MEK1(EE) (Mouse), Active

Full-length recombinant protein expressed in E. coli cells

Cat# CY-SPM02

Lot No. K055-1
5 µg 0.1 µg/µl

Background:

MEK1 is a member of the dual specificity protein kinase family that acts as a mitogen-activated protein kinase (MAPK) kinase. MEK1 lies upstream of MAPK/ERK and stimulates the enzymatic activity of MAPK/ERK upon a wide variety of extra- and intracellular signals. As an essential component of MAPK/ERK signal transduction pathway, MEK1 is involved in many cellular processes such as proliferation, differentiation, transcription regulation and development (1). Constitutive activation of MEK1 results in cellular transformation. Thus, MEK1 represents a likely target for pharmacologic intervention in proliferative disease such as cancer (2).

Product Description:

Recombinant full-length mouse MEK1 (S218E, S222E) was expressed in E. coli cells using an N-terminal GST tag. The gene accession number is NM_008927.

Gene Aliases:

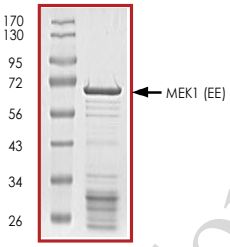
MAP2K1; MKK1; MAPKK1; PRKMK1

Formulation:

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Purity & Molecular Weight:

The purity was determined to be >70% by densitometry. Approx. MW 69kDa.



Storage:

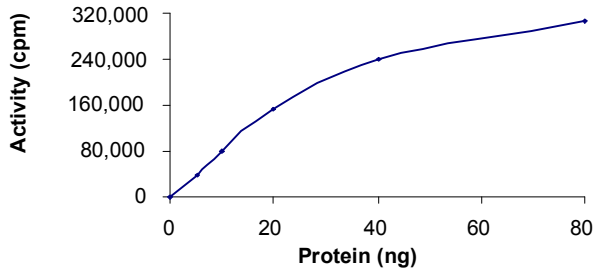
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

1 year at –70 °C from date of shipment.

Specific Activity:

The specific activity was determined to be 326 nmol /min/mg as per Activity Assay Protocol.



Activity Assay Protocol:

Assay 1st reaction of the MEK1 (EE) activity in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 10 µL of 0.2µg/µL 1st step Substrate Solution, 5 µL of the diluted MEK1 and 5 µL of 250 µM ATP solution. Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Then assay 2nd reaction in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 5 µL of 1 mg/ml 2nd step Substrate Solution, 5 µL of 1st reaction mixture, 5 µL of distilled H_2O and 5 µL of 250 µM ATP solution containing [gamma ^{32}P] ATP (0.167 µCi/µL). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate 2nd reaction by spotting 20 µL of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

Substrate Solution:

0.2 µg/µL of unactive ERK1 as 1st substrate for the MEK1 (EE) and Myelin Basic Protein (MBP) diluted in distilled H_2O to a final concentration of 1 mg/ml was used as 2nd substrate for the activated ERK1.

5 X Kinase Assay Buffer:

25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

1.Seger, R. et al: The MAPK signaling cascade. FASEB J. 9: 726-735, 1995.
2.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.

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