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ProductInformation

Anti-phospho- MEK3 [pSer^{189}/pThr^{193}]/MEK6 [pSer^{207}/pThr^{211}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **M 5193**

Product Description

Anti-phospho- (MAP/Erk kinase-1 MEK3 [pSer^{189}/pThr^{193}]/MEK6 [pSer^{207}/pThr^{211}] was developed in rabbit using a synthetic phosphopeptide derived from a region of human MEK 3 that contains serine 189 and threonine 193 as immunogen. The serum is affinity purified using sequential epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity towards the non-phosphorylated MEK 3 protein. The final product is generated by affinity chromatography using a MEK3-derived peptide that is phosphorylated at serine 189 and threonine 193.

The antibody specifically detects human MEK 3 and MEK 6 (93% homologous). Other species of MEK 3 and MEK 6 have not been tested, but are expected to cross react. This antibody shows some cross-reactivity with MEK 4 when tested in a system with high MEK 4 expression levels. It does not react with any other MEK isoforms. It has been used in immunoblotting applications.

Mitogen-Activated Protein Kinase Kinases 3 and 6 (MEK3/6 or MKK3/6) are 42 kDa members of a tyrosine/threonine protein kinase family that activate p38, which is part of the inflammation/ stress-signaling pathway. Phosphorylation of MEK 3 and 6 by MEKK1 on serine 189 and threonine 193 (serine 207 and threonine 211 for MEK 6) in the catalytic domain activates the proteins and enables them to phosphorylate p38.

Reagent

The antibody 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 Mal-E-tagged fusion protein expressing MEK 6, in inactive form or activated by adding MEKK.

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 background extracts with Mal-E-tagged fusion protein expressing MEK 6 in inactive form (Lane 6) or activated by addition of MEKK (Lanes 1-5) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
3. Membranes were pre-incubated with different peptides, as follows:
   Lane 1and 6 – no peptide
   Lane 2 – the non-phosphorylated peptide corresponding to immunogen
   Lane 3 – a generic peptide containing phosphoserine
   Lane 4 – generic peptide containing phosphothreonine
   Lane 5 – immunogen MEK 3/6 double phosphorylated
4. Subsequently, membranes were incubated with 0.50 µg/mL MEK3 [pSer^{189}/pThr^{193}]/MEK6 [pSer^{207}/pThr^{211}] antibody for two hours at room temperature in a 3% BSA-TBST buffer.
5. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase and signals were detected using the Tropix WesternStar method.

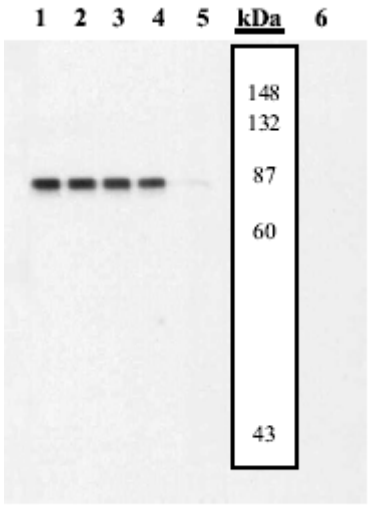

Figure 1 Peptide Competition

The data shows that only the peptide corresponding to MEK3/6 [pSpT$^{189/193}$]/[pSpT$^{207/211}$] blocks the antibody signal, thereby demonstrating the specificity of the antibody. The Mal-E-tagged fusion protein expressing MEK 6 runs at ~81 kDa.

References

1. Chen, Z., and M.H. Cobb Regulation of stress-responsive mitogen-activated protein (MAP) kinase pathways by TAO2. J. Biol. Chem., **276**, 16070-16075 (2001).
2. Nishio, H., et al. Immunolocalization of the mitogen-activated protein kinase signaling pathway in Hassall's corpuscles of the human thymus. Acta. Histochem., **103**, 89-98.
3. Browning, D.D., et al., Activation of p38 mitogen-activated protein kinase by lipopolysaccharide in human neutrophils requires nitric oxide-dependent cGMP accumulation. J. Biol. Chem., **274,** 537-542 (1999).
4. Stein, B., et al. Cloning and characterization of MEK 6, a novel member of the mitogen activated protein kinase kinase cascade. J. Biol. Chem., **271**, 11427-11433 (1996).

AH/JK 4/16/2004