

Product Information

MEKK1 (900-1748), active, GST tagged, human PRECISIO® Kinase recombinant, expressed in Sf9 cells

Catalog Number **SRP5047**
Storage Temperature -70°C

Synonyms: MAP3K1, MEKK, MAPKKK1

Product Description

MEKK1 or MEK kinase, is a serine/threonine kinase that is downstream of mitogenic and metabolic stimuli, including insulin and many growth factors. MEKK1 functions not only as an upstream activator of ERK and JNK through its kinase domain, but also as an E3 ligase through its PHD domain, providing a negative regulatory mechanism for decreasing ERK1/ERK2 activity by ubiquitination and degradation.¹ MEKK1 $-/-$ embryonic stem cells from mice show loss or altered responses of JNK to microtubule disruption and cold stress.² Furthermore, activation of JNK is lost and that of ERK is diminished in response to hyperosmolarity and serum factors in MEKK1 $-/-$ cells.

Recombinant human MEKK1 (900-1748) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_005921. Recombinant protein stored in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~155 kDa

Purity: 70–95% (SDS-PAGE, see Figure 1)

Specific Activity: 697–943 nmole/min/mg (see Figure 2)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at -70°C is recommended. After opening, aliquot into smaller quantities and store at -70°C . Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot
70–95% (densitometry)

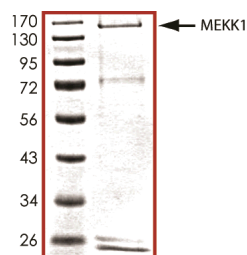
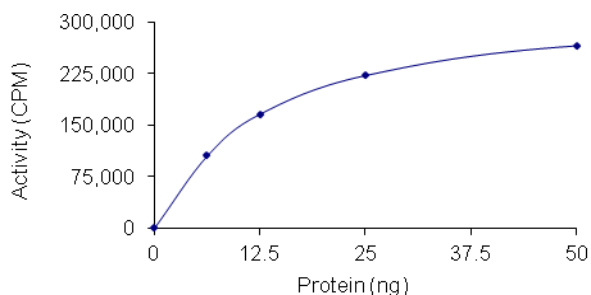


Figure 2.
Specific Activity of Typical Lot
697–943 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl_2 , 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/ μL BSA solution.

Kinase Solution – Dilute the active MEKK1, (0.1 $\mu\text{g}/\mu\text{L}$) with Kinase Dilution Buffer to the desired concentration.
Note: The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active MEKK1 kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 µl aliquots at –20 °C.

γ -³³P-ATP Assay Cocktail (250 µM) – Combine 5.75 ml of Kinase Assay Buffer, 150 µl of 10 mM ATP Stock Solution, 100 µl of γ -³³P-ATP (1 mCi/100 µl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Unactive MEK1 and ERK1 were activated in a coupled reaction. Myelin Basic Protein diluted in distilled water to a final concentration of 1 mg/ml was subsequently used as a substrate for the activated ERK1.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay

This assay involves the use of the ³³P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active MEKK1, Kinase Assay Buffer, unactive MEK1, unactive ERK, and Kinase Dilution Buffer on ice. The γ -³³P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 µl:
 - 10 µl of Kinase Solution
 - 1 µl of unactive MEK1 (0.2 µg/µl)
 - 1 µl of unactive ERK (0.2 µg/µl)
 - 8 µl of Kinase Dilution Buffer
3. Initiate the reaction by adding 5 µl of ATP (250 µM) and incubate at 30 °C water bath for 25 minutes.
4. After 25 minutes, remove 5 µl of reaction mixture to add to the following components:
 - 5 µl of reaction mixture
 - 10 µl cold water (4 °C)
 - 5 µl of MBP substrate on ice (1 mg/ml)
5. Set up a blank control as outlined in step 4, substituting 5 µl of cold water (4 °C) for the Substrate Solution.
6. Initiate each reaction with the addition of 5 µl of the γ -³³P-ATP Assay Cocktail, bringing the final reaction volume to 25 µl. Incubate the mixture in a water bath at 30 °C for 15 minutes.
7. After the 15 minute incubation, stop the reaction by spotting 20 µl of the reaction mixture onto an individually pre-cut strip of phosphocellulose P81 paper.

8. Air dry the pre-cut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
9. Set up a radioactive control to measure the total γ -³³P-ATP counts introduced into the reaction. Spot 5 µl of the γ -³³P-ATP Assay Cocktail on a pre-cut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
10. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
11. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of 5 } \mu\text{l of } \gamma\text{-}^{33}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

cpm – value from control (step 7)
nmole – 1.25 nmole (5 µl of 250 µM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)
Δcpm = cpm of the sample – cpm of the blank (step 3)
25 = total reaction volume
20 = spot volume
T = reaction time (minutes)
E = amount of enzyme (mg)

References

1. Lu, Z., et al, The PHD domain of MEKK1 acts as an E3 ubiquitin ligase and mediates ubiquitination and degradation of ERK1/2. *Molec. Cell*, **9**, 945-956, (2002).
2. Yujiri, T., et al., Role of MEKK1 in cell survival and activation of JNK and ERK pathways defined by targeted gene disruption. *Science*, **282**, 1911-1914 (1998).

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