

Product Information

**MEK2, active, GST-tagged, human
PRECISIO® Kinase
recombinant, expressed in Sf9 cells**

Catalog Number **M1198**
Lot Number 041M1003
Storage Temperature $-70\text{ }^{\circ}\text{C}$

Synonyms: MAP2K2, MKK2, PRKMK2, MAPKK2

Product Description

MEK2 is a member of the MAPK kinase (MAPKK) family of signaling protein kinases. MEK2 is a dual-specificity kinase that activates the extracellular signal-regulated kinase (ERK) and mitogen-activated protein (MAP) kinase upon agonist binding to receptors. MEK2 plays a key role in the Ras/Raf/MEK/ERK mitogen-activated protein kinase (MAPK) signaling pathways.¹ Approximately 30% of all human cancers have a constitutively activated MAPK pathway and constitutive activation of MEK2 results in cellular transformation. The ERK/MAP kinase cascade regulates cell growth and differentiation.²

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 030662. 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: ~71 kDa

Purity: $\geq 70\%$ (SDS-PAGE, see Figure 1)

Specific Activity: 190–258 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 $-70\text{ }^{\circ}\text{C}$ is recommended. After opening, aliquot into smaller quantities and store at $-70\text{ }^{\circ}\text{C}$. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Lot Number 041M1003:
>90% (densitometry)

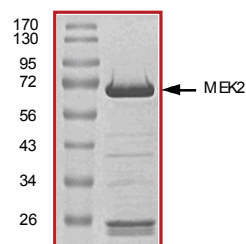
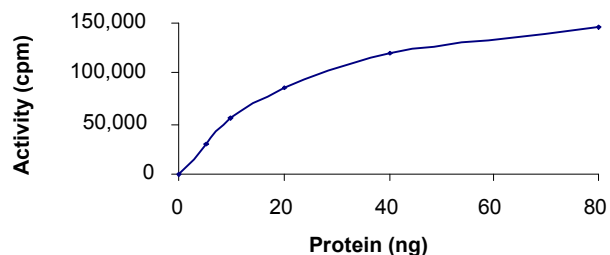


Figure 2.
Specific Activity of Lot Number 041M1003:
224 nmole/min/mg



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 MEK2 (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 MEK2 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 – Inactive ERK2 (0.2 µg/ml); 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 ³²P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active MEK2, Kinase Assay Buffer, Inactive ERK2, 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, prepare an activation mixture with a volume of 20 µl:
 - 5 µl of Kinase Solution
 - 10 µl of Inactive ERK2 (0.2 µg/µl)
 - 5 µl of Kinase Assay Buffer
3. Start the activation reaction by adding 5 µl of 250 µM ATP and incubate in a water bath at 30 °C for 15 minutes.
4. In a microcentrifuge tube, add the following solutions to a volume of 20 µl:
 - 5 µl of activated mixture (step 3)
 - 5 µl of MBP Substrate Solution
 - 10 µl of cold water (4 °C)
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-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.

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-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.
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)

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

$$\begin{aligned} \text{cpm} &= \text{value from control (step 7)} \\ \text{nmole} &= 1.25 \text{ nmole (5 } \mu\text{l of 250 } \mu\text{M ATP Assay Cocktail)} \end{aligned}$$

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. Shuichan, X. et al., Mol. Endocrinol., **11**, 1618-1625 (1997).
2. Louis-François, B, et al., Mol. Cellular Biol., **23**, 4778-4787 (2003).

PRECISIO is a registered trademark of Sigma-Aldrich® Biotechnology LP and Sigma-Aldrich Co.

TD,MAM 05/11-1

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.