#9220 Store at -20°C

PhosphoPlus® ATF-2 (Thr71) Antibody Kit

☑ 10 mini-blots

Cell Signaling TECHNOLOGY®

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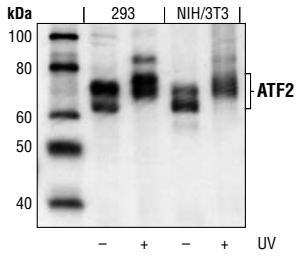
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Products Included	Product #	Quantity	Applications	Species Cross-Reactivity	Mol. Wt.	Source
Phospho-ATF-2 (Thr71) Antibody	9221	100 µl	W, IP, IHC-P, IHC-F, IF-IC, F	H, M, R, Mk	70 kDa	Rabbit
ATF-2 (20F1) Rabbit mAb	9226	100 µl	W, IP, IHC-P	H, M, R, Mk	65 to 75 kDa	Rabbit
Anti-rabbit IgG, HRP-linked Antibody	7074	50 µl				Goat
Anti-biotin, HRP-linked Antibody	7075	100 µl				Goat
Biotinylated Protein Ladder Detection Pack	7727	100 µl				
20X LumiGLO® Reagent and 20X Peroxide	7003	5 ml				
ATF-2 Control Cell Extracts	9223	50 µl				

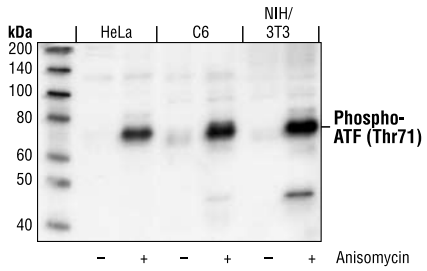
Background: The transcription factor ATF-2 (also called CRE-BP1) binds to both AP-1 and CRE DNA response elements and is a member of the ATF/CREB family of leucine zipper proteins (1). ATF-2 interacts with a variety of viral oncoproteins and cellular tumor suppressors and is a target of the SAPK/JNK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

Specificity/Sensitivity: Phospho-ATF-2 (Thr71) Antibody detects endogenous levels of ATF-2 only when phosphorylated at Thr71. It recognizes this site regardless of the phosphorylation state of Thr69. ATF-2 (20F1) Rabbit mAb detects endogenous levels of total ATF-2 protein. Neither antibody cross-reacts with c-Jun, CREB or other transcription factors.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Thr71 of human ATF-2 (Phospho-ATF-2 (Thr71) Antibody), or with a synthetic peptide (KLH-coupled) derived from the amino terminal sequence of human ATF-2 (ATF-2 (20F1) Rabbit mAb). Antibodies are purified by protein A and peptide affinity chromatography.



*Western blot analysis of extracts from 293 and NIH/3T3 cells, untreated or UV-treated, using **ATF-2 (20F1) Rabbit mAb #9226.***



*Western blot analysis of extracts from HeLa, C6 and NIH/3T3 cells, untreated or anisomycin-treated, using **Phospho-ATF-2 (Thr71) Antibody #9221.***

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.*

Recommended Antibody Dilutions:

Western blotting	1:1000

See www.cellsignal.com for individual component dilutions and additional application protocols.

Companion Products:

PhosphoPlus® c-Jun (Ser63) II and c-Jun (Ser73) Antibody Kit #9260

Phospho-c-Jun (Ser73) Antibody #9164

PhosphoPlus® p38 MAP Kinase (Thr180/Tyr182) Antibody Kit #9210

Phospho-p38 MAP Kinase (Thr180/Tyr182) Antibody #9211

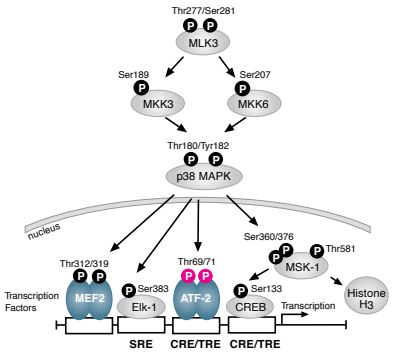
PhosphoPlus® MKK3/MKK6 (Ser189/207) Antibody Kit #9230

Phospho-MKK3/MKK6 (Ser189/207) Antibody #9231

Phospho-SEK1/MKK4 (Thr261) Antibody #9151

Prestained Protein Marker, Broad Range (Premixed Format) #7720

ATF-2 (20F1) Rabbit mAb #9226

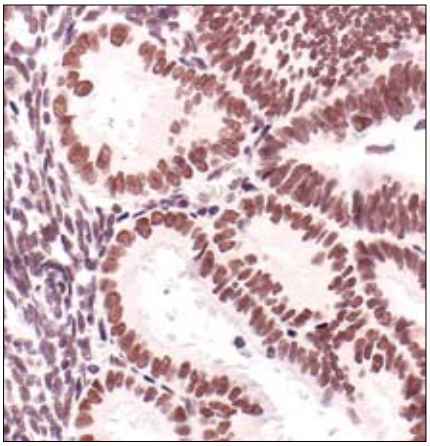


ATF-2 Signaling Pathway

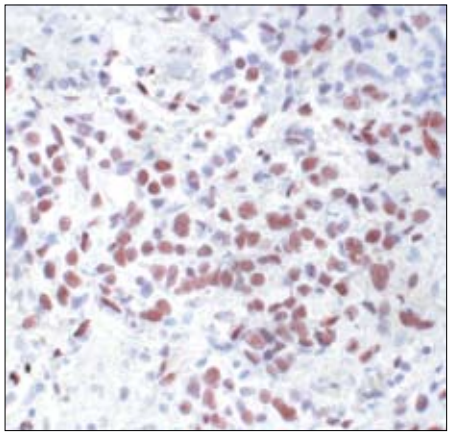
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **IC**—Immunocytochemistry **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **D**—DELFIA®

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **X**—Xenopus **Z**—zebra fish **B**—bovine **All**—all species expected

Species enclosed in parentheses are predicted to react based on 100% sequence homology.



Immunohistochemical analysis of paraffin-embedded human endometrial carcinoma, using ***ATF-2 (20F1) Rabbit mAb #9226.***



Immunohistochemical staining of paraffin-embedded human breast carcinoma, using ***Phospho-ATF-2 (Thr71) Antibody #9221*** *showing nuclear localization of phosphorylated ATF-2.*

Selected Application References:

de Ruiter, 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. Applications: W.

Kacharmina, J.E. et al. (2000) Stimulation of glutamate receptor protein synthesis and membrane insertion within isolated neuronal dendrites. *Proc. Natl. Acad. Sci. USA* 97, 11545–11550. Applications: IC-ABC.

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. Applications: 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. Applications: 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.

Western Immunoblotting Protocol (Primary Ab Incubation In BSA)

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.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope®-HRP Western Blot Detection System** #7071**:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.