#9230 Store at -20°C

PhosphoPlus® MKK3/MKK6 (Ser189/207) Antibody Kit

☑ 1 Kit
10 western blots

Cell Signaling TECHNOLOGY®

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Support ■ 877-678-TECH (8324)
info@cellsignal.com
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rev. 04/04/16

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-MKK3 (Ser189)/MKK6 (Ser207) (D8E9) Rabbit mAb	12280	100 µl	38 kDa MKK6, 40 kDa MKK3	Rabbit IgG
MKK3 (D4C3) Rabbit mAb	8535	100 µl	38-40 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-biotin, HRP-linked Antibody	7075	100 µl		Goat
20X LumiGLO® Reagent and 20X Peroxide	7003	5 ml		
Biotinylated Protein Ladder	7727	100 µl		
MKK3/MKK6 Control Cell Extracts	9233	200 µl (10 western blots)		

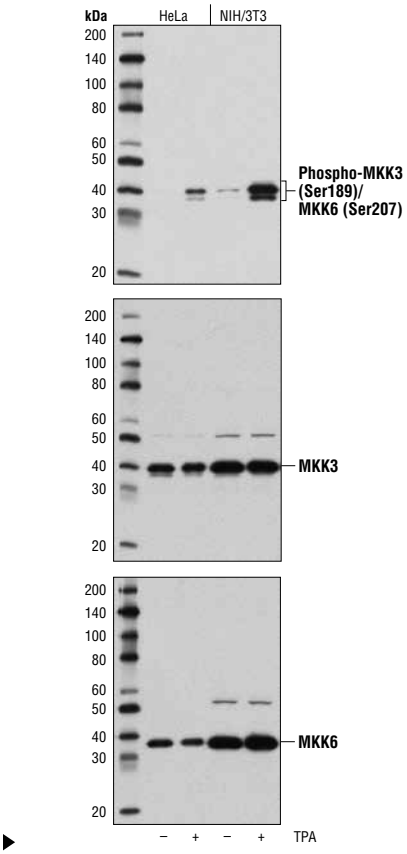
See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The PhosphoPlus® MKK3/MKK6 (Ser189/207) Antibody Kit provides reagents and protocols for the rapid analysis of the phosphorylation status of MKK3/MKK6 at Ser189/207.

Background: MKK3 and MKK6 are two closely related dual-specificity protein kinases that activate p38 MAP kinase (1-5). MKK3 and MKK6 both phosphorylate and activate p38 MAP kinase at its activation site Thr-Gly-Tyr but do not phosphorylate or activate Erk1/2 or SAPK/JNK. Phosphorylation of p38 MAP kinase dramatically stimulates its ability to phosphorylate protein substrates such as ATF-2 and Elk-1. MKK3 and MKK6 are both activated by different forms of cellular stress and inflammatory cytokines (4,5). Activation of MKK3 and MKK6 occurs through phosphorylation of serine and threonine residues at sites Ser189 and Thr193 for MKK3 (2) and Ser207 and Thr211 for MKK6 (4,5).

Specificity/Sensitivity: Phospho-MKK3 (Ser189)/MKK6 (Ser207) (D8E9) Rabbit mAb recognizes endogenous levels of MKK3 and MKK6 proteins only when phosphorylated at Ser189 (MKK3) or Ser207 (MKK6). MKK3 (D4C3) Rabbit mAb recognizes endogenous levels of total MKK3 protein. This antibody cross-reacts slightly with MKK6 but demonstrates an approximate 10-fold preference for MKK3 using recombinant, purified proteins.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser189 of human MKK3 protein and Ser207 of human MKK6 protein and with a synthetic peptide corresponding to residues near the carboxy terminus of human MKK3 protein.



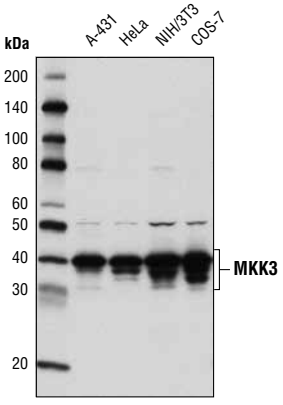
*Western blot analysis of extracts from HeLa and NIH/3T3 cells, untreated (-) or treated with TPA #4174 (200 nM, 15 min; +), using **Phospho-MKK3 (Ser189)/MKK6 (Ser207) (D8E9) Rabbit mAb #12280** (upper), MKK3 (D4C3) Rabbit mAb #8535 (middle), or MKK6 (D31D1) Rabbit mAb #8550 (lower).*

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

Background References:

(1) Derijard, B. et al. (1995) *Science* 267, 682–685.
(2) Raingeaud, J. et al. (1995) *J. Biol. Chem.* 270, 7420–7426.
(3) Sluss, H.K. et al. (1994) *Mol. Cell. Biol.* 14, 8376–8384.
(4) Raingeaud, J. et al. (1996) *Mol. Cell. Biol.* 16(3), 1247–1255.
(5) Han, J. et al. (1996) *J. Biol. Chem.* 271, 2886–2891.



*Western blot analysis of extracts from various cell lines using **MKK3 (D4C3) Rabbit mAb #8535.***

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.

#9230

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.

MSDS

Material Safety Data Sheet (MSDS) for Antibodies

Cell Signaling TECHNOLOGY®

rev. 08/09/07

I. Identification:

Product name: Antibodies
Product Catalog Number: Includes antibodies within the following range of catalog numbers: 2000-5999, 7000-7999 and 9000-9999.
CAS number: None

Manufacturer Supplier: Cell Signaling Technology
3 Trask Lane
Danvers, MA 01923 USA
1-978-867-2300 TEL
1-978-867-2400 FAX
1-978-578-6737 Emergency Phone

II. Composition/Information on Ingredients:

This product is composed of antibodies in aqueous buffer solution. According to 29 CFR 1910.1200(d), hazardous ingredients at less than <1% and carcinogens at less than < 0.1% are considered non-hazardous. Any hazardous or carcinogenic ingredients exceeding these criteria are listed below.

This product may contain the following hazardous ingredients.

Ingredient	CAS#	Percent
Glycerol	56-81-5	50%

III. Hazard Identification:

Emergency Overview of Hazardous ingredient: Glycerol (CAS# 56-81-5)
Caution: Avoid contact and inhalation.
Target Organ: Kidneys.

NFPA Rating:

Health Rating:	1
Flammability Rating:	0
Reactivity Rating:	0

IV. First Aid Measures:

Inhalation: If inhaled, remove to fresh air. If breathing is difficult, get medical attention.
Ingestion: If swallowed and person is conscious, rinse out mouth with water. Get medical attention.
Skin Exposure: In case of contact, wash skin with soap and water.
Eye Exposure: In case of contact with eyes, immediately flush eyes water for at least 15 minutes. Get medical attention.

V. Fire Fighting Measures:

Flash Point: Data not available.
Autoignition Temperature: Data not available.
Fire Extinguishing Media: Water spray, dry chemical, foam, or carbon dioxide.
Firefighting: Wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes.

VI. Accidental Release Measures:

Absorb liquid with an absorbent material. Transfer contaminated absorbent to a chemical waste container for disposal.

VII. Handling And Storage:

Avoid inhalation and contact with eyes and skin. Avoid prolonged or repeated exposure. Store at –20°C in tightly closed container.

VIII. Exposure Controls/Personal

Engineering Controls: Maintain adequate ventilation, eye wash and quick-drench facilities in work area.
Personal Protective Equipment: Lab coat, chemical resistant gloves and chemical safety glasses.
Occupational Exposure Limits: Data not available.

IX. Exposure Controls/Personal Protection:

Physical State:	Colorless liquid.
Odor:	Odorless.
Boiling Point:	Data not available.
Melting Point:	Data not available.
Volatile Organic Compound:	Data not available.
Solubility in water:	Readily miscible in water.

X. Stability and Reactivity:

Stability: Stable.
Hazardous Decomposition: May form carbon dioxide and carbon monoxide.
Conditions to avoid: Strong oxidizing agents

XI. Toxicological Information:

May cause skin irritation.
May be toxic if absorbed through skin or ingested.
May cause eye irritation.

Target Organs: Kidneys
Prolonged exposure may cause nausea, headache, and vomiting.

XII. Ecological Information:

Data not available.

XIII. Disposal Considerations:

Dispose of in accordance with federal, state and local environmental regulations.

XIV. Transport Information:

D.O.T.: This substance is considered non-hazardous for transport.
IATA: This substance is considered non-hazardous for air transport.

XV. Regulatory Information:

EU Regulation/Classification/Labeling Information: Not available for this product.

Chemical Inventory Status:
SARA Listed Component: None.
TSCA Listed Component: None.
Canada (WHMIS): DSL No, NDSL No.

XVI. Other Information:

This compound is sold only for research use by personnel familiar with chemicals and who are well trained in good laboratory habits, such as avoiding spills, keeping hands clean at all times and not rubbing eyes with hands while working in the laboratory.

This solution is sold only in microliter quantities for use in life sciences research. No other use is intended, and any other use may involve substantive hazards.

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide for experienced personnel. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product. The burden of safe use of this material rests entirely with the user.