Revision 7

Store at -20C

#5726

Phospho-p44/42 MAPK (Erk1) (Tyr204)/(Erk2) (Tyr187) (D1H6G) Mouse mAb

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IF-IC, FC-FP	H M R Mk	Endogenous	42, 44	Mouse IgG2a	#P27361, #P28482	5595, 5594

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunofluorescence (Immunocytochemistry)	1:200
Flow Cytometry (Fixed/Permeabilized)	1:400 - 1:1600

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.

For a carrier free (BSA and azide free) version of this product see product #89967.

Specificity / Sensitivity

Phospho-p44/42 MAPK (Erk1) (Tyr204)/(Erk2) (Tyr187) (D1H6G) Mouse mAb recognizes endogenous levels of p44/42 MAPK/Erk protein when phosphorylated at Tyr204 of p44 MAPK/Erk1 (Tyr187 of p42 MAPK/Erk2). This antibody detects dual-phosphorylated p44 MAPK/Erk1 (Thr202/Tyr204)/p42 MAPK/Erk2 (Thr185/Tyr187), but does not detect threonine mono-phosphorylated p44/42 MAPK/Erk. This antibody does not cross-react with any other MAP kinases.

Species predicted to react based on 100% sequence homology:

Chicken, D. melanogaster, Xenopus, Zebrafish, Bovine, C. elegans

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr187 of human Erk2 protein.

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 research investigators consider it 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.

The "activation loop" of MAPK family members contains two phosphorylation sites, typically a threonine and a tyrosine separated by a single amino acid, designated the T-x-Y motif. Phosphorylation on both residues has been shown to be required for full activation of kinase activity, but it has been appreciated for some time that mono-phosphorylation of the T-x-Y motif occurs, resulting in partial activation of catalytic acitvity and priming for subsequent, dual-phosphorylation (11,12). The crystal structures of non-, mono-, and dual-phospho MAPK/Erk demonstrate that each phospho-isomer assumes an independent conformation (13). In addition, mono-phosphorylation of MAPK/Erk2 at Tyr187 reveals that phosphorylation at this site serves to configure the ATP binding site, while phosphorylation of both Tyr and Thr residues is required to completely stabilize the substrate binding site (14). Furthermore, T-x-Y mutational analysis of members of the Erk and p38 MAP kinases appears to suggest that mono-phosphorylation of the T-x-Y motif confers differential activity and substrate preference (15,16). Taken together, these data suggest an important and underappreciated role for Thr- and Tyr- mono-phosphorylation of the T-x-Y motif among MAP kinases.

Background References

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Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

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

Applications Key

WB: Western Blotting **IF-IC:** Immunofluorescence (Immunocytochemistry)
FC-FP: Flow Cytometry (Fixed/Permeabilized)

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **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
GP: Guinea Pig **Rab:** rabbit **All:** all species expected

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