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# ProductInformation

**Anti-phospho-MEK 2 [pThr$^{394}$], Murine**
Developed in Rabbit, Affinity Isolated Antibody

Product Number **M 2693**

## Product Description

Anti-phospho-MEK 2 (Mitogen-Activated Protein Kinase 2) [pThr$^{394}$] was developed in rabbit using a synthetic phosphopeptide derived from the region of murine MEK 2 that contains threonine 394 as immunogen. The serum is affinity purified using sequential epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity towards the non-threonine phosphorylated MEK 2 protein.

Anti-phospho-MEK 2 [pThr$^{394}$] recognizes specifically mouse and rat MEK 2. It does not cross react with human MEK 2. 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. Two cDNAs, MEK 1 and MEK 2, were cloned and sequenced. MEK 1 and MEK 2 encode 393 and 400 amino acid residues, respectively. The human MEK 1 shares 99% amino acid sequence identity with the murine MEK 1 and 80% with human MEK 2. The purified MEK 2 protein stimulated threonine and tyrosine phosphorylation on ERK1 and concomitantly activated ERK1 kinase activity more than 100-fold. Inhibition of MEK 2 blocks p53-induced NF-kappa-B activation and apoptosis but not cell cycle arrest. MEK 1&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 2 is phosphorylated by PAK1, which promotes MEK 2 binding to c-Raf and its subsequent phosphorylation of MEK 2 leading to activation. Threonine 292 of MEK 1 is phosphorylated by ERK2, which serves as a negative feedback loop by suppressing activation of MEK 1. Threonine 394 of MEK 2 is retro-phosphorylated by ERK2, which serves as a negative feedback loop by suppressing activation of MEK 2.[1-4]

Constitutive activation of MEK 2 results in cellular transformation. This protein kinase therefore represents a likely target for pharmacological intervention in proliferative disease. MEK inhibitors represent a promising, non-cytotoxic approach to the clinical management of colon cancer. [5-6]

## Reagent

Anti-phospho-MEK 2 [pThr$^{394}$] is supplied as a solution in Dulbecco's phosphate buffered saline (without $Mg^{2+}$ and $Ca^{2+}$), pH 7.3, containing 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 hazardous and safe handling practices.

## Storage/Stability

Store at –70°C. For extended storage, upon initial thawing, freeze in working aliquots. Avoid repeated freezing and thawing to prevent denaturing the antibody. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 6 months when stored appropriately.

## Product Profile

A recommended working concentration of 0.1-1.0 µg/mL is determined by immunoblotting using extracts prepared from NIH3T3 cells treated with PDGF.

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

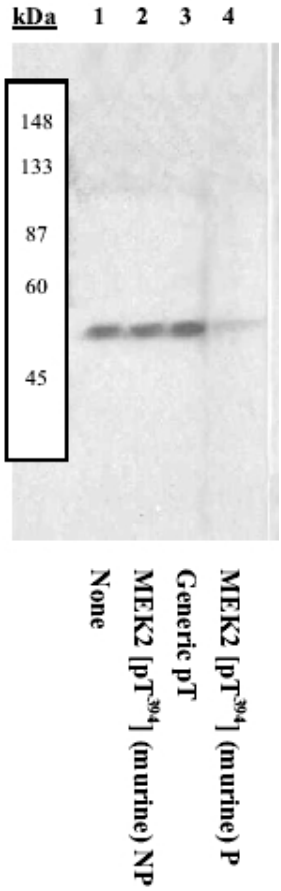
## Results

Peptide Competition

1. Extracts from NIH3T3 cells were treated with PDGF (Lane 1-4)
2. After the treatment extracts were pre-incubated with different peptides, as follows:
   Lane 1 – no peptide
   Lane 2 – non-phosphorylated peptide (murine) corresponding to immunogen MEK 2

Lane 3 – generic threonine phosphorylated MEK 2
Lane 4 – immunogen murine MEK 2 [pThr$^{394}$)

3. The extracts were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C
4. All lanes were incubated with 0.50 µg/mL MEK 2 [pThr$^{394}$] murine antibody for two hours at room temperature.
5. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase.
6. Signals were detected using the Tropix WesternStar method.
7. The data show that only the peptide corresponding to MEK 2 [pThr$^{394}$] (murine) blocks the antibody signal, but the corresponding non-phosphopeptide does not, thereby demonstrating the specificity of the antibody.




## References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