Background: Mitogen-activated protein kinases (MAPKs) are a widely conserved family of serine/threonine protein kinases involved in many cellular processes 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 (MAP3K) 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 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 MEK1 or 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.

Species/Sensitivity: p44/42 MAP Kinase (137F5) Rabbit mAb detects endogenous levels of total p44/42 MAP kinase (Erk1/Erk2) protein. The antibody does not cross-react with JNK/SAPK or p38 MAP kinase.

Source/ Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the C-terminus of human p44 MAP kinase.

Western blot analysis of extracts from HeLa, NIH/3T3 and C6 cells, using p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb.

Immunohistochemical analysis of paraffin-embedded human breast carcinoma, showing cytoplasmic and nuclear localization, using p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb.

Recommended Antibody Dilutions:
- Western blotting: 1:1000
- Immunoprecipitation: 1:50
- Immunohistochemistry (Paraffin): 1:250
- Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.
- Unmasking buffer: Citrate
- Antibody diluent: SignalStain® Antibody Diluent #8114
- Detection reagent: SignalStain® Boost (HRP, Rabbit) #8114
- Immunofluorescence (IF-IC): 1:100
- Flow Cytometry: 1:100

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.

Background References:

Storage: Supplied in 10 mM sodium HEPS (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.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween at 4°C with gentle shaking, overnight.
Immunohistochemical analysis of paraffin-embedded human breast carcinoma, using p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb in the presence of control peptide (left) or #1240 p44/42 MAPK (Erk1/2) Blocking Peptide (#4695 Specific) (right).

Flow cytometric analysis of Jurkat cells, U0126-treated (blue) or PMA-treated (green), using p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb compared to a nonspecific negative control antibody (red).

Confocal immunofluorescent analysis of NIH/3T3 cells, treated with either U0126 (MEK1/2 Inhibitor) #9903 (left) or PDGF (right), using p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red).

Confocal immunofluorescent analysis of HT-1080 cells, serum-starved (left) or treated with phorbol di-butyrate (PDBu) (100 nM for 15 min) (right) using p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red).