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 4 [pSer^{257}/Thr^{261}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **M 4943**

Product Description

Anti-phospho-MEK 4 (Mitogen-Activated Protein Kinase 4) [pSer^{257}/pThr^{261}] was developed in rabbit using a synthetic MEK 4-derived peptide that is phosphorylated at serine 257 and threonine 261 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 4 protein. This antibody specifically recognizes MEK 4 double phosphorylated at serine 257 and threonine 261.

The antibody reacts with human MEK 4. Mouse and frog (100% homologous) have not been tested, but are predicted to cross react. It has been used in immunoblotting applications.

Mitogen-Activated Protein Kinase Kinase 4 (MEK 4 or MKK4) is a 42 kDa member of a tyrosine/threonine protein kinase family that activates the c-Jun NH2-terminal kinases (JNK), which is part of the inflammation/stress signaling pathway. The mutation of the MEK 4 gene in some carcinomas indicates that it may also have a role as a tumor suppressor.[1-2]

Phosphorylation of MEK4 by MEKK1 on serine 257 and threonine 261 in the catalytic domain activates the protein and enables MEK 4 to phosphorylate JNK.[3]

Reagent

Anti-phospho- MEK 4 [pSer^{257}/pThr^{261}] 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 GST-tagged fusion protein expressing MEK 4.

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 GST-tagged fusion protein expressing MEK 4 added 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 1 – 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 4 double phosphorylated
4. Subsequently, membranes were incubated with 0.50 µg/mL MEK 4 [pSer^{271}/pThr^{275}] 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.

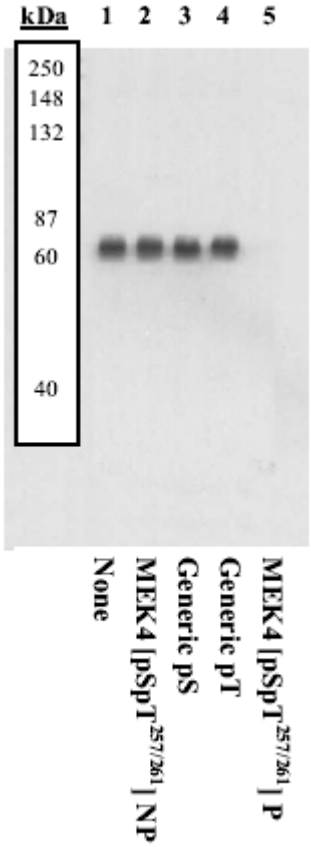

Figure 1 Peptide Competition

The data (Figure 1) show that only the peptide corresponding to MEK 4 [pSer^{257}/pThr^{261}] blocks the antibody signal, but the corresponding non-phosphopeptide does not, thereby demonstrating the specificity of the antibody.

References

1. Hagemann, C. and Blank, J.L., The ups and downs of MEK kinase interactions. Cell Signal. **13**, 863-875 (2001).
2. Cuenda, A., Mitogen-activated protein kinase kinase 4 (MKK4). Int. J. Biochem. Cell. Biol., **32**, 581-587 (2000).
3. Davis, R.J. Signal transduction by the c-Jun N-terminal kinase. Biochem. Soc. Symp. **64**, 1-12 (1999).

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