

MAPK Family Antibody Sampler Kit

1 Kit
 (3 x 40 µl)

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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
p44/42 MAP Kinase (137F5) Rabbit mAb	4695	40 µl	42, 44 kDa	Rabbit IgG
SAPK/JNK (56G8) Rabbit mAb	9258	40 µl	46, 54 kDa	Rabbit IgG
p38 MAP Kinase Antibody	9212	40 µl	43 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

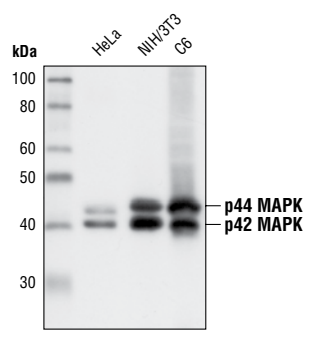
See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The MAPK Family Antibody Sampler Kit provides an economical means of evaluating total levels of p38, p44/42, and SAPK/JNK mitogen-activated protein kinases. The kit contains enough primary and secondary antibody to perform four western blot experiments.

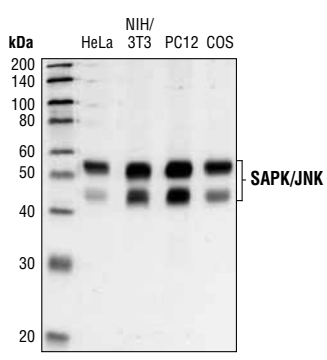
Background: p44/42 MAPK (Erk1/2), SAPK/JNK, and p38 MAPK function in protein kinase cascades that play a critical role in the regulation of cell growth, differentiation, and control of cellular responses to cytokines and stress. p44/42 MAPK is activated by growth and neurotrophic factors. Activation occurs through phosphorylation of threonine and tyrosine residues (Thr202 and Tyr204 in human Erk1) at the sequence T*EY* by a single upstream MAP kinase (MEK). SAPK/JNK and p38 MAPK are activated by inflammatory cytokines and by a wide variety of cellular stresses. Activation of SAPK/JNK occurs via phosphorylation at Thr183 and Tyr185 by the dual specificity enzyme SEK/MKK4. Both MKK3 and SEK phosphorylate p38 MAPK on tyrosine and threonine at the sequence T*GY* to activate p38 MAP kinase (1-5).

Specificity/Sensitivity: Each antibody in the MAPK Family Antibody Sampler Kit recognizes only its specific target. The antibodies do not cross-react with other MAP kinases.

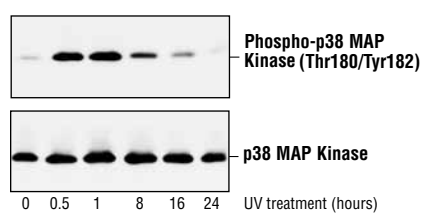
Source/Purification: p44/42 MAP Kinase Antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the C-terminus of rat p44 MAP kinase. SAPK/JNK (56G8) Rabbit mAb is produced by immunizing animals with a human JNK2/MBP fusion protein. p38 MAP Kinase Antibody is produced by immunizing animals with a synthetic peptide corresponding to the sequence of human p38 MAP kinase. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from HeLa, NIH/3T3 and C6 cells, using p44/42 MAP Kinase (137F5) Rabbit mAb #4695.



Western blot analysis of extracts from HeLa, NIH/3T3, PC12 and COS cells using SAPK/JNK (56G8) Rabbit mAb #9258.



Western blot analysis of extracts from UV-treated NIH/3T3 cells, using Phospho-p38 MAP Kinase (Thr180/Tyr182) Antibody #9211 (upper) or control p38 MAP Kinase Antibody #9212 (lower).

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 antibodies.

Recommended Antibody Dilutions:
Western blotting 1:1000

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

Background References:

- (1) Lewis, T. S. et al. (1998) *Adv. Cancer Res.* 74, 49–139.
- (2) Garrington, T.P. and Johnson, G.L. (1999) *Curr. Opin. Cell Biol.* 11, 211–218.
- (3) Schaeffer, H.J. and Weber, M.J. (1999) *Mol. Cell. Biol.* 19, 2435–2444.
- (4) Whitmarsh, A.J. and Davis, R.J. (1998) *Trends Biochem. Sci.* 23, 481–485.
- (5) Cobb, M.H. (1999) *Prog. Biophys. Mol. Biol.* 71, 479–500.

Western Immunoblotting Protocol (Primary Antibody 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.

- 1X Phosphate Buffered Saline (PBS)
- 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 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).
- Nonfat Dry Milk (weight to volume [w/v])
- 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%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- 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%).
- 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.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 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.

- 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 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.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- 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.

- 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.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- 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.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- 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.

- 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.