3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Anti-phospho-MEK 1 (pSer^{298})
Developed in Rabbit, Affinity Isolated Antibody

Product Number **M 2568**

Product Description

Anti-phospho-MEK 1 (MAP/Erk kinase-1) [pSer^{298}] was developed in rabbit using a synthetic peptide derived from the region of MEK 1 that contains serine 298 as immunogen. The serum is affinity purified using sequential epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity towards the non-tyrosine phosphorylated MEK 1 protein.

Anti-phospho-MEK 1[pSer^{298}] specifically recognizes human, mouse and rat MEK 1 phosphorylated at serine 298. Other species and MEK 2 (80% homology) have not been tested. It is 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. A mouse cDNA, denoted as Mek1 (for Map/Erk kinase-1), is a 393-amino acid, 43.5-kD 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 MEK 1 was cloned in 1995 and shares 99% amino acid identity with murine MEK 1 and 80% homology with human MEK 2. Inhibition of MEK 1 blocks p53-induced NF-kappa-B 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 MEK 1 binding to c-Raf and its subsequent phosphorylation of MEK 1, leading to activation. Threonine 386 of MEK1 is phosphorylated by ERK2, which serves as a negative feedback loop by suppressing activation of MEK1.[4-6]

Reagent

Anti-phospho-MEK 1 [pSer^{298}] is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 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, freeze in working aliquots. Avoid repeated freezing and thawing to prevent denaturing of the antibody. Do not store in a frost-free freezer. The antibody is stable for at least 12 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.

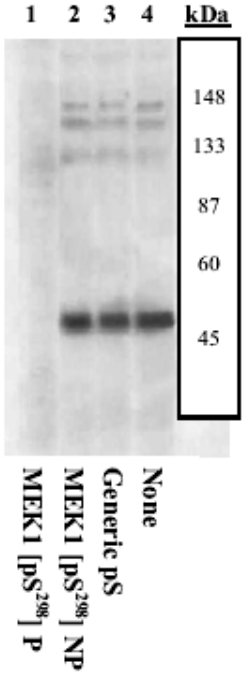
Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

Results

Peptide Competition

1. Extracts from NIH3T3 cells were left untreated (Lane 1) or treated with PDGF (Lane 2-5)
2. After the treatment extracts were pre-incubated with different peptides, as follows:
   Lane 1 – the immunogen MEK 1 [pSer^{298}]
   Lane 2 – non-phosphorylated MEK 1
   Lane 3 – generic serine phosphorylated MEK 1
   Lane 4 – no peptide

3. The extracts were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF.
4. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C
5. All lanes were incubated with 0.50 µg/mL MEK1 [pSer^{298}] antibody for two hours at room temperature.
6. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase.
7. Signals were detected using the Tropix WesternStar method.
8. The data show that only the peptide corresponding to MEK1 [pSer^{298}] blocks the antibody signal, but the corresponding non-phosphopeptide does not, thereby demonstrating the specificity of the antibody.



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