Store at -20°C

#9820

p38 MAP Kinase Assay Kit (Nonradioactive)

☑ 40 assays

Cell Signaling TECHNOLOGY®

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

Description: Nonradioactive p38 MAP Kinase Assay Kit provides all the reagents necessary to measure p38 MAP kinase activity in cells. First, Immobilized Phospho-p38 MAPK (Thr180/Tyr182) mAb is used to immunoprecipitate p38 MAP kinase, then an *in vitro* kinase assay is performed using ATF-2 as a substrate. ATF-2 phosphorylation is detected by western blotting using Phospho-ATF-2 (Thr71) Antibody.

Species Cross Reactivity: H, M, R

Molecular Weight: 34kDa

Kit Components:

Immobilized Phospho-p38 MAP Kinase (Thr180/Tyr182) Antibody
Monoclonal antibody is produced by immunizing mice with a synthetic phosphopeptide corresponding to residues around Thr180/Tyr182 of human p38 MAP Kinase. The phospho-p38 MAP Kinase (Thr180/Tyr182) mAb was immobilized by crosslinking to agarose hydrazide beads via carbohydrate linkages and is supplied in 50% Glycerol. Store at -20oC. Do not aliquot the immobilized antibody.

Phospho-ATF-2 (Thr71) Antibody (rabbit polyclonal IgG, peptide affinity purified)
Phospho-ATF-2 (Thr71) Antibody detects ATF-2 only when phosphorylated at Thr71. Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-Thr71 peptide corresponding to residues around Thr71 of human ATF-2. Polyclonal antibodies are purified sequentially by protein A and peptide affinity chromatography and supplied in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% Glycerol. Store at –20°C.

ATF-2 Fusion protein: A recombinant protein fusion containing ATF-2 residues 19-96. Produced from E. Coli expressing pGEXKG (1).

10X Kinase Buffer: 1X concentration: 25 mM Tris (pH 7.5), 5 mM β-glycerophosphate, 2 mM DTT, 0.1 mM Na_3VO_4, 10 mM $MgCl_2$.

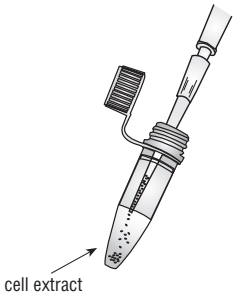
10X Cell Lysis Buffer: 1X concentration: 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 µg/ml Leupeptin.

10 mM ATP (50 µL): Adenosine-5' triphosphate (ATP) supplied as a 10 µM solution in doubly distilled water as a disodium salt.

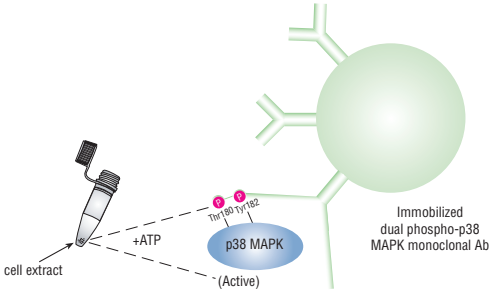
Products Included	Product #	Kit Quantity
Immobilized Phospho-p38 MAPK (Thr180/Tyr182) Mouse mAb	9219	400 µl
Phospho-ATF-2 (Thr71) Antibody	9221	100 µl
ATF-2 Fusion Protein	9224	40 µg
Kinase Buffer (10X)	9802	15 ml
Cell Lysis Buffer (10X)	9803	15 ml
ATP (10 mM)	9804	50 µl
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl
Anti-biotin, HRP-linked Antibody	7075	100 µl
Biotinylated Protein Ladder Detection Pack	7727	100 µl
20X LumiGLO® Reagent and 20X Peroxide	7003	5 ml each

p38 Kinase Assay Kit Overview

Step 1: Selective IP of p38 using Immobilized Phospho p38 (Thr180/Tyr182) mAb.

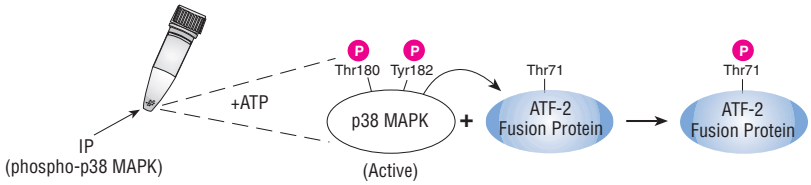


a) Add immobilized Phospho-p38 (Thr180/Tyr182) mAb

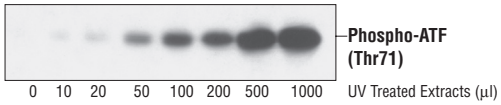


b) IP p38 from cell extracts using Immobilized Phospho p38 (Thr180/Tyr182) mAb

Step 2: Incubate IP pellets in Kinase Buffer containing ATF-2 fusion protein and cold ATP.



Step 3: Detect ATF-2 phosphorylation using phospho-antibodies by Western blotting and chemiluminescent detection.



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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 **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Background: p38 MAP kinase (MAPK), also called RK (1) or CSBP (2), is the mammalian orthologue of the yeast HOG kinase which participates in a signaling cascade controlling cellular responses to cytokines and stress (1-4). Four isoforms of p38 MAP kinase, p38α, β, γ (also known as ERK6 or SAPK3) and δ (also known as SAPK4) have been identified. Similar to the SAPK/JNK pathway, p38 MAP kinase is activated by a variety of cellular stresses including osmotic shock, inflammatory cytokines, lipopolysaccharides (LPS), UV light and growth factors (1-5). MKK3, MKK6 and SEK activate p38 MAP kinase by phosphorylation at Thr180 and Tyr182. Activated p38 MAP kinase has been shown to phosphorylate and activate MAPKAP kinase 2 (3) and to phosphorylate the transcription factors ATF-2 (5), Max (6) and MEF2 (5-8).

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Rouse, J. et al. (1994) *Cell* 78, 1027–1037.

(2) Han, J. et al. (1994) *Science* 265, 808–811.

(3) Lee, J.C. et al. (1994) *Nature* 372, 739–746.

(4) Freshney, N.W. et al. (1994) *Cell* 78, 1039–1049.

(5) Raingeaud, J. et al. (1995) *J. Biol. Chem.* 270, 7420–7426.

(6) Zervos, A.S. et al. (1995) *Proc. Natl. Acad. Sci. USA* 92, 10531–10534.

(7) Zhao, M. et al. (1999) *Mol. Cell. Biol.* 19, 21–30.

(8) Yang, S.H. et al. (1999) *Mol. Cell. Biol.* 19, 4028–4038.

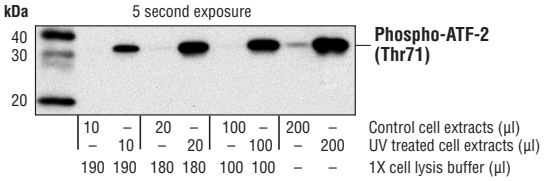
Recommended Antibody Dilutions:

Immobilized Phospho-p38 MAPK (Thr180/Tyr182) mAb:

Western blotting	N/A
Immunoprecipitation	10 µl of resuspended slurry/IP

Phospho-ATF-2 (Thr71) Antibody:

Western blotting	1:1000
Immunoprecipitation	N/A



Analysis of p38 MAP Kinase activity of UV-treated NIH-3T3 cells by western blot using Phospho-ATF-2 (Thr71) Antibody. Cell extracts were incubated overnight with Immobilized p38 MAPK (Thr180/Tyr182) mAb. Kinase reaction was performed in the presence of 100 µM of cold ATP and 1 µg of ATF-2 fusion protein. Phosphorylation of ATF-2 at Thr71 was measured by western blot using Phospho-ATF-2 (Thr71) Antibody.

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Nonradioactive IP-Kinase Assay Protocol

A Solutions and Reagents

1. **Note:** Prepare solutions with Milli-Q or equivalently purified water.
2. **1X Cell Lysis Buffer:** May be stored at 4°C for short-term use (1–2 weeks). Note: Supplied 10X Cell Lysis Buffer should be vortexed before being used to make 1X solution.
3. **1X Kinase Buffer:** Store at –20°C. May be stored at 4°C for short-term use (1–2 weeks).
4. **ATF-2 Fusion Protein:** (1 µg/assay)
5. **10 mM ATP** #9804. 2-(Methylthio) adenosine 5'-triphosphate tetra-sodium salt.
6.* **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5).
7.* **3X SDS Sample Buffer:** 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v sodium dodecyl sulfate (SDS), 30% glycerol, 150 mM dithiothreitol (DTT), 0.03% w/v bromophenol blue. For 100 mL, use 2.27 g Tris-HCl, 6g SDS, 30 mL glycerol and 30 mg w/v bromophenol blue or bromophenol blue dye. Store at –20°C. Add DTT fresh just before use.
8.* **10X Tris-Buffered Saline with Tween®20 (TBS/T):** 0.2 M Tris base, 1.36 M NaCl, 1.0% Tween®20. To prepare 1 liter, dissolve 24.2 g Tris, 80 g NaCl in dH_2O and adjust pH to 7.6 with HCl. Store at room temperature.
9.* **Blocking Buffer:** 1X TBS/T with 5% w/v nonfat dry milk. For 150 mL, dissolve 7.5 g nonfat dry milk in 15 mL 10X TBS/T and 135 mL dH_2O. Mix well. Prepare freshly for each experiment.
10.* **Wash Buffer:** 1X TBS, 0.1% Tween®20 (TBS/T). Store at room temperature.
11.* **Primary Antibody Dilution Buffer:** 1X TBS/T with 5% BSA.
12. **Phototope-HRP Western Blot Detection System #7071:** Includes biotinylated protein marker, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), Anti-biotin, HRP-linked Antibody (#7075), 20X LumiGLO® chemiluminescent reagent and 20X peroxide (#7003).
13. **LumiGLO® Substrate #7003:** 0.5 mL 20X LumiGLO®, 0.5 mL 20X peroxide and 9.0 mL Milli-Q water.

B Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under **nondenaturing conditions**, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 mL ice-cold 1X Cell Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm^2) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice four times for 5 seconds each (dependent on sonicator used).
6. Microcentrifuge for 10 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C.

C IP with Immobilized Antibodies

1. For immunoprecipitations with immobilized Phospho-p38 MAPK (Thr180/Tyr182) mAb primary antibody: Dilute by adding 10 µl of immobilized antibody bead slurry in 200 µL cell lysate; incubate with gentle rocking overnight at 4°C.
2. **Instructions for Use:** Prior to use, put the tube on ice for 5 minutes to lower viscocity of buffer: Then the beads should be resuspended to a 50% slurry by inversion or gentle vortexing.

D Kinase Assay

1. Microcentrifuge cell lysate/immobilized antibody at 14,000 x G for 30 seconds at 4°C. Wash pellet two times with 500 µL of 1X Cell Lysis Buffer. Keep on ice during washes.
2. Wash pellet twice with 500 µL of 1X Kinase Buffer. Keep on ice.
3. Suspend pellet in 50 µL of 1X Kinase Buffer supplemented with 200 µM ATP and appropriate quantity of kinase substrate (1 µl).
4. Incubate for 30 minutes at 30°C.
5. Terminate reaction with 25 µL 3X SDS Sample Buffer. Vortex, then microcentrifuge for 30 seconds at 14,000 x G.

E Western Immunoblotting

1. Heat the sample to 95–100°C for 2–5 minutes.
2. Load 30 µl sample on SDS-PAGE gel.
3. **Note:** CST recommends loading prestained molecular weight markers (#7720, 15 µL/lane) to verify electrotransfer and biotinylated protein marker (#7727, 10 µL/lane) to estimate molecular weights.
4. Run SDS-page and electrotransfer to nitrocellulose or PVDF membrane.
5. **Note:** Volumes for all the following steps are for 10 cm x 10 cm membrane; for different sized membranes, adjust volumes accordingly.
6. **(Optional)** After transfer, wash nitrocellulose membrane with 25 mL TBS for 5 minutes at room temperature.
7. Incubate membrane in 10 mL Blocking Buffer for 1-2 hours at room temperature.
8. Wash three times for 5 minutes each with 15 mL Wash Buffer.
9. Incubate membrane and Phospho-ATF-2 (Thr71) Antibody (1:1000 dilution) in 10 mL Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
10. Wash three times for 5 minutes each with 15 mL Wash Buffer.
11. 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.
12. Wash three times for 5 minutes each with 15 mL Wash Buffer.
13. Incubate membrane with 10 mL LumiGLO® Substrate with gentle agitation for 1 minute at room temperature.
14. Drain membrane of excess LumiGLO® Substrate (but 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.
15. **Note:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours. LumiGLO® Substrate can be further diluted if signal response is too fast.

Reagent not provided in kit.