

Product Information

MEKK3, active, GST-tagged, human PRECISIO® Kinase

recombinant, expressed in baculovirus infected Sf9 cells

Catalog Number **M0449**

Lot Number 051M0819

Storage Temperature -70 °C

Synonyms: MAP3K3, MAPKKK3

Product Description

MEKK3 is a member of the MEKK family of protein kinases that directly regulate the SAPK and ERK pathways but not the p38 pathway. MEKK3 modulates SAPK and ERK activity by activating the upstream targets SEK and MEK1/2, respectively.¹ KSR2 can regulate the activity of MEKK3 and can reduce MEKK3-mediated ERK activation. Knockout of the MEKK3 gene in mice leads to disruption of blood vessel development and the structural integrity of the yolk sac.² The blockage of angiogenesis in these mice is not a result of decreased VEGF1 expression but rather due to an intrinsic defect in Mekk3 -/- endothelial cells. Thus, MEKK3 is necessary for blood vessel development and may be a possible target for drugs that control angiogenesis.

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 002401. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~105 kDa

Purity: ≥70% (SDS-PAGE, see Figure 1)

Specific Activity: 17–23 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 Lot Number 051M0819
>90% (densitometry)

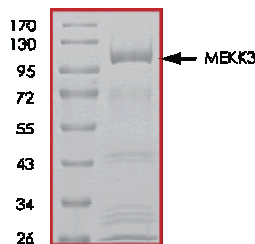
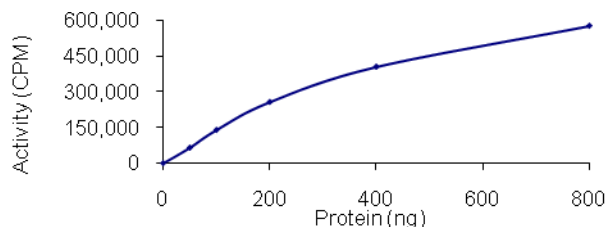


Figure 2.
Specific Activity of Lot Number 051M0819
23 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl₂, 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 MEKK3 (0.1 µg/µl) 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 MEKK3 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-³²P-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-³²P-ATP (1 mCi/100 µl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Prepare Myelin basic protein (MBP) in water at a final concentration of 1 mg/ml.

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 ³²P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active MEKK3, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-³²P-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
 - 5 µl of Substrate Solution
 - 5 µl of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5 µl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 µl of the γ-³²P-ATP Assay Cocktail, bringing the final reaction volume to 25 µl. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. 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.

6. 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.
7. Set up a radioactive control to measure the total γ-³²P-ATP counts introduced into the reaction. Spot 5 µl of the γ-³²P-ATP Assay Cocktail on a pre-cut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. 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)

$$SR = \frac{\text{cpm of 5 } \mu\text{l of } \gamma\text{-}^{32}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

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. Ellinger-Ziegelbauer, H. et al., Direct activation of the stress-activated protein kinase (SAPK) and extracellular signal-regulated protein kinase (ERK) pathways by an inducible mitogen-activated protein kinase/ERK kinase kinase 3 (MEKK) derivative. *J. Biol. Chem.*, **272**, 2668-2674 (1997).
2. Yang, J. et al., Mek3 is essential for early embryonic cardiovascular development. *Nature Genet.*, **24**, 309-313 (2000).

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