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ProductInformation

Anti-phospho-MEK1 [pThr^{292}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **M 2943**

Product Description

Anti-phospho-MEK1 (Map/Erk Kinase-1; MAP2K1) [pThr^{292}] was developed in rabbit using a synthetic phosphopeptide derived from the region of human MEK1 that contains threonine 292 as immunogen. The antibody is preadsorbed to remove any reactivity towards the non-tyrosine phosphorylated MEK1 protein.

Anti-phospho-MEK1 [pThr^{292}] specifically recognizes human, mouse, and rat MEK1 phosphorylated at threonine 292. Other species and MEK2 (80% homology) have not been tested. It has been used in immunoblotting applications.

Mitogen-activated protein (MAP) kinases, also known as extracellular signal-regulated kinases (ERKs), act as an integration point for multiple biochemical signals because they are activated by a wide variety of extracellular signals, are rapidly phosphorylated on threonine and tyrosine residues, and are highly conserved in evolution. A critical protein kinase lies upstream of MAP kinase and stimulates the enzymatic activity of MAP kinase. Denoted as MEK1 (for Map/Erk Kinase-1), it is a 393-amino acid, 43.5 kDa protein most closely related in size and sequence to the product encoded by the byr1 gene of *S. pombe*. MEK1 protein expressed in bacteria phosphorylates the ERK gene product *in vitro*. The MEK1 gene is highly expressed in murine brain.[1] A human cDNA corresponding to MEK1 was cloned in 1995 and shares 99% amino acid identity with murine MEK1 and 80% homology with human MEK2. Inhibition of MEK1 blocks p53-induced NF-κB activation and apoptosis but not cell cycle arrest.[2] Constitutive activation of MEK1 results in cellular transformation. This protein kinase therefore represents a likely target for pharmacological intervention in proliferative disease, specifically in colon cancer.[3]

MEK1&2 are also activated by dual-phosphorylation, which occurs on serines 218 and 222, in the activation loop of the MEKs. Serine 298 of MEK 1 is phosphorylated by PAK1, which promotes MEK1 binding to c-Raf and its subsequent phosphorylation of MEK 1, leading to activation. Threonine 386 and 292 of MEK1 are phosphorylated by ERK2, which serves as a negative feedback loop by suppressing activation of MEK1.[4-6]

Reagent

The antibody is supplied in 100 µL of Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 50% glycerol, 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °C. Due to the presence of 50% glycerol the antibody will remain in solution. For extended storage, centrifuge the vial briefly before opening and prepare working aliquots. To ensure accurate dilutions mix gently, remove excess solution from pipette tip with clean absorbent paper, pipette slowly. The antibody is stable for at least six months when stored appropriately. Working dilutions should be discarded if not used within 12 hours.

Product Profile

The supplied reagent is sufficient for 10 immunoblots.

A recommended working dilution of 1:1000 is determined by immunoblotting.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

Results

Peptide Competition

1. Extracts prepared from NIH3T3 cells left untreated (1) or treated with PDGF (2-5)
2. The extracts were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. Membranes were blocked with a 4% BSA-TBST buffer for 1 hour at room temperature.
3. After the treatment extracts were pre-incubated with different peptides, as follows:
   - Lane 1, 2 no peptide
   - Lane 3 a generic phosphothreonine containing peptide
   - Lane 4 the non-phosphopeptide corresponding to the immunogen
   - Lane 5 immunogen MEK 1 [pThr^{292}] peptide
4. All lanes were incubated with MEK1 [pThr^{292}] antibody in a 1% BSA-TBST for two hours at room temperature.
5. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase. Signals were detected using the Pierce SuperSignal®. method.

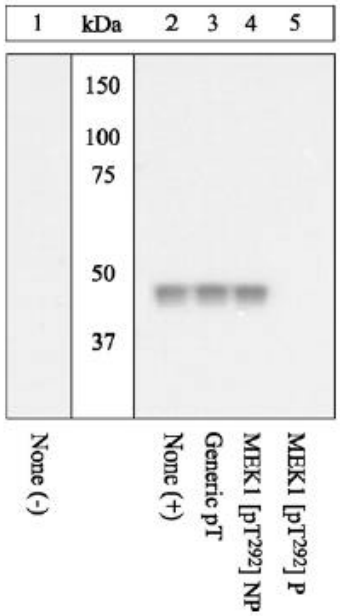

Figure 1 Peptide competition

The data show that only the peptide corresponding to MEK1 [pThr^{292}] blocks the antibody signal, but the corresponding non-phosphopeptide does not, thereby demonstrating the specificity of the antibody.

References

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3. Sebolt-Leopold, J. S., et al., Blockade of the MAP kinase pathway suppresses growth of colon tumors *in vivo*. Nature Med., **5**, 810-816 (1999).
4. Coles, L.C., and Shaw, P.E. PAK1 primes MEK1 for phosphorylation by Raf-1 kinase during cross-cascade activation of the ERK pathway. Oncogene, **21**, 2236-2244 (2002).
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