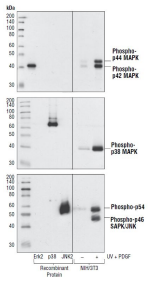
Product Data Sheet: Phospho-ERK1/ERK2 (p44/p42 MAPK) (T202/Y204)

Cat. No:	MAB-94112
Conjugate:	Unconjugated
Size:	200 ul
Clone:	197G2
Concentration:	1mg/ml
Host:	Rb
Isotype:	IgG
Reactivity:	Hu, Ms, Rt
Applications:	Western blotting 1:1000 Immunohistochemistry 2,5 ug/mL .
Molecular Weight:	42, 44 kDa
Purification:	Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr202/Tyr204 of human p44 MAP kinase.
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.Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (197G2) Rabbit mAb detects endogenous levels of p44 and p42 MAP Kinase (Erk1 and Erk2) when dually phosphorylated at Thr202 and Tyr204 of Erk1 (Thr185 and Tyr187 of Erk2), and singly phosphorylated at Tyr204. The antibody does not cross-react with the corresponding phosphorylated residues of either JNK/SAPK or p38 MAP kinase.
Form:	liquid
Buffer:	Supplied in phosphate buffered saline with 5% trehalose
Storage:	At +4°C for short term. At20°C for longer term Avoid Freezing and Thawing cycles.



Western blot analysis of purified MAPK phospho-proteins or extracts from NIH/3T3 cells treated with UV light and PDGF, using Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (197G2) Rabbit mAb (upper), Phospho-p38 MAPK (Thr180/Tyr182) (3D7) Rabbit mAb 5 (middle), and Phospho-SAPK/JNK (Thr183/Tyr185) (98F2) Rabbit (lower).

Western Blot Protocol

1. Transfer the electrophoresed proteins to Immobilon membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 0.1µg/mL rabbit anti-human/mouse/rat phosphoERK1/2.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a HRP-conj. anti-goat IgG secondary antibody.
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with ECL Reagent (Amersham).

Cell lysates for Western blottings

To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 m M NaF and bromophenyl blue) at 2 x 106 – 1 x 107 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 – 4 bursts of 5 – 10 seconds each. Samples are diluted with 1x SDS sample buffer to the desired concentration. Note: Anti Rabbit HRP secondary antibody must be used for Western Blot (cat. IS1054P Gt Anti Rb IgG)