Recombinant Human Active MEK1 Kinase

Catalog Number: 11584-ME

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived human MEK1 protein Met1-Val393 with an N-terminal GST tag Accession # Q02750.2
N-terminal Sequence Analysis	Ser of GST-tag
Predicted Molecular Mass	69 kDa

SPECIFICATIONS

SDS-PAGE	62-68 kDa, under reducing conditions
Activity	Measured by its ability to hydrolyze the 5'-phosphate groups from the substrate adenosine-5'-triphosphate (ATP). The orthophosphate product is measured by a Malachite Green Phosphate Detection Kit (Catalog # DY996). The specific activity is >7 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 20 mM $MgCl_2$, 5 mM $MnCl_2$, 0.1 mg/ml BSA, pH 7.5
- Recombinant Human MEK1 (rhMEK1) (Catalog # 11584-ME)
- Adenosine triphosphate (ATP), 10 mM stock in deionized water
- Malachite Green Phosphate Detection Kit (Catalog # DY996)
- Clear 96-well Plate (Catalog # DY990)
- Plate Reader with Absorbance Read Capability

Assay

1. Prepare a standard curve from the 1 M Phosphate Standard (supplied in kit) by combining 10 µL of the 1 M Phosphate Standard to 990 µL of Assay Buffer for a 10 mM stock. Then, combine 10 µL of the 10 mM phosphate stock to 990 µL of Assay Buffer for a 100 µM stock. This is the first dilution to use as a standard.
2. Continue standard curve by performing six one-half serial dilutions of the 100 µM phosphate stock in Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
3. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
4. Dilute ATP to 400 µM in Assay Buffer.
5. Dilute rhMEK1 to 20 µg/mL in Assay Buffer.
6. Load 25 µL of 20 µg/mL rhMEK1 into the plate. Include a Control containing 25 µL of Assay Buffer.
7. Start the reaction by adding 25 µL of 400 µM ATP to the wells, excluding the standard curve and curve blank.
8. Seal plate and incubate at 37 °C for 3 hours.
9. Add 30 µL of the Malachite Green Reagent A (supplied in kit) to all wells. Mix briefly.
10. Add 100 µL of deionized water to all wells. Mix briefly.
11. Add 30 µL of the Malachite Green Reagent B (supplied in kit) to all wells. Mix briefly and incubate for 20 minutes at room temperature.
12. Read plate at 620 nm (absorbance) in endpoint mode.
13. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control

Final Assay Conditions

Per Reaction:

- rhMEK1: 0.5 µg
- ATP: 200 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

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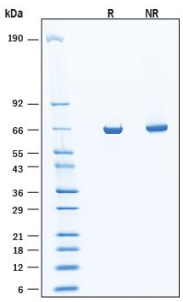
DATA

SDS-PAGE



Recombinant Human Active MEK1 Kinase Protein SDS-PAGE. 2 µg/lane of Recombinant Human Active MEK1 Kinase Protein (Catalog # 11584-ME) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 62-68 kDa, under reducing conditions.

BACKGROUND

MEK1 (MAPK/ERK kinase 1), also referred to as dual specificity mitogen-activated protein kinase kinase 1 (MAP2K1, MAPKK1) and ERK activator kinase 1 (ERK kinase 1), is a widely expressed cytoplasmic kinase from the protein kinase MAP kinase family. MEK1 and MEK2 are dual specificity enzymes that can phosphorylate threonine and tyrosine residues in their substrates (1,2). Although they are 86% identical in their catalytic domain, there is evidence that they may be regulated differentially (2-4). MEK1 contains an N-terminus with an inhibitory segment, a kinase domain with an ATP-binding site and a unique inhibitor binding pocket adjacent (5), and a C-terminal region. MEK1 is activated through its phosphorylation by upstream kinases such as RAF kinases which leads to conformational changes in the kinase that allow it to phosphorylate ERK1/2 with high substrate specificity (2,6). As a critical component of the MAP kinase signal transduction pathway, MEK1 participates in signaling cascades that transmit a variety of extra- and intracellular signals to mediate diverse biological functions such as cell growth, adhesion, survival, and differentiation through regulation of transcription, metabolism, and cytoskeletal rearrangements (2,6,7). Constitutive activation of MEK1 was shown to stimulate cell proliferation directly and mitigate dependence on growth factors (2,8). Significant research has been performed to discover inhibitors of MEK1 as important research tools to facilitate understanding of ERK biology and also to target MEK1 for pharmacological intervention in cancer therapy and other diseases associated with MEK1 and MAP kinase pathway dysfunction (2,6,9-11).

References:

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