PhosphoPlus® ATF-2 (Thr71) Antibody Kit

- 10 mini-blots

Store at -20°C

**Products Included**

<table>
<thead>
<tr>
<th>Product #</th>
<th>Quantity</th>
<th>Applications</th>
<th>Species Cross-Reactivity</th>
<th>Mol. Wt.</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>9221</td>
<td>100 µl</td>
<td>W, IP, IHC-P, IHC-F, IF-IC, F</td>
<td>H, M, R, Mk</td>
<td>70 kDa</td>
<td>Rabbit</td>
</tr>
<tr>
<td>9226</td>
<td>100 µl</td>
<td>W, IP, IHC-P</td>
<td>H, M, R, Mk</td>
<td>65 to 75 kDa</td>
<td>Rabbit</td>
</tr>
<tr>
<td>7074</td>
<td>50 µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7075</td>
<td>100 µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7727</td>
<td>100 µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7003</td>
<td>5 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9223</td>
<td>50 µl</td>
<td></td>
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</tbody>
</table>

**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.

**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
- PhosphoPlus® Phospho-p38 MAP Kinase (Thr180/Tyr182) Antibody Kit #9260
- Phospho-MKK3/MKK6 (Ser189/207) Antibody Kit #9230
- Phospho-SEK1/MKK4 (Thr261) Antibody #9151
- Prestained Protein Marker, Broad Range (Premixed Format) #7720

**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.

**Applications Key:**
- Western blots
- Immunoprecipitation
- Immunohistochemistry
- Immunocytochemistry
- Immunofluorescence
- Flow cytometry
- ELISA
- DELFIA®

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

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

**Companion Products:**
- ATF-2 (20F1) Rabbit mAb #9226
- ATF-2 Control Cell Extracts

**ATF-2 Signaling Pathway**

- ATF-2 (20F1) Rabbit mAb #9226

**Support:**
- 877-616-CELL (2355)
- orders@cellsignal.com
- 877-678-TECH (8324)
- info@cellsignal.com
- www.cellsignal.com

**Orders:**
- 877-616-CELL (2355)
- orders@cellsignal.com

**Web:**
- www.cellsignal.com
Selected Application References:


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.


Background References:
# 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 cm²) 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.