Store at –80°C

#7465

MEK1 Kinase

☑5 µg

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New 03/08

Description: Purified recombinant full-length human MEK1 (Met1-Val393) kinase, supplied as a GST fusion protein.

Background: MEK1 and MEK2, also called MAPK or Erk kinases, are dual-specificity protein kinases that function in a mitogen activated protein kinase cascade controlling cell growth and differentiation (1-3). Activation of MEK1 and MEK2 occurs through phosphorylation of two serine residues at positions 217 and 221 (in the activation loop of subdomain VIII) by Raf-like molecules. MEK1/2 is activated by a wide variety of growth factors and cytokines and also by membrane depolarization and calcium influx (1-4). Constitutively active forms of MEK1/2 are sufficient for the transformation of NIH/3T3 cells or the differentiation of PC12 cells (4). MEK activates p44 and p42 MAP kinase by phosphorylating both threonine and tyrosine residues at sites located within the activation loop of kinase subdomain VIII.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length human MEK1 (Met1-Val393) (GenBank Accession No. NM_002755) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The theoretical molecular weight of the MEK1 protein is 72 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. MEK1 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

(1) Crews, C.M. et al. (1992) *Science* 258, 478–480.

(2) Alessi, D.R. et al. (1994) *EMBO J.* 13, 1610–1619.

(3) Rosen, L.B. et al. (1994) *Neuron* 12, 1207–1221.

(4) Cowley, S. et al. (1994) *Cell* 77, 841–852.

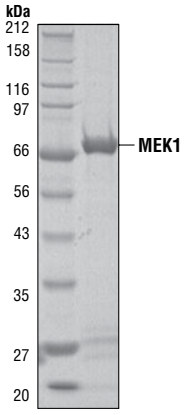

Figure 1. The purity of the GST-MEK1 protein was analyzed using SDS/PAGE followed by Coomassie stain.

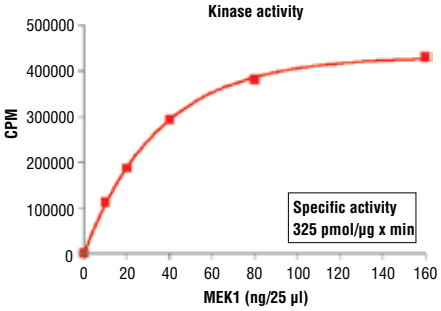

Figure 2. MEK1 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM $MgCl_2$, 0.05 mM DTT, 50 µM ATP, Substrate: unactive ERK1 (0.2 µg/µl) as the first substrates and MBP (200 ng/µl) as the final substrate, and variable amounts of recombinant MEK1.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione.

Store at -80° C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

Protocol for MEK1 Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. **Kinase Buffer (10X)**
   50 mM MOPS, pH 7.2
   25 mM β-glycerophosphate
   10 mM EGTA
   4 mM EDTA
   50 mM $MgC1_2$
   0.5 mM DTT
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. MBP (2.0 µg/µl)
5. Inactive p38γ (0.2 µg/µl)

B Suggested Protocol

1. Dilute 10X assay buffer to make 2X and 1X assay buffer.
2. Dilute 10 mM ATP with 1X assay buffer by 1:40 to make 250 µM ATP.
3. Dilute [^{32}P] ATP to 0.16 µCi/µl [^{32}P] ATP with 250 µM ATP solution.
4. Dilute MEK1 protein to 20 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. Initiate the activation reaction by combining these components together:
   a. 10 µl of diluted active MEK1
   b. 5 µl of inactive ERK1 (0.2 µg/µl)
   c. 5 µl 2X assay buffer
   d. 5 µl ATP (250 µM) (without [^{32}P] ATP)
   Incubate at room temperature for 15 minutes.
6. Initiate the secondary reaction by combining these components together:
   a. 5 µl of reaction mixture from Step 5
   b. 5 µl of MBP substrate (1.0 mg/ml)
   c. 10 µl 2X assay buffer
   d. 5 µl of 0.16 µCi/µl [^{32}P] ATP solution

Final Assay Conditions
5 mM MOPS, pH 7.2
2.5 mM β-glycerophosphate
1 mM EGTA
4 mM $MgCl_2$
0.05 mM DTT
200 ng/µl MBP

7. After 15 minutes terminate reaction by spotting 20 µl of the reaction mixture onto phosphocellulose P81 paper.
8. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
9. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
10. Count samples in a scintillation counter.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: drugdiscovery@cellsignal.com.