Recombinant Human Active MEK2 Protein, CF

Catalog Number: 2855-KS-010
Lot Number: 1767580

SPECIFICATIONS AND USE

Source	Recombinant human MEK2 was expressed by baculovirus in *Sf9* insect cells using an N-terminal GST tag. The gene accession number is NM_030662.
Molecular Mass	The approximate molecular weight is 71 kDa.
Purity	The purity was determined to be > 80% by densitometry.
Formulation	Supplied in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of MEK2 was determined to be 190 nmol/min/mg using an ERK2 substrate and a Myelin Basic Protein (MBP) substrate.
Storage	This product is stable at ≤ -70 °C for up to one year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

BACKGROUND

MEK2 is the member of 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).

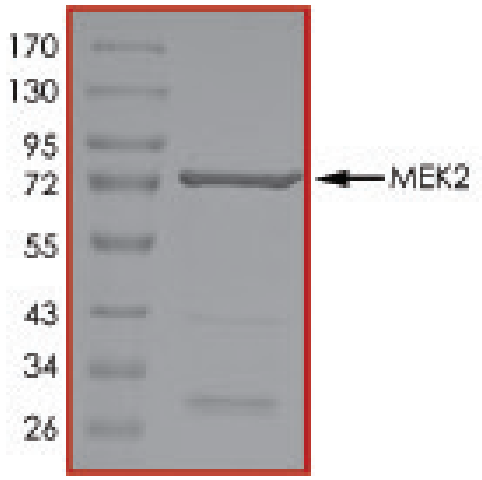

Figure 1: The approximate molecular weight is 71 kDa and the purity is > 80%.

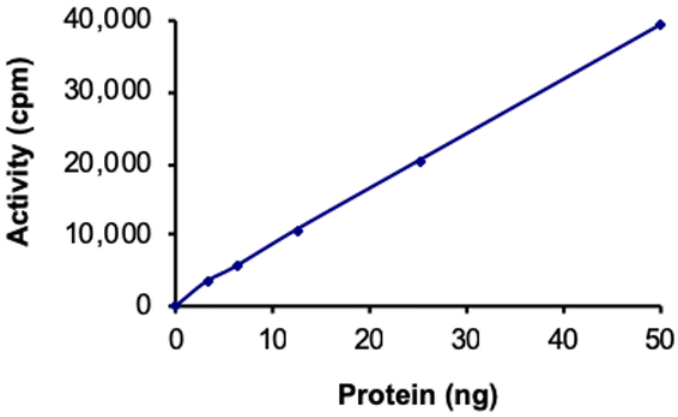

Figure 2: The specific activity of this lot of MEK2 was determined to be 190 nmol/min/mg in a coupled assay as per the Activity Assay Protocol.

REFERENCES

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

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ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - Active MEK2 (0.1 µg/µL) diluted with Kinase Dilution Buffer III as outlined in Sample Activity Plot.
Note: *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*

Kinase Assay Buffer I - 25 mM MOPS pH 7.2, 12.5 mM β-glycerolphosphate, 25 mM $MgCl_2$, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer I prior to use.

Kinase Dilution Buffer III - Kinase Assay Buffer I diluted at a 1:4 ratio (5X dilution) with 50 ng/µL BSA solution.

[^{32}P]-ATP Assay Cocktail - Prepare 250 µM [^{32}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 µL of 10 mM ATP Stock Solution, 100 µL [^{33}P]-ATP, 5.75 mL of Kinase Assay Buffer I. Store 1 mL aliquots at ≤ -20 °C.

10 mM ATP Stock Solution - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I. Store 200 µL aliquots at ≤ -20 °C.

Substrate - Unactive ERK2 was activated using MEK2 and a Myelin Basic Protein (MBP) diluted in 100 mM MOPS, pH 6.5 buffer to a final concentration of 0.2 mg/mL was used as a substrate.

ASSAY PROCEDURE

1. Thaw the Active MEK2, Kinase Assay Buffer I, and unactive ERK2 on ice. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 µL.

Reaction Component	Amount
Diluted active MEK2	5 µL
Unactive ERK2 (0.2 µg/µL	10 µL
Kinase Assay Buffer	5 µL

2. Start the reaction with the addition of 5 µL ATP (250 µM) and incubate in a water bath at 30 °C for 15 minutes.
3. After the 15 minute incubation, remove 5 µL and add it to the following reaction components on ice, bringing the initial reaction volume up to 20 µL.

Reaction Component	Amount
Reaction Mixture	5 µL
MBP Substrate (1 mg/mL; on ice)	5 µL
Distilled water (2-8 °C)	10 µL

4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled water.
5. Thaw the [^{32}P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area. Initiate the reaction with the addition of 5 µL [^{32}P]-ATP Assay Cocktail, bringing the final volume up to 25 µL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
6. After the 15 minute incubation, terminate the reaction by spotting 20 µL of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10 mL of phosphoric acid to 990 mL of distilled water to make a 1L solution) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
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 (step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [^{32}P]-ATP Specific Activity (SA) (cpm/pmol)

Specific Activity (SA) = cpm for 5 µL [^{32}P]-ATP / pmole of ATP (in 5 µL of a 250 µM ATP stock solution, i.e. 1250 pmoles)

Calculation of Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{32}P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in µg or mg)] x [(Reaction volume) / (Spot Volume)]