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Product Information

MEKK2, active, GST-tagged, human
PRECISIO® Kinase
recombinant, expressed in *Sf*9 cells

Catalog Number **M0324**
Storage Temperature –70 °C

Synonyms: mitogen-activated protein kinase (MAPK) kinase kinase 2, MAP3K2, MEKK2B

Product Description
MEKK2 is a major upstream activator of the JNK/MAPK cascade.[1] MEKK2 can mediate *in vitro* phosphorylation of MKK4 and MKK7, which are key MAPKs that activate JNK. In addition, MEKK2 immunoprecipitates activated JUN in an IL1-dependent manner. Rheumatoid arthritis and osteoarthritis synovial tissues show high levels of MEKK2 indicating it may be an important activator of the JNK pathway in arthritis.[2] Endogenous MEKK2 is localized mainly in the cytosol of unstimulated cells.

This recombinant product was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST-tag. The gene accession number is NM 006609. 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: ~115 kDa

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% (SDS-PAGE, densitometry)

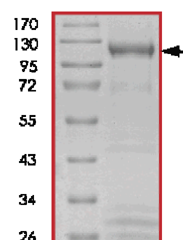
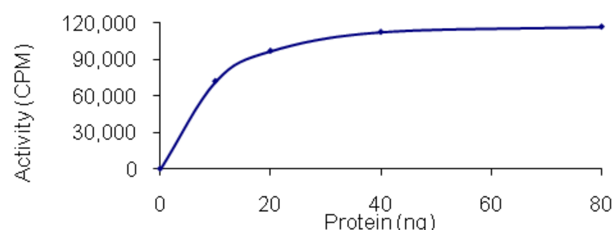


Figure 2.
Specific Activity of Typical Lot:
192–260 nmole/min/mg



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 water.

Kinase Solution – Dilute the active MEKK2 (0.1 μg/μl) with Kinase Dilution Buffer to the desired concentration. Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active MEKK2 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-^{32}P-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-^{32}P-ATP (1 mCi/100 μl). Store in 1 ml aliquots at –20 °C.

Substrate Solutions – Inactive MEK1 (0.2 μg/μl), Inactive ERK1 (0.2 μg/μl), and Myelin Basic Protein (MBP) diluted 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 ^{32}P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active MEKK2, Kinase Assay Buffer, Inactive ERK1, and Kinase Dilution Buffer on ice. The γ-^{32}P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, prepare an activation mixture with a final volume of 15 μl:
   - 10 μl of Kinase Solution
   - 1 μl of Inactive ERK1 (0.2 μg/μl)
   - 1 μl of Inactive MEK1 (0.2 μg/μl)
   - 3 μl of water
3. Set up a blank control as outlined in step 2, substituting 10 μl of Kinase dilution buffer for the Kinase Solution.
4. Initiate each reaction with the addition of 5 μl of the γ-^{32}P-ATP Assay Cocktail, bringing the reaction volume to 20 μl. Incubate the mixture in a water bath at 30 °C for 30 minutes.
5. In a microcentrifuge tube, add the following solutions to a volume of 25 μl:
   - 20 μl of the activated mixture (step 4)
   - 5 μl of MBP Substrate Solution
6. 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 precut strip of phosphocellulose P81 paper.
8. Air dry the precut 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-^{32}P-ATP counts introduced into the reaction. Spot 5 μl of the γ-^{32}P-ATP Assay Cocktail on a precut 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)

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

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. Blank, J.L. et al., J. Biol. Chem., **271**, 5361-5368 (1996).
2. Hammaker, D.R. et al., J. Immun., **172**, 1612-1618 (2004).

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