

# Phospho-ATF-2 (Thr71) Antibody

- Small 100 µl (10 Western mini-blot)
- Large 300 µl (30 Western mini-blot)

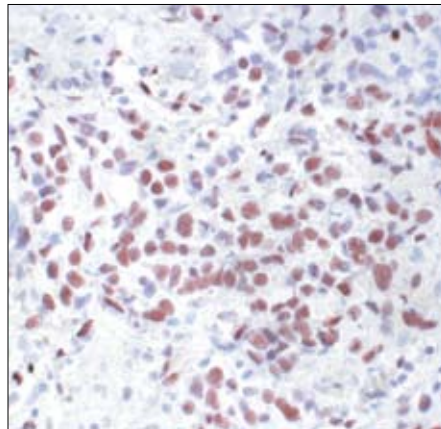
**Orders** ■ 877-616-CELL (2355)  
orders@cellsignal.com  
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This product is for *in vitro* research use only and is not intended for use in humans or animals. This product is not intended for use as a therapeutic or in diagnostic procedures.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, IHC-P, IHC-F, IF-IC, F Endogenous	H, M, R, Mk	70 kDa	Rabbit**

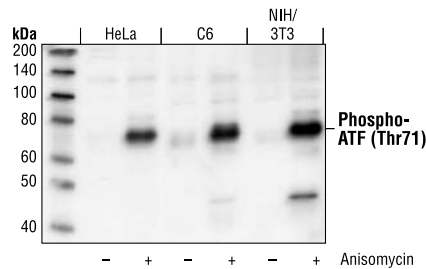
**Background:** The transcription factor ATF-2 (also called CRE-BP1) binds to both AP-1 and CRE DNA response elements and is a member of the ATF/CREB family of leucine zipper proteins (1). ATF-2 interacts with a variety of viral oncoproteins and cellular tumor suppressors and is a target of the SAPK/JNK 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).



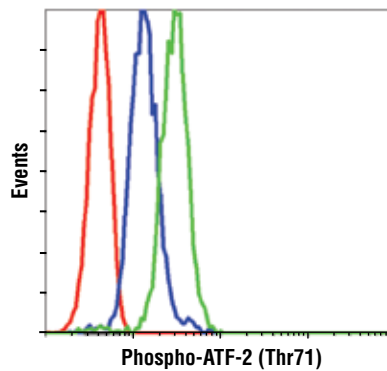
Immunohistochemical analysis of paraffin-embedded human breast carcinoma, showing nuclear localization, using Phospho-ATF-2 (Thr71) Antibody.

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

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Thr71 of human ATF2. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from HeLa, C6 and NIH/3T3 cells, untreated or anisomycin-treated, using Phospho-ATF-2 (Thr71) Antibody.



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

Entrez-Gene ID #1386  
Swiss-Prot Acc. #P15336

**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.

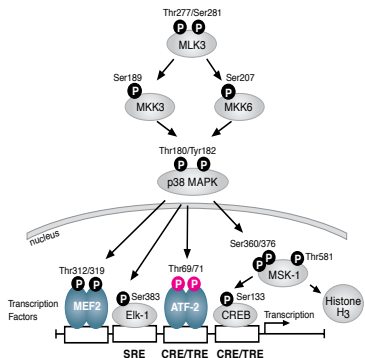
\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.

**Recommended Antibody Dilutions:**

Western blotting	1:1000
Immunoprecipitation	1:250
Immunohistochemistry (Paraffin)	1:50
IHC protocol: Unmasking buffer/Antibody diluent Citrate/TBST-5%NGS	
Immunohistochemistry (Frozen)	1:50
Fixative	10% Neutral buffered formalin
Immunofluorescence (IF-IC)	1:50
Flow Cytometry	1:50

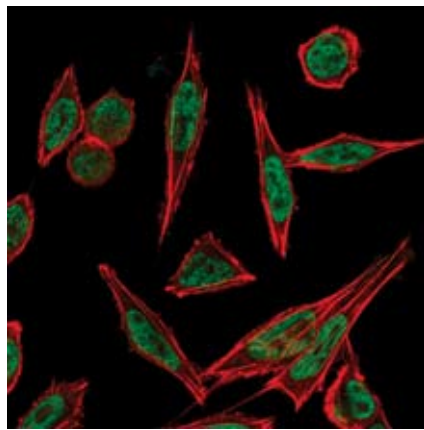
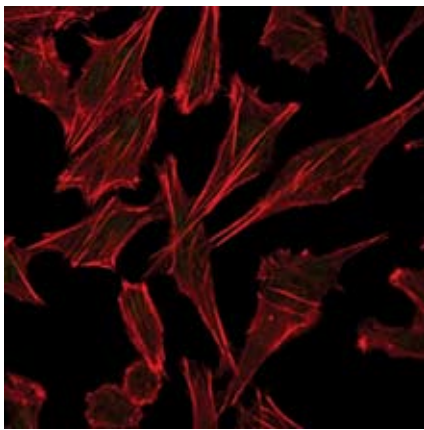
For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

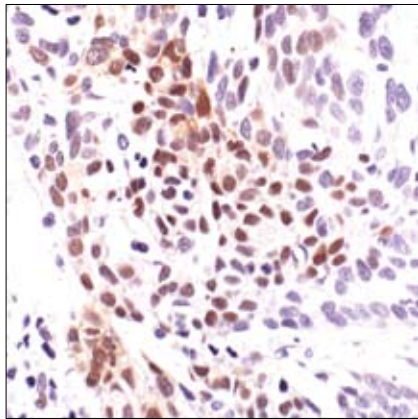


**IMPORTANT:** For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

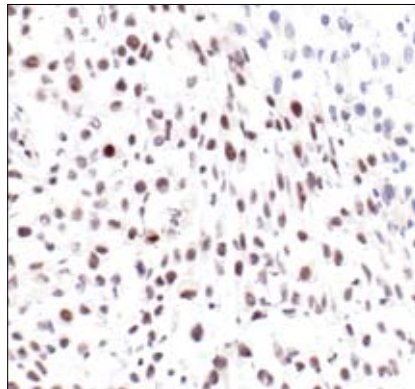
**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish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



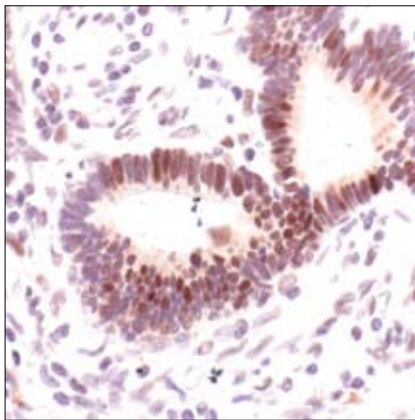
Confocal immunofluorescent analysis of HeLa cells, either untreated (left) or anisomycin-treated (right), using Phospho-ATF-2 (Thr71) Antibody (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red).



Immunohistochemical analysis of paraffin-embedded human lung carcinoma using Phospho-ATF-2 (Thr71) Antibody.



Immunohistochemical analysis of frozen H1650 xenograft using Phospho-ATF-2 (Thr71) Antibody.



Immunohistochemical analysis of paraffin-embedded human colon carcinoma using Phospho-ATF-2 (Thr71) Antibody.

#### Selected Application References:

deRuiter, N.D. et al. (2000) Ras-dependent regulation of c-Jun phosphorylation is mediated by the Ral guanine nucleotide exchange factor-Ral pathway. *Mol. Cell. Biol.* 20, 8480–8488. Application: W.

Hocevar, B.A. et al. (1999) TGF- $\beta$  induces fibronectin synthesis through a c-Jun amino-terminal kinase-dependent, Smad4-independent pathway. *EMBO J.* 18, 1345–1356. Application: W.

Hou, N. et al. (2008) Reactive oxygen species-mediated pancreatic beta-cell death is regulated by interactions between stress-activated protein kinases, p38 and c-Jun N-terminal kinase, and mitogen-activated protein kinase phosphatases. *Endocrinology* 149, 1654–65. Application: W.

Marinissen, M.J. et al. (2001) Regulation of gene expression by the small GTPase Rho through the ERK6 (p38  $\gamma$ ) MAP kinase pathway. *Genes Dev.* 15, 535–553. Application: W.

Ouwens, D.M. et al. (2002) Growth factors can activate ATF2 via a two-step mechanism: phosphorylation of Thr71 through the Ras-MEK-ERK pathway and of Thr69 through RalGDS-Src-p38. *EMBO J.* 21, 3782–3793. Application: W.

Suh, Y. et al. (2000) Differential activation of c-Jun NH2-terminal kinase and p38 mitogen-activated protein kinases by methyl methanesulfonate in the liver and brain of rats: implication for organ-specific carcinogenesis. *Cancer Res.* 60, 5067–5073. Application: W.

Zhang, S. and Kaplan, M.H. (2000) The p38 mitogen-activated protein kinase is required for IL-12-induced IFN- $\gamma$  expression. *J. Immunol.* 165, 1374–1380. Application: W.

Ogino, T. et al. (2009) Activation of c-Jun N-terminal kinase is essential for oxidative stress-induced Jurkat cell apoptosis by monochloramine. *Leuk Res* 33, 151–8. Application: W.

#### Background References:

- (1) Abdel-Hafiz, H.A. et al. (1992) *Mol. Endocrinol.* 6, 2079–2089.
- (2) Gupta, S. et al. (1995) *Science* 267, 389–393.
- (3) van Dam, H. et al. (1995) *EMBO J.* 14, 1798–1811.
- (4) Livingstone, C. et al. (1995) *EMBO J.* 14, 1785–1797.