Anti-ATF5 Antibody [SD2099]

ET1612-38

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 31 kDa
Clone number:	SD2099

Description: Eukaryotic gene transcription is regulated by sequence-specific transcription factors that bind modular cis acting promoter and enhancer elements. The ATF/CREB transcription factor family binds the palindromic cAMP response element (CRE) octanucleotide TGACGTCA. The best characterized members of this gene family include CREB-1, CREB-2 (also designated ATF-4), CRE-BPa, LZIP (also designated CREB3 and Luman), CREM-1, CREM-2, ATF-1, ATF-2, ATF-3, ATF-5, ATF-6 and ATF-7. This family of proteins contain highly divergent N-terminal domains, but share a C-terminal leucine zipper for dimerization and DNA binding. ATF-5 (ATFx), which can localize to the cytoplasm or the nucleus, binds DNA as a dimer. It interacts with CCND3 and PTP4A1.

Immunogen: Synthetic peptide within Human ATF5 aa 203-252 / 282.

Positive control: HEK-293 cell lysate, Jurkat cell lysate, NIH/3T3 cell lysate, RAW264.7 cell lysate, C6 cell lysate, PC-12 cell lysate, HeLa, RAW264.7, C6, human liver tissue, human stomach tissue, mouse liver tissue, mouse stomach tissue, rat liver tissue, rat stomach tissue.

Subcellular location: Centrosome, Nucleus, Cytoplasm.

Database links: SwissProt: Q9Y2D1 Human | O70191 Mouse | Q6P788 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100
IF-Tissue	1:50-1:200
IHC-P	1:200-1:1,000
IP	1-2μg/sample
FC	1:1,000

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

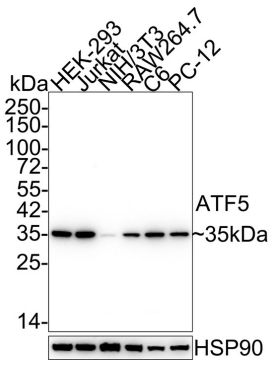


Fig1: Western blot analysis of ATF5 on different lysates with Rabbit anti-ATF5 antibody (ET1612-38) at 1/2,000 dilution.

Lane 1: HEK-293 cell lysate
Lane 2: Jurkat cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: RAW264.7 cell lysate
Lane 5: C6 cell lysate
Lane 6: PC-12 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 31 kDa
Observed band size: 35 kDa

Exposure time: 28 seconds;

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 (ET1612-38) at 1/2,000 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.

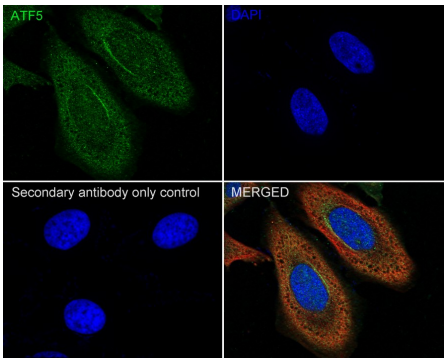


Fig2: Immunocytochemistry analysis of HeLa cells labeling ATF5 with Rabbit anti-ATF5 antibody (ET1612-38) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-ATF5 antibody (ET1612-38) at 1/100 dilution in 1% BSA in PBST 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.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

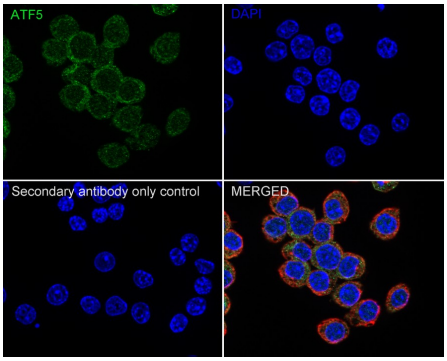


Fig3: Immunocytochemistry analysis of RAW264.7 cells labeling ATF5 with Rabbit anti-ATF5 antibody (ET1612-38) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-ATF5 antibody (ET1612-38) at 1/100 dilution in 1% BSA in PBST 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.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

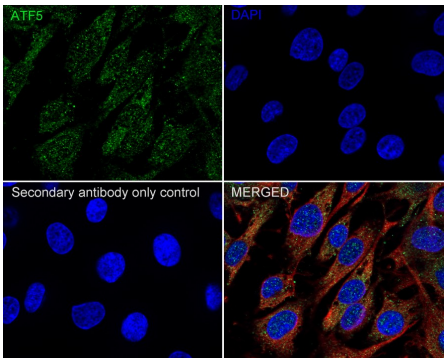


Fig4: Immunocytochemistry analysis of C6 cells labeling ATF5 with Rabbit anti-ATF5 antibody (ET1612-38) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-ATF5 antibody (ET1612-38) at 1/100 dilution in 1% BSA in PBST 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.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

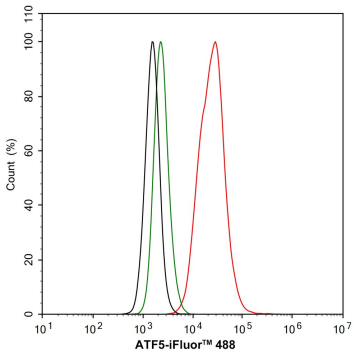


Fig5: Flow cytometric analysis of HeLa cells labeling ATF5.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1612-38, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

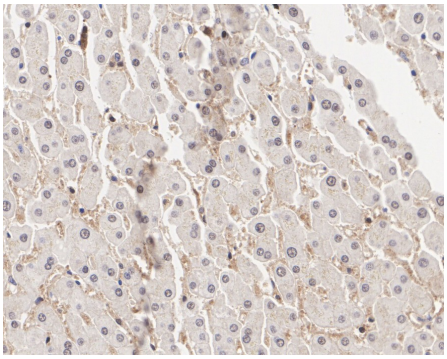


Fig6: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-ATF5 antibody (ET1612-38) at 1/200 dilution.

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 (ET1612-38) 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.

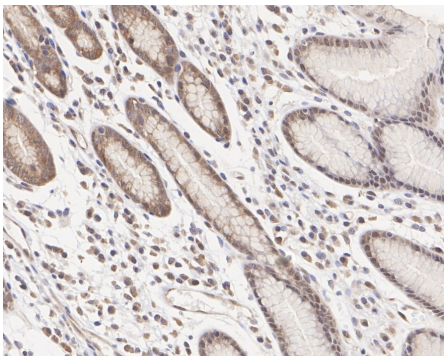


Fig7: Immunohistochemical analysis of paraffin-embedded human stomach tissue with Rabbit anti-ATF5 antibody (ET1612-38) at 1/1,000 dilution.

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 (ET1612-38) at 1/1,000 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.

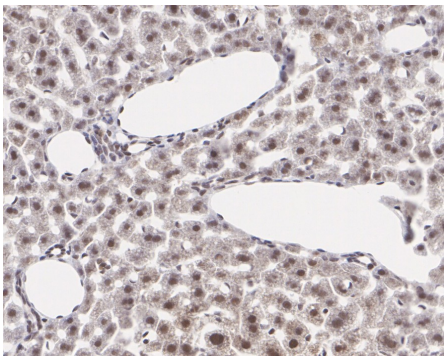


Fig8: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-ATF5 antibody (ET1612-38) at 1/1,000 dilution.

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 (ET1612-38) at 1/1,000 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.

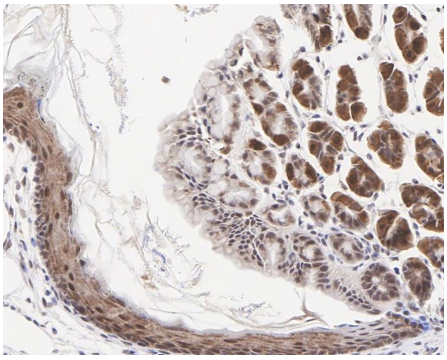


Fig9: Immunohistochemical analysis of paraffin-embedded mouse stomach tissue with Rabbit anti-ATF5 antibody (ET1612-38) at 1/1,000 dilution.

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 (ET1612-38) at 1/1,000 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.

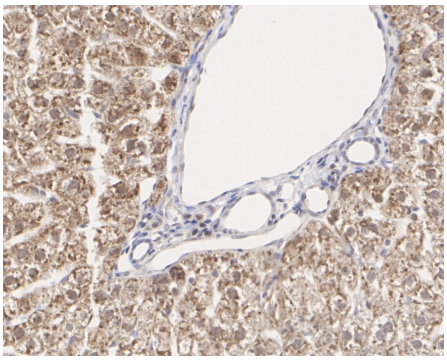


Fig10: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-ATF5 antibody (ET1612-38) at 1/1,000 dilution.

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 (ET1612-38) at 1/1,000 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.

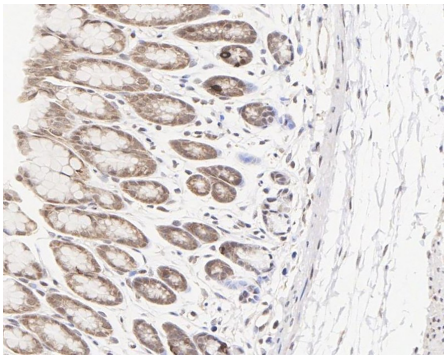


Fig11: Immunohistochemical analysis of paraffin-embedded rat stomach tissue with Rabbit anti-ATF5 antibody (ET1612-38) at 1/1,000 dilution.

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 (ET1612-38) at 1/1,000 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.

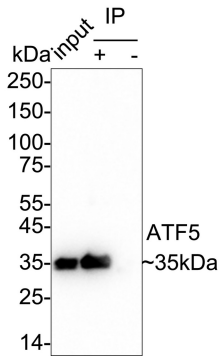


Fig12: ATF5 was immunoprecipitated from 0.2 mg Jurkat cell lysate with ET1612-38 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using ET1612-38 at 1/2,000 dilution. Anti-Rabbit IgG for IP, AlpSdAbs® VHH(HRP) (025-100-005) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Jurkat cell lysate (input)
Lane 2: ET1612-38 IP in Jurkat cell lysate
Lane 3: Rabbit IgG instead of ET1612-38 in Jurkat cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 8 seconds; ECL: K1801

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Liu X et al. Nucleophosmin (NPM1/B23) Interacts with Activating Transcription Factor 5 (ATF5) Protein and Promotes Proteasome- and Caspase-dependent ATF5 Degradation in Hepatocellular Carcinoma Cells. J Biol Chem 287:19599-609 (2012).
2. Dluzen D et al. BCL-2 is a downstream target of ATF5 that mediates the prosurvival function of ATF5 in a cell type-dependent manner. J Biol Chem 286:7705-13 (2011).