



Product Specification Sheet

ATF4 antibody

<input type="checkbox"/> Cat# ATF411-A	Rabbit Anti-Mouse ATF4 antibody	SIZE: 100 µg
<input type="checkbox"/> Cat# ATF412-A	Rabbit Anti-Mouse ATF4 antibody #2	SIZE: 100 µg
<input type="checkbox"/> Cat# ATF411-C	Recombinant ATF4 control for Western blotting	SIZE: 100 µl

Activating transcription factor 4 (tax-responsive enhancer element B67), also known as ATF4, is a transcription factor, that accumulates predominantly in osteoblasts, where it regulates terminal osteoblast differentiation and bone formation. As a basic leucine-zipper (bZip) transcription factor, ATF4 can regulate amino acid metabolism, cellular redox state, and anti-stress responses. It also regulates age-related and diet-induced obesity and glucose homeostasis in mammals and has conserved metabolic functions in flies. Due to its location at chromosome 22q13, a region linked to schizophrenia, ATF4 is considered as a positional candidate gene for schizophrenia. Otherwise, since ATF4 is induced by tumor microenvironmental factors, and regulates processes relevant to cancer progression, it might serve as a potential therapeutic target in cancer.

Source of Antigen and Antibodies

Uniprot: Q06507

Host: Rabbit

Clonality: Polyclonal

Immunogen: Both antibodies were generated with a synthetic peptide within region 250-300 derived from Mouse ATF4

Purification: Ammonium sulfate followed by peptide affinity purification

Species Reactivity: Mouse

Cross reactivity: The peptides used as immunogens exhibit 100% homology with Rat, Human, Non-human Primates, Bovine, Equine, Dog, Cat, Sheep, Pig, Danio rerio and more species not listed. If your species of interest is not listed above, contact ADI to check for sequence homology. Reactivity has only been confirmed in Mouse and Human samples.

Alternative names: cAMP-dependent transcription factor ATF-4, C/EBP-related ATF, C/ATF

Subcellular Location: Nucleus, Centrosome, Cell membrane, Cytoskeleton, and Cytoplasm

Recommended Secondary Antibody: Goat anti-Rabbit IgG-HRP (ADI cat#20320)

Form & Storage of Antibodies

Affinity pure IgG Solution

Concentration: 0.5 mg/ml Volume: 200 µl

Supplied in PBS, pH 7.4

The antibody can be made available carrier free or conjugated to HRP, Biotin, or FITC on request

Lyophilized powder

Lyophilized from a formulation containing PBS, pH 7.4 +3% Trehalose. Reconstitute in 200 µl PBS, 0.05% tween-20, 0.1% BSA, and a preservative to 0.5 mg/ml.

Storage:

Short-term: 4°C for 1 month

Long-term: at -20°C or below in suitable aliquots after reconstitution for 1 year. Do not expose to multiple freeze/thaw cycles or store working, diluted solutions. Glycerol may be added to a final concentration of 50% and antibodies can be stored un-aliquoted at -20°C.

Recommended Usage

Western Blotting: 0.5-1.0 µg/ml

Observed band size: 49 kDa

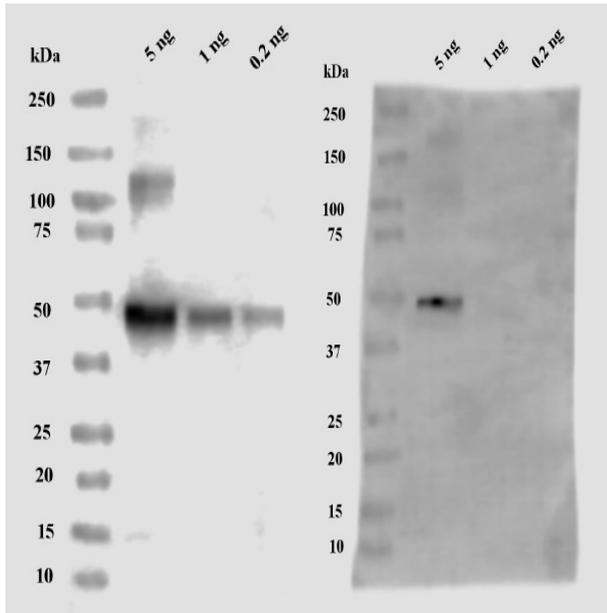
IHC-P: 1-10 µg/ml. QC tested using 10 mM sodium citrate, pH 6 antigen retrieval buffer. The antibody may work better with no retrieval or alternative retrieval solutions.

The above concentrations are a *suggestion*, user's must optimize their assay based on their own conditions. The antibody may work in other applications such as Immunocytochemistry or IP. These methods have not been tested by ADI.

***This product is for In vitro research use only.**

Related materials available from ADI

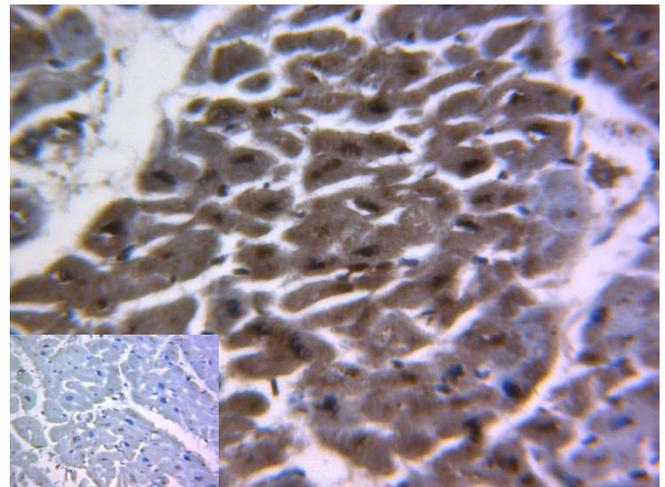
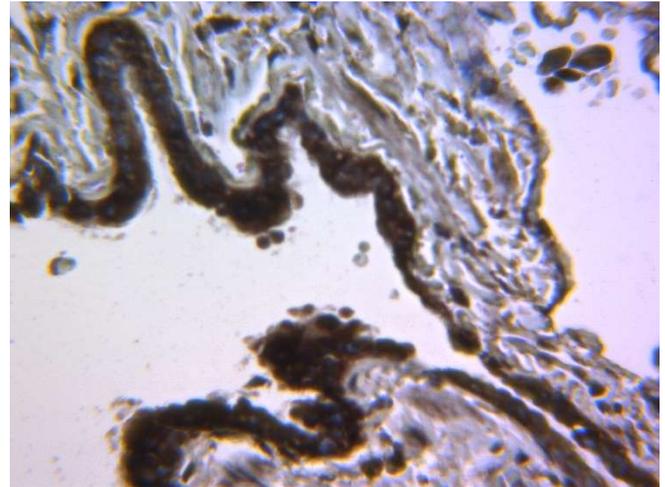
Catalog#	Description
BCL11-A	Rabbit Anti-Human BCL-2 antibody
BCL21-A	Rabbit Anti-Mouse BCL-2 antibody
BCL2-C	Recombinant BCL-2 control for Western blotting
AKT1-A	Rabbit anti-Human AKT1 antibody
AKT1-C	Recombinant AKT1 control for Western blotting
CASP3-A	Rabbit anti-Mouse Caspase 3 antibody
ATF411-A	1907311A



Western blotting: 5, 1, and 0.2 ng of recombinant ATF4 (**ATF411-C**) was heated for 5 minutes at 95°C then electrophoretically separated on a 4-20% SDS-PAGE gel. The gel was run at 100V for ~1 hour and 30 minutes then transferred to a 0.2 µm nitrocellulose membrane using the 'Mixed MW' settings on a Transblot Turbo (Biorad). The blot was blocked for 1 hour at room temperature with 1% Casein. **ATF411-A (right)** and **ATF412-A (left)** were diluted with TBST+0.1% BSA to 1 µg/ml and incubated overnight at 4°C. The blot was washed with TBS-T 3 times for 5 minutes each. Goat anti-rabbit IgG HRP (**ADI cat#20320**) was diluted in TBST+0.1% BSA at a 1:1,000 dilution (500 ng/ml) then incubated for 1 hour at room temperature. The blot was washed 3 times with TBS-T for 5 minutes each. The blot was then incubated with regular strength ECL substrate for 1 minute and imaged on a CCD imaging system (C-DiGit, LI-COR).

Note: ATF412-A exhibits much higher sensitivity in Western blotting than ATF411-A

ATF411-C: Contains a recombinant *E.coli* expressed full length Human ATF4 protein at a concentration of 1 ng/µl in Laemmli buffer (62.5 mM Tris-HCL, pH 6.8, 2% SDS, 10% glycerol, 5% BME, and 0.002% bromphenol blue). Heat for 5 minutes at 95°C then load 1-5 µl. Store at -20°C in suitable size aliquots, do not expose to multiple freeze/thaw cycles.
Note: Due to the addition of tags, the control will appear larger than native protein.



Immunohistochemistry: FFPE Human Heart and Lung slides were heated for 20 minutes at 60°C then deparaffinized. Antigen retrieval was performed for 10 minutes at 95°C in a microwave using 10 mM pH 6, sodium citrate buffer. The slide was then cooled for 20 minutes at room temperature before being blocked for 30 minutes with 2.5% normal goat serum. **ATF412-A** was diluted to 5 µg/ml in TBST+0.1% BSA and incubated overnight at 4°C (Inset represents recombinant ATF4 absorbed antibody incubation). The slides were then washed twice and incubated with 3% hydrogen peroxide for 10 minutes to quench endogenous peroxidase. The slide was washed then incubated with Goat anti-Rabbit IgG HRP polymer detection reagent for 30 minutes at room temperature. The slide was washed twice, incubated with DAB for 3 minutes, washed with distilled water, then counterstained for 1 minute with Gil's II Hematoxylin before being cover-slipped.