

Phospho-MKK3 (Ser189)/MKK6 (Ser207) (D8E9) Rabbit mAb

✓ 100 µl
 (10 western blots)



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Entrez-Gene ID #5606, 5608
UniProt ID #P46734, P52564

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC Endogenous	H, M, R, Mk, (Z, B, Guinea Pig)	38 kDa MKK6, 40 kDa MKK3	Rabbit IgG**

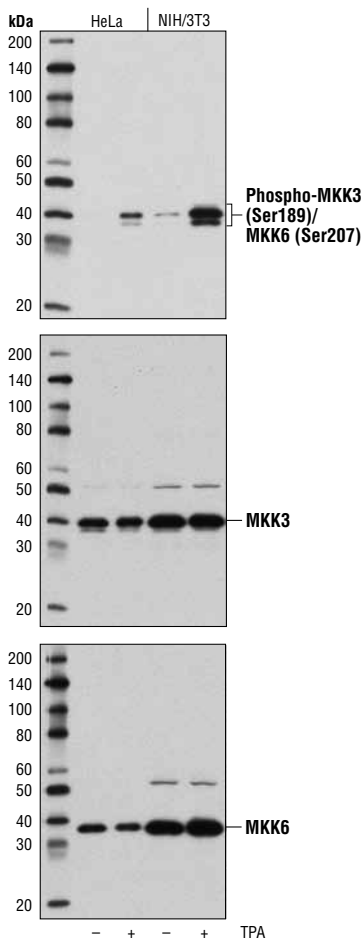
Background: MKK3 and MKK6 are two closely related dual-specificity protein kinases that activate p38 MAP kinase (1-5). MKK3 and MKK6 both phosphorylate and activate p38 MAP kinase at its activation site, Thr-Gly-Tyr, but do not phosphorylate or activate Erk1/2 or SAPK/JNK. Phosphorylation of p38 MAP 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 at Ser189 and Thr222 on MKK3 (2) and Ser207 and Thr211 on 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).

Source/Purification: Monoclonal antibody is produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser189 of human MKK3 protein and Ser207 of human MKK6 protein.

Background References:

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



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 (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, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

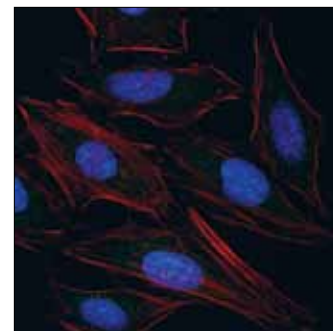
Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:100
Immunofluorescence (IF-IC)	1:400

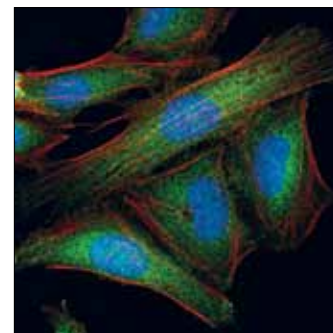
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Please visit www.cellsignaling.com for a complete listing of recommended companion products.

Untreated



UV-treated



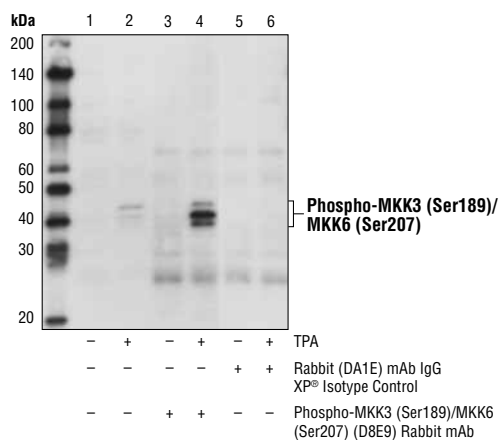
Confocal immunofluorescent analysis of HeLa cells, untreated (upper) or UV-treated (40 mJ/cm² with 30 min recovery; lower), using Phospho-MKK3 (Ser189)/MKK6 (Ser207) (D8E9) Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

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 DyLight is a trademark of Thermo Fisher Scientific Inc. and its subsidiaries.

Tween is a registered trademark of ICI Americas, Inc.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

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.



Immunoprecipitation of phospho-MKK3 (Ser189)/MKK6 (Ser207) from HeLa cells, untreated or treated with TPA #4174 (200 nM, 15 min), using Phospho-MKK3 (Ser189)/MKK6 (Ser207) (D8E9) Rabbit mAb (lanes 3 and 4) or Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lanes 5 and 6). Lanes 1 and 2 are 10% input. Western blot analysis was performed using Phospho-MKK3 (Ser189)/MKK6 (Ser207) Antibody #9231. Mouse Anti-rabbit IgG (Conformation Specific) (L27A9) mAb #3678 was used as a secondary antibody.