

#9790 Store at -20°C

# p38 MAPK Isoform Activation Antibody Sampler Kit

✓ 1 Kit  
(5 x 20 µl)



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

rev. 06/16

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-p38 MAPK (Thr180/Tyr182) (D3F9) XP™ Rabbit mAb	4511	20 µl	43 kDa	Rabbit IgG
p38α MAPK Antibody	9218	20 µl	40 kDa	Rabbit IgG
p38β MAPK (C28C2) Rabbit mAb	2339	20 µl	43 kDa	Rabbit IgG
p38γ MAPK Antibody	2307	20 µl	46 kDa	Rabbit IgG
p38δ MAPK (10A8) Rabbit mAb	2308	20 µl	43 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions, and additional application protocols.

**Description:** The p38 MAPK Isoform Activation Antibody Sampler Kit provides an economical means to evaluate the activation status of individual isoforms of p38 MAPK through immunoprecipitation of the phosphorylated p38 MAPK followed by western blot using isoform specific antibodies. 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 that 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). SB203580 (4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)imidazole) is a selective inhibitor of p38 MAPK. This compound inhibits the activation of MAPKAP-2 by p38 and thereby phosphorylation of HSP27 (9). SB203580 inhibits p38 catalytic activity by binding to the ATP binding pocket, but does not inhibit phosphorylation of p38 by upstream kinases (10).

**Specificity/Sensitivity:** Each isoform-specific antibody in the p38 MAPK Isoform Activation Antibody Sampler Kit recognizes the appropriate isoform of p38 MAPK. The Phospho-p38 MAPK (Thr180/Tyr182) (D3F9) XP™ Rabbit mAb recognizes all four isoforms of p38 MAPK when phosphorylated at the specified sites.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to carboxy-terminal residues of human p38α MAPK or human p38γ MAPK. Antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with a synthetic

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibodies.

**Recommended Antibody Dilutions:**  
Western blotting 1:1000

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

peptide corresponding to residues surrounding the carboxy-terminus of p38β MAPK or p38δ MAPK and surrounding Thr180/Tyr182 of human p38 MAPK.

**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.

© 2014 Cell Signaling Technology, Inc. Cell Signaling Technology® is a trademark of Cell Signaling Technology, Inc.

**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.

U.S. Patent No. 5,675,063

# 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.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH<sub>2</sub>O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH<sub>2</sub>O, mix.
- 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<sub>2</sub>O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH<sub>2</sub>O, mix.
- 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<sub>2</sub>O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH<sub>2</sub>O, mix.
- Nonfat Dry Milk:** (#9999)
- 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.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- 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.
- Biotinylated Protein Ladder Detection Pack:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

## B. Protein Blotting

**A general protocol for sample preparation.**

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- 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.
- Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- 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.
- Electrotransfer to nitrocellulose membrane (#12369).

## C. Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

### I. Membrane Blocking

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

### II. Primary Antibody Incubation

- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

## D. Detection of Proteins

- 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.
- 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.

LumiGLO® is a registered trademark of Kirkegaard & Perry Laboratories. Tween® is a registered trademark of ICI Americas, INC. SignalFire™ is a trademark of Cell Signaling Technology, INC.

Orders ■ 877-616-CELL (2355) orders@cellsignal.com Support ■ 877-678-TECH (8324) info@cellsignal.com Web ■ www.cellsignal.com