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#24329

# Phospho-ATF-2 (Thr71)/ATF-7 (Thr53) Antibody



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**Entrez-Gene ID** #1386, 11016  
**UniProt ID** #P15336, P17544

rev. 05/10/19

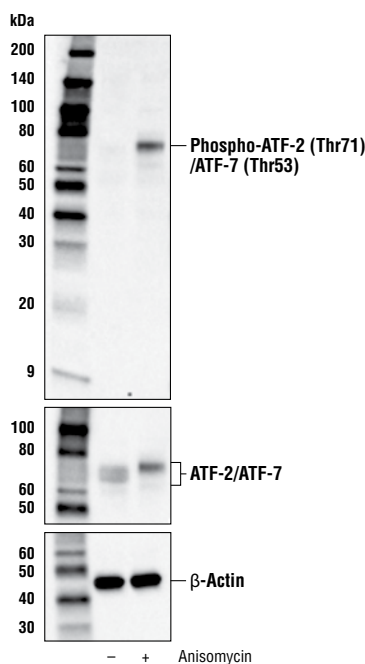
**For Research Use Only. Not For Use In Diagnostic Procedures.**

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, IHC-P, IF-IC, F Endogenous	H, M, R, Mk	65,75 kDa	Rabbit**

**Background:** The transcription factor ATF-2 (also called CRE-BP1) binds to both AP-1 and CRE DNA response elements and is a member of the ATF/CREB family of leucine zipper proteins (1). ATF-2 interacts with a variety of viral oncoproteins and cellular tumor suppressors and is a target of the SAPK/JNK and p38 MAP kinase signaling pathways (2-4). Various forms of cellular stress, including genotoxic agents, inflammatory cytokines, and UV irradiation, stimulate the transcriptional activity of ATF-2. Cellular stress activates ATF-2 by phosphorylation of Thr69 and Thr71 (2-4). Both SAPK and p38 MAPK have been shown to phosphorylate ATF-2 at these sites *in vitro* and in cells transfected with ATF-2. Mutations of these sites result in the loss of stress-induced transcription by ATF-2 (2-4). In addition, mutations at these sites reduce the ability of E1A and Rb to stimulate gene expression via ATF-2 (2). ATF-7 is another member of the ATF/CREB family of leucine zipper proteins (5). Similarly, Thr51 and Thr53 (corresponding to Thr69 and Thr71 of ATF-2, respectively) can be phosphorylated under different conditions (6, 7).

**Specificity/Sensitivity:** Phospho-ATF-2 (Thr71)/ATF-7 (Thr53) Antibody detects endogenous levels of ATF-2 and ATF-7 only when phosphorylated at threonine 71 and threonine 53, respectively. This antibody does not cross-react with phosphorylated c-Jun, CREB, or other transcription factors. It recognizes Thr69/Thr71 dually phosphorylated ATF-2, Thr51/Thr53 dually phosphorylated ATF-7, Thr71 singly phosphorylated ATF-2, and Thr53 singly phosphorylated ATF-7 equally well.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr71 of human ATF-2. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from 3T3 cells, untreated (-) or treated with Anisomycin (25 µg/ml, 30 min; +), using Phospho-ATF-2 (Thr71)/ATF-7 (Thr53) Antibody (upper), ATF-2/ATF-7 (A9G1M) Rabbit mAb #82870 (middle), or β-Actin (D6A8) Rabbit mAb #8457 (lower).

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

\*Species cross-reactivity is determined by western blot.

\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.

#### Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:250
Immunohistochemistry (Paraffin)	1:50

Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.

Unmasking buffer: SignalStain® Citrate Unmasking Solution (10X) #14746

Antibody diluent: SignalStain® Antibody Diluent #8112  
Detection reagent: SignalStain® Boost (HRP, Rabbit) #8114

Immunofluorescence (IF-IC) 1:50

Fixative: 4% Formaldehyde  
Permeabilization: 0.3% Triton X-100

Flow Cytometry 1:50

**For product specific protocols and a complete listing of recommended companion products please see the product web page at [www.cellsignal.com](http://www.cellsignal.com).**

#### Background References:

- (1) Abdel-Hafiz, H.A. et al. (1992) *Mol Endocrinol* 6, 2079-89.
- (2) Gupta, S. et al. (1995) *Science* 267, 389-93.
- (3) van Dam, H. et al. (1995) *EMBO J* 14, 1798-811.
- (4) Livingstone, C. et al. (1995) *EMBO J* 14, 1785-97.
- (5) Peters, C.S. et al. (2001) *J Biol Chem* 276, 13718-26.
- (6) Camuzeaux, B. et al. (2008) *J Mol Biol* 384, 980-91.
- (7) Maekawa, T. et al. (2010) *EMBO J* 29, 196-208.

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**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.**

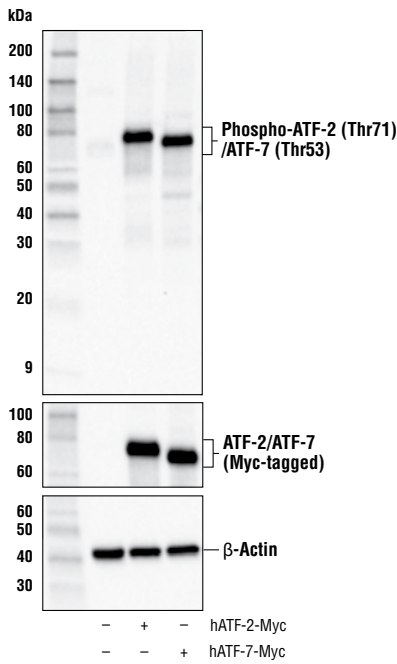
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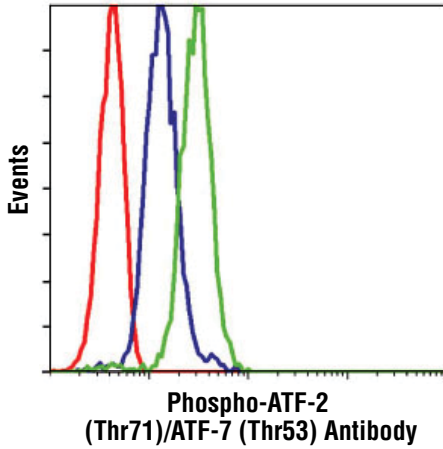
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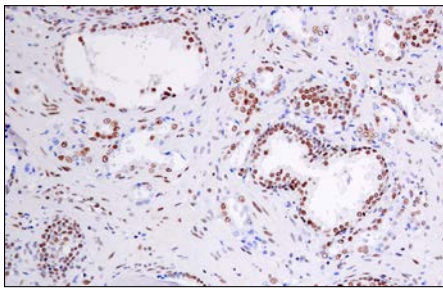
Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



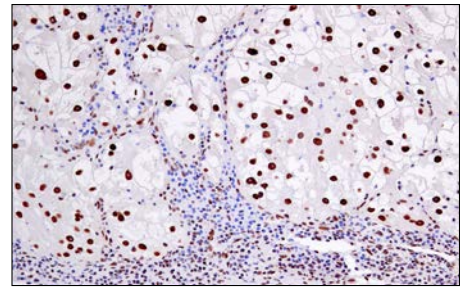
Western blot analysis of extracts from HeLa cells, mock transfected (-) or transfected with a construct expressing Myc-tagged full-length human ATF-2 protein (hATF-2-Myc; +) or a construct expressing Myc-tagged full-length human ATF-7 protein (hATF-7-Myc; +), using Phospho-ATF-2 (Thr71)/ATF-7 (Thr53) Antibody (upper), Myc-Tag (71D10) Rabbit mAb #2278 (middle), and  $\beta$ -Actin (D6A8) Rabbit mAb #8457 (lower).



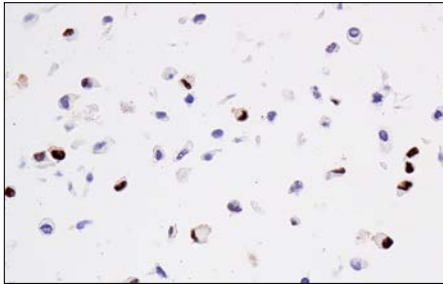
Flow cytometric analysis of Jurkat cells, untreated (blue) or Anisomycin-treated (green), using Phospho-ATF-2 (Thr71)/ATF-7 (Thr53) Antibody compared to a nonspecific negative control antibody (red).



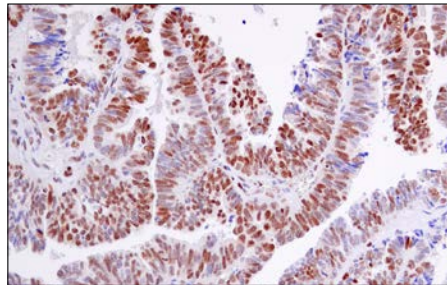
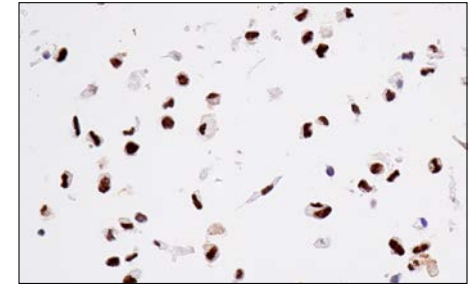
Immunohistochemical analysis of paraffin-embedded human prostate carcinoma using Phospho-ATF-2 (Thr71)/ATF-7 (Thr53) Antibody.



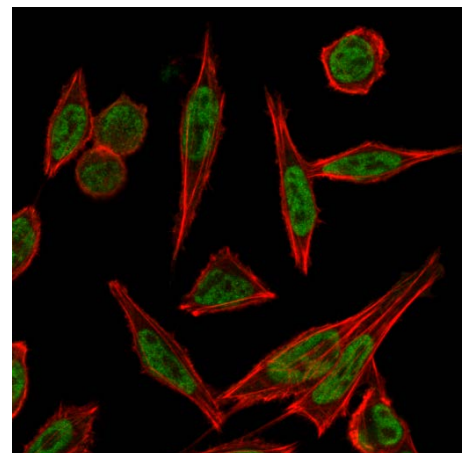
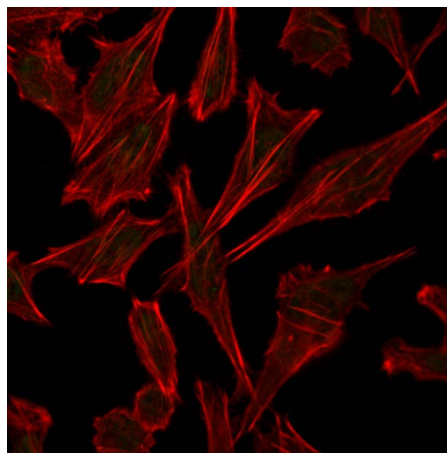
Immunohistochemical analysis of paraffin-embedded human renal cell carcinoma using Phospho-ATF-2 (Thr71)/ATF-7 (Thr53) Antibody.



Immunohistochemical analysis of paraffin-embedded 3T3 cell pellet, untreated (left) or treated with Anisomycin #2222 (right), using Phospho-ATF-2 (Thr71)/ATF-7 (Thr53) Antibody.



Immunohistochemical analysis of paraffin-embedded human serous papillary carcinoma of the ovary using Phospho-ATF-2 (Thr71)/ATF-7 (Thr53) Antibody.



Confocal immunofluorescent analysis of HeLa cells, either untreated (left) or treated with Anisomycin #2222 (25  $\mu$ g/mL, 30 min; right), using Phospho-ATF-2 (Thr71)/ATF-7 (Thr53) Antibody (green). Actin filaments have been labeled with Alexa Fluor<sup>®</sup> 555 Phalloidin #8953 (red).

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