

# Anti-ATF4 Antibody [SD20-92]

ET1612-37



<b>Product Type:</b>	Recombinant Rabbit monoclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human, Mouse, Rat
<b>Applications:</b>	WB, IF-Cell, IF-Tissue, IHC-P
<b>Molecular Wt:</b>	Predicted band size: 39 kDa
<b>Clone number:</b>	SD20-92

**Description:** Eukaryotic gene transcription is regulated by sequence-specific transcription factors which bind modular cis-acting promoter and enhancer elements. The cAMP response element (CRE), one of the best studied of such elements, consists of the palindromic octanucleotide TGACGTCA. Several CRE binding proteins have been identified within the ATF/CREB family, the best characterized of which include CREB-1, CREB-2 (also designated ATF-4), ATF-1, ATF-2 and ATF-3. These proteins share highly related COOH terminal leucine zipper dimerization and basic DNA binding domains but are highly divergent in their amino terminal domains. Although each of the ATF/CREB proteins appear capable of binding CRE in its homodimeric form, certain of these also bind as heterodimers, both within the ATF/CREB family and even with members of the AP-1 transcription factor family.

**Immunogen:** Recombinant protein within Human ATF4 aa 1-220 / 351.

**Positive control:** HeLa cell lysate, HL-60 cell lysate, Mouse testis tissue lysate, Rat testis tissue lysate, Neuro-2a, human colon carcinoma tissue, human liver carcinoma tissue, human thyroid tissue, human prostate carcinoma tissue, human skin tissue, human breast carcinoma tissue, human stomach carcinoma tissue, human small intestine tissue, mouse colon tissue.

**Subcellular location:** Nucleus, Nucleus speckle, Cell membrane, Cytoplasm, Centrosome.

**Database links:** SwissProt: P18848 Human | Q06507 Mouse | Q9ES19 Rat

**Recommended Dilutions:**

<b>WB</b>	1:2,000-1:5,000
<b>IF-Cell</b>	1:100-1:200
<b>IF-Tissue</b>	1:200-1:500
<b>IHC-P</b>	1:200-1:1,000

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

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

**Purity:** Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

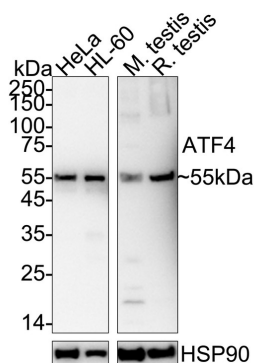
Technical:0086-571-89986345

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

## Images



**Fig1:** Western blot analysis of ATF4 on different lysates with Rabbit anti-ATF4 antibody (ET1612-37) at 1/5,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)

Lane 2: HL-60 cell lysate (20 µg/Lane)

Lane 3: Mouse testis tissue lysate (40 µg/Lane)

Lane 4: Rat testis tissue lysate (40 µg/Lane)

Predicted band size: 39 kDa

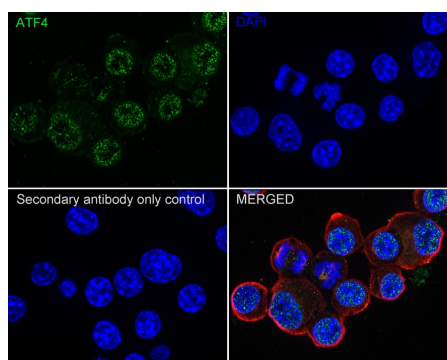
Observed band size: 55 kDa

Exposure time: 1 minute; ECL: K1801;

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 (ET1612-37) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4 °C 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 Neuro-2a cells labeling ATF4 with Rabbit anti-ATF4 antibody (ET1612-37) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-ATF4 antibody (ET1612-37) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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 (HA601187, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

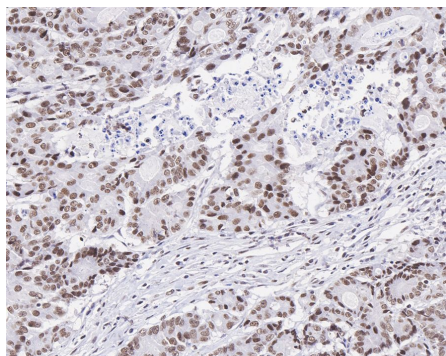
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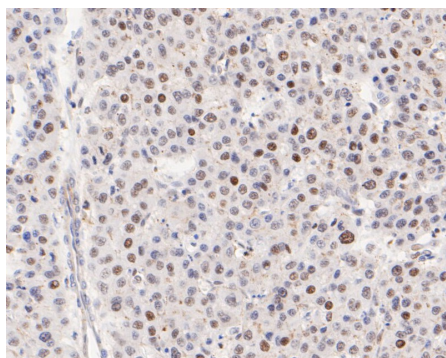
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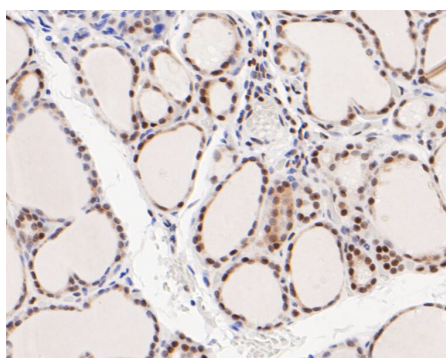
**Fig3:** Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-ATF4 antibody (ET1612-37) at 1/1,000 dilution.

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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-37) 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.



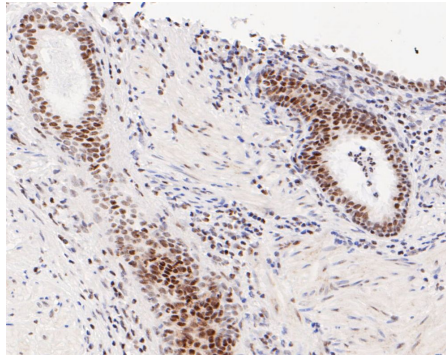
**Fig4:** Immunohistochemical analysis of paraffin-embedded human liver carcinoma tissue with Rabbit anti-ATF4 antibody (ET1612-37) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-37) 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.



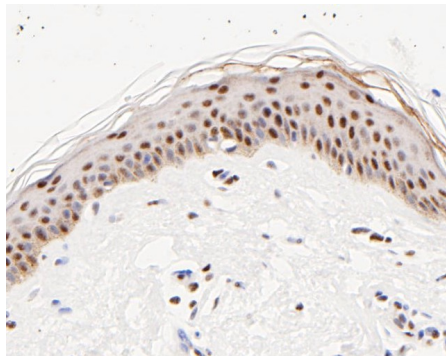
**Fig5:** Immunohistochemical analysis of paraffin-embedded human thyroid tissue with Rabbit anti-ATF4 antibody (ET1612-37) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-37) 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.



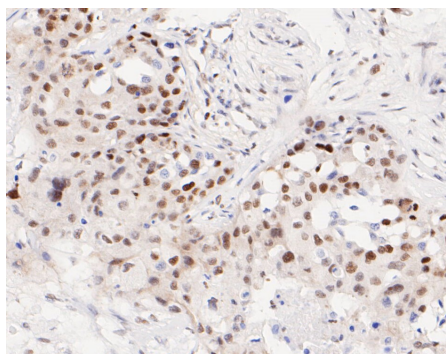
**Fig6:** Immunohistochemical analysis of paraffin-embedded human prostate carcinoma tissue with Rabbit anti-ATF4 antibody (ET1612-37) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-37) 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 skin tissue with Rabbit anti-ATF4 antibody (ET1612-37) at 1/200 dilution.

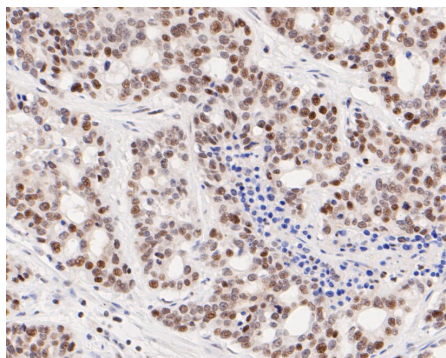
The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-37) at 1/200 dilution for 1 hour 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.



**Fig8:** Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-ATF4 antibody (ET1612-37) at 1/200 dilution.

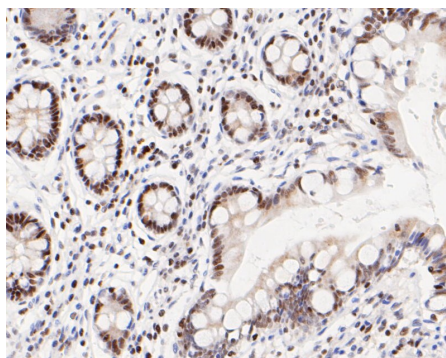
The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-37) at 1/200 dilution for 1 hour 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.





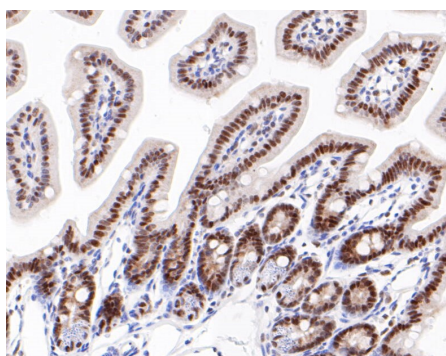
**Fig9:** Immunohistochemical analysis of paraffin-embedded human stomach carcinoma tissue with Rabbit anti-ATF4 antibody (ET1612-37) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-37) 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.



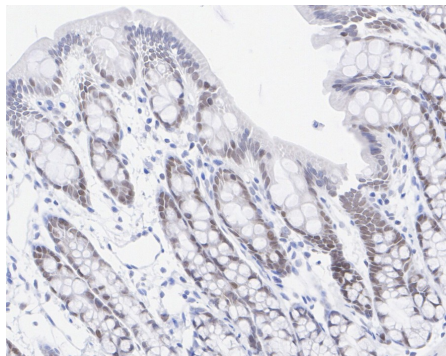
**Fig10:** Immunohistochemical analysis of paraffin-embedded human small intestine tissue with Rabbit anti-ATF4 antibody (ET1612-37) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-37) 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 mouse colon tissue with Rabbit anti-ATF4 antibody (ET1612-37) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-37) 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 rat colon tissue with Rabbit anti-ATF4 antibody (ET1612-37) 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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1612-37) 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.

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

### Background References

1. Zhu H et al. Activating transcription factor 4 promotes esophageal squamous cell carcinoma invasion and metastasis in mice and is associated with poor prognosis in human patients. PLoS One 9:e103882 (2014).
2. Lenna S et al. HLA-B35 and dsRNA induce endothelin-1 via activation of ATF4 in human microvascular endothelial cells. PLoS One 8:e56123 (2013).

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