated with or without untreated anisomycin(250ng/ml 30min) with Rabbit anti-Phospho-ATF2 (T71) antibody (ET1610-30) at 1/1,000 dilution.

Lysates/proteins at 20 µg/Lane.

Predicted band size: 70 kDa
Observed band size: 70 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1610-30) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

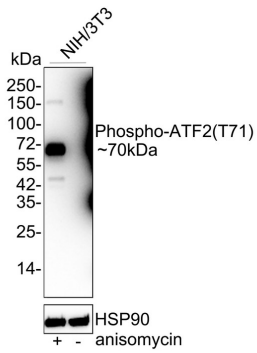

Fig2: Western blot analysis of Phospho-ATF2 (T71) on NIH/3T3 cell lysates treated with or without untreated anisomycin(250ng/ml 30min) with Rabbit anti-Phospho-ATF2 (T71) antibody (ET1610-30) at 1/1,000 dilution.

Lysates/proteins at 20 µg/Lane.

Predicted band size: 70 kDa
Observed band size: 70 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1610-30) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

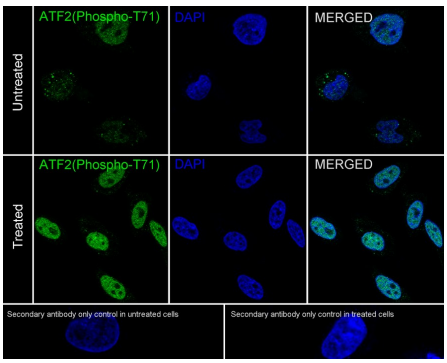

Fig3: Immunocytochemistry analysis of Hela cells treated with or without 250ng/mL anisomycin for 30 minutes labeling Phospho-ATF2 (T71) with Rabbit anti-Phospho-ATF2 (T71) antibody (ET1610-30) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Phospho-ATF2 (T71) antibody (ET1610-30) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

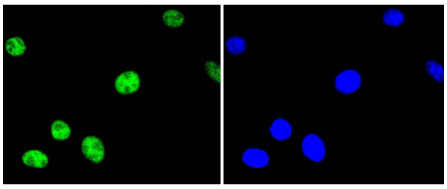

Fig4: ICC staining of Phospho-ATF2 (T71) in SH-SY5Y cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1610-30, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

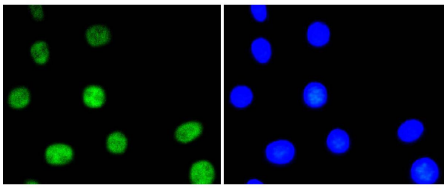

Fig5: ICC staining of Phospho-ATF2 (T71) in NIH/3T3 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1610-30, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

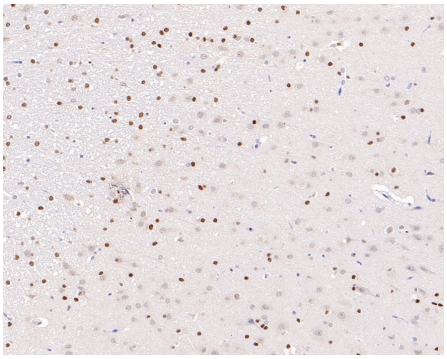

Fig6: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Phospho-ATF2 (T71) antibody (ET1610-30) at 1/800 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-30) at 1/800 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

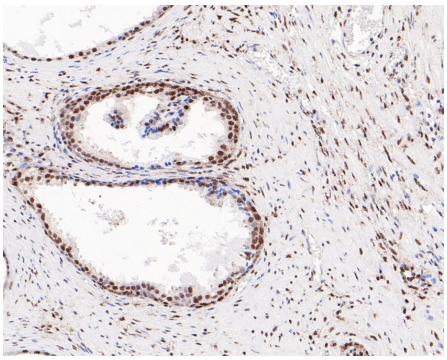

Fig7: Immunohistochemical analysis of paraffin-embedded human prostate tissue using anti-Phospho-ATF2 (T71) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-30, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

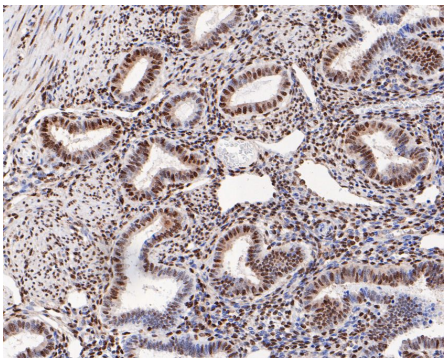

Fig8: Immunohistochemical analysis of paraffin-embedded human uterus tissue using anti-Phospho-ATF2 (T71) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-30, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

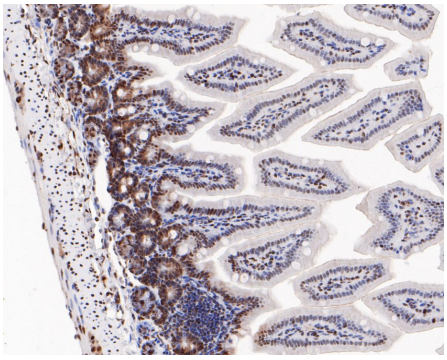

Fig9: Immunohistochemical analysis of paraffin-embedded mouse colon tissue using anti-Phospho-ATF2 (T71) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-30, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

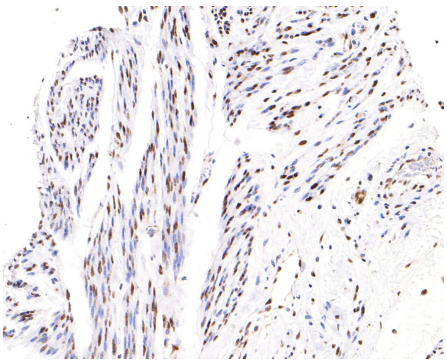

Fig10: Immunohistochemical analysis of paraffin-embedded human cervix tissue with Rabbit anti-Phospho-ATF2 (T71) antibody (ET1610-30) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-30) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

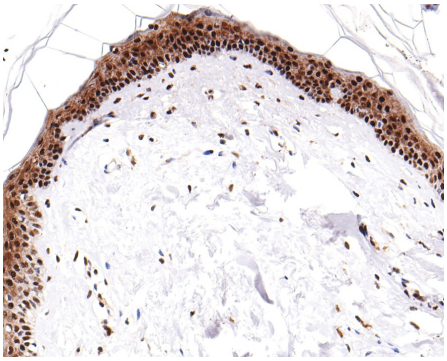

Fig11: Immunohistochemical analysis of paraffin-embedded human skin tissue with Rabbit anti-Phospho-ATF2 (T71) antibody (ET1610-30) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-30) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

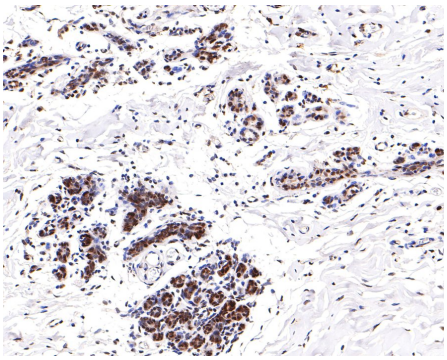

Fig12: Immunohistochemical analysis of paraffin-embedded human breast tissue with Rabbit anti-Phospho-ATF2 (T71) antibody (ET1610-30) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-30) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

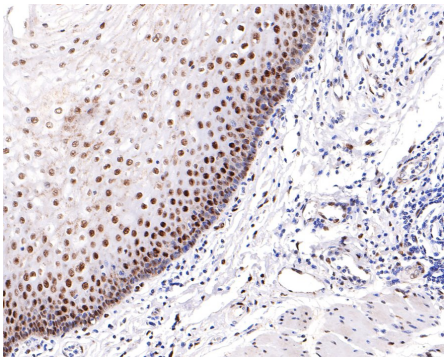

Fig13: Immunohistochemical analysis of paraffin-embedded human esophagus tissue with Rabbit anti-Phospho-ATF2 (T71) antibody (ET1610-30) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-30) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

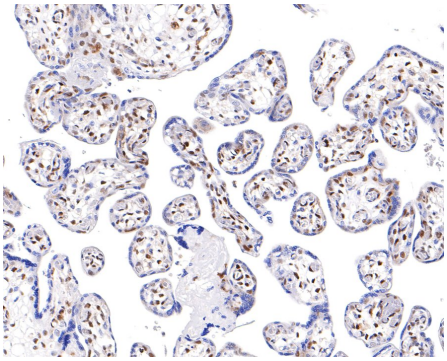

Fig14: Immunohistochemical analysis of paraffin-embedded human placenta tissue with Rabbit anti-Phospho-ATF2 (T71) antibody (ET1610-30) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-30) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

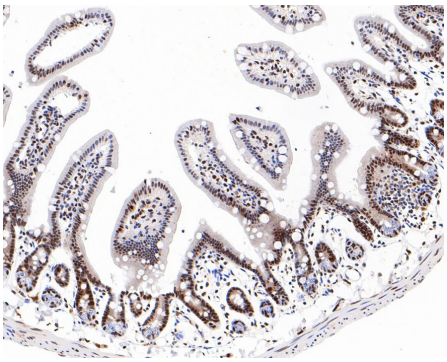

Fig15: Immunohistochemical analysis of paraffin-embedded mouse large intestine tissue with Rabbit anti-Phospho-ATF2 (T71) antibody (ET1610-30) at 1/800 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-30) at 1/800 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

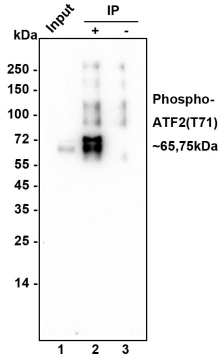

Fig16: Phospho-ATF2 (T71) was immunoprecipitated in 0.2mg HeLa cell treated anisomycin(25ug/ml 30min) lysate with (ET1610-30) at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using (ET1610-30) at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: (ET1610-30) IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of (ET1610-30) in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 3 mintues

Background References

1. Namachivayam, K. et al. 2015. All-Trans Retinoic Acid Induces TGF-β2 in Intestinal Epithelial Cells via RhoA- and p38α MAPK-Mediated Activation of the Transcription Factor ATF2. PloS one. 10: e0134003.
2. Lindaman, LL. et al. 2013. Phosphorylation of ATF2 and interaction with NFY induces c-Jun in the gonadotrope. Molecular and cellular endocrinology. 365: 316-26.