Store at –20°C

#9101

Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody

☐ Small 200 µl
(20 Western mini-blots)

☐ Large 600 µl
(60 Western mini-blots)

Cell Signaling TECHNOLOGY®

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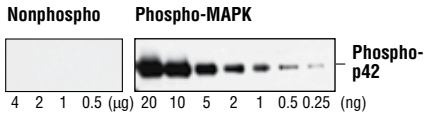
Entrez-Gene ID #5595, 5594
Swiss-Prot Acc. #P27361, P28482

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, IF-IC, F Endogenous	H, M, R, Mk, Mi, Pg, C, Hm, B, Dm, Z	42, 44 kDa	Rabbit**

Background: Mitogen-activated protein kinases (MAPKs) are a widely conserved family of serine/threonine protein kinases involved in many cellular programs such as cell proliferation, differentiation, motility, and death. The p44/42 MAPK (ERK1/2) signaling pathway can be activated in response to a diverse range of extracellular stimuli including mitogens, growth factors, and cytokines (1-3) and is an important target in the diagnosis and treatment of cancer (4). Upon stimulation, a sequential three-part protein kinase cascade is initiated, consisting of a MAP kinase kinase kinase (MAPKKK or MAP3K), a MAP kinase kinase (MAPKK or MAP2K), and a MAP kinase (MAPK). Multiple p44/42 MAP3Ks have been identified, including members of the Raf family as well as Mos and Tpl2/Cot. MEK1 and MEK2 are the primary MAPKKs in this pathway (5,6). MEK1 and MEK2 activate p44 and p42 through phosphorylation of activation loop residues Thr202/Tyr204 and Thr185/Tyr187, respectively. Several downstream targets of p44/42 have been identified, including p90RSK (7) and the transcription factor Elk-1 (8,9). p44/42 are negatively regulated by a family of dual-specificity (Thr/Tyr) MAPK phosphatases, known as DUSPs or MKPs (10), along with MEK inhibitors such as U0126 and PD98059.



Specificity and sensitivity of Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody. The antibody reacts specifically with as little as 0.25 ng of phosphorylated p42 MAP kinase and does not cross-react with up to 4 µg of nonphosphorylated p42 MAP kinase.

Specificity/Sensitivity: Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody detects endogenous levels of p44 and p42 MAP Kinase (Erk1 and Erk2) when phosphorylated either individually or dually at Thr202 and Tyr204 of Erk1 (Thr185 and Tyr187 of Erk2). The antibody does not cross-react with the corresponding phosphorylated residues of either JNK/SAPK or p38 MAP Kinase, and does not cross-react with non-phosphorylated Erk1/2.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Thr202/Tyr204 of human p44 MAP kinase. Antibodies are purified by protein A and peptide affinity chromatography.

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.*

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

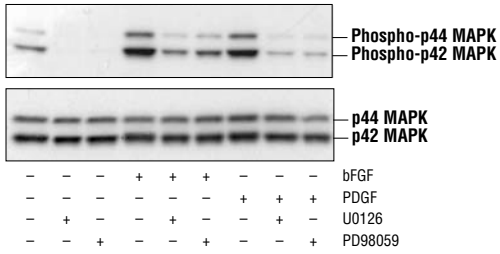
****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

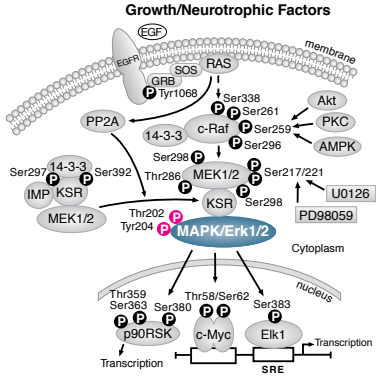
Western Blotting	1:1000
Immunoprecipitation	1:50
Immunofluorescence (IF-IC)	1:1000
Flow Cytometry	1:200

For application specific protocols please see the web page for this product at www.cellsignal.com.

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



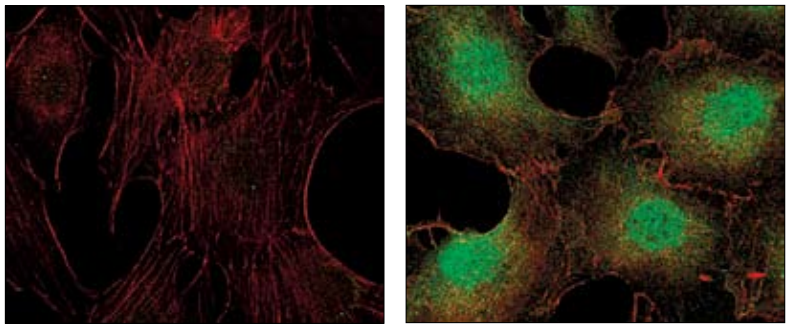
◀ *Western blot analysis of whole-cell extracts from unstarved wild-type mouse embryonic fibroblasts (MEFs) treated with the indicated combinations of basic Fibroblast Growth Factor (bFGF #9952, 100 ng/ml for 30 minutes), Platelet-Derived Growth Factor (PDGF #9909, 100 ng/ml for 30 minutes), MEK1 Inhibitor (PD98059 #9900, 50 µM, 2 hour pre-treatment), and MEK1/2 Inhibitor (U0126 #9903, 10 µM, 2 hour pre-treatment), using Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody #9101 (upper panel) and p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb #4695 (lower panel).*



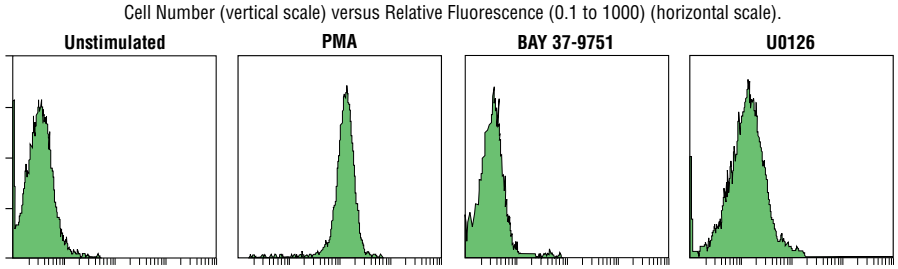
IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

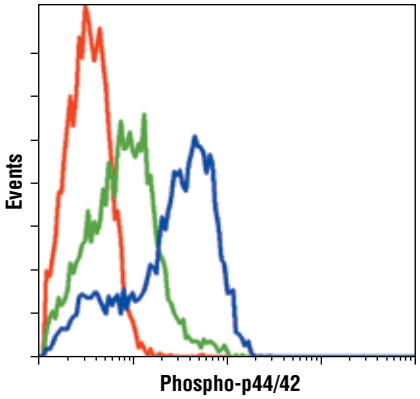
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebra fish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.



Confocal immunofluorescent analysis of NIH/3T3 cells either U0126-treated (left) or PDGF-treated (right) and labeled with Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody (green). Actin filamentshave been labeled with Alexa Fluor® 555 phalloidin (red).



Phosphorylated MEK and Erk were assayed in human peripheral blood lymphocytes stimulated with PMA in the presence or absence of the Raf inhibitor BAY 37-9751 or the MEK inhibitor U0126 #9903. BAY 37-951 blocked PMA-stimulated phosphorylation of both MEK and Erk, consistent with inhibition at the level of Raf, while U0126 blocked phosphorylation of Erk only, consistent with inhibition at the level of MEK. From Chow, S. et al. (2001) Cytometry 46, 72-78.



Flow cytometric analysis of Jurkat cells, untreated (green) or PMA-treated (blue), using Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody compared to a nonspecific negative control antibody (red).

Selected Application References:

Schulze, A. et al. (2001) Analysis of the transcriptional program induced by Raf in epithelial cells. *Genes. Dev.* 15, 981–94. Application: W.

Zimmermann, S. and Moelling, K. (1999) Phosphorylation and regulation of Raf by Akt (protein kinase B). *Science* 286, 1741–4. Application: W.

Zhang, Y.W. et al. (2003) Hepatocyte growth factor/scatter factor mediates angiogenesis through positive VEGF and negative thrombospondin 1 regulation. *Proc. Natl Acad. Sci. USA* 100, 12718–23. Application: W.

Patrucco, E. et al. (2004) PI3Kγ modulates the cardiac response to chronic pressure overload by distinct kinase-dependent and -independent effects. *Cell* 118, 375–87. Application: W.

Yu, C. et al. (2002) Pharmacologic mitogen-activated protein/extracellular signal-regulated kinase kinase/mitogen-activated protein kinase inhibitors interact synergistically with STI571 to induce apoptosis in Bcr/Abl-expressing human leukemia cells. *Cancer Res.* 62, 188–99. Application: W.

Dai, Y. et al. (2001) Pharmacological inhibitors of the mitogen-activated protein kinase (MAPK) kinase/MAPK cascade interact synergistically with UCN-01 to induce mitochondrial dysfunction and apoptosis in human leukemia cells. *Cancer Res.* 61, 5106-15. Application: Western Blotting.

Chow, S. et al. (2001) Measurement of MAP kinase activation by flow cytometry using phospho-specific antibodies to MEK and ERK: potential for pharmacodynamic monitoring of signal transduction inhibitors. *Cytometry* 46, 72–8. Application: Flow Cytometry.

Chen, H. et al. (2005) A cell-based immunocytochemical assay for monitoring kinase signaling pathways and drug efficacy. *Anal. Biochem.* 338, 136–42. Application: In Cell Western (LI-COR).

Background References:

(1) Roux, P.P. and Blenis, J. (2004) *Microbiol Mol Biol Rev* 68, 320–44.

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(8) Marais, R. et al. (1993) *Cell* 73, 381–93.

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