#9913 Store at -20°C

Phospho-p38 MAPK Pathway Antibody Sampler Kit

1 Kit
(6 x 20 µl)

Cell Signaling TECHNOLOGY®

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

Products Included	Product #	Quantity	Mol. Wt.	Source
Phospho-MSK1 (Thr581) Antibody	9595	20 µl	90 kDa	Rabbit IgG
Phospho-p38 MAPK (Thr180/Tyr182) (D3F9) XP® Rabbit mAb	4511	20 µl	43 kDa	Rabbit IgG
Phospho-MKK3 (Ser189)/MKK6 (Ser207) (D8E9) Rabbit mAb	12280	20 µl	38 kDa MKK6, 40 kDa MKK3	Rabbit IgG
Phospho-ATF-2 (Thr71)/ATF-7 (Thr53) (A8J7P) Rabbit mAb	15411	20 µl	65, 75 kDa	Rabbit IgG
Phospho-HSP27 (Ser82) (D1H2) XP® Rabbit mAb	9709	20 µl	27 kDa	Rabbit IgG
Phospho-MAPKAPK-2 (Thr334) (27B7) Rabbit mAb	3007	20 µl	49 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

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

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

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Phospho-p38 MAPK Pathway Antibody Sampler Kit provides an economical means to evaluate the activation status of multiple members of the p38 MAPK pathway, including phosphorylated MSK1, p38 MAPK, MKK3/MKK6, ATF-2, HSP27 and MAPKAPK-2. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

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).

Four residues (Thr25, Thr222, Ser272 and Thr334) of MAPKAPK-2 are phosphorylated by p38 in an *in vitro* kinase assay (3). Phosphorylation at Thr222, Ser272 and Thr334 seems to be essential for the activity of MAPKAPK-2 (3). Activated MAPKAPK-2 can in return phosphorylate HSP27 at serines 15, 78 and 82 (3,9). Phosphorylation of HSP27 causes a change in the tertiary structure of HSP27, which shifts from large homotypic multimers to dimmers and monomers (10). It has been illustrated that phosphorylation and increased concentration of HSP27 modulate actin polymerization and reorganization (11,12).

Sensitivity/Specificity: Each antibody in the Phospho-p38 MAPK Pathway Antibody Sampler Kit recognizes only the phosphorylated form of its specific target.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Thr581 of human MSK1, Thr180/Tyr182 of human p38 MAPK, Ser189 of human MKK3, Thr71 of human ATF-2, Ser82 of human HSP27 or Thr334 of human MAPKAPK-2.

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.
(9) Landry et al. (1992) *J. Biol. Chem.* 267, 794–803.
(10) Rogalla, . et al. (1999) *J. Biol. Chem.* 274, 1 8947–18956.
(11) Lavoie, J. et al. (1993) *J. Biol. Chem.* 268, 24210–24214.
(12) Rousseau, S. et al. (1997) *Oncogene* 15, 2169–2177.

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.

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

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.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.