Recombinant Human Active MEK2 Kinase His-tag

Catalog Number: 11825-ME

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived human MEK2 protein Leu2-Val400, with an N-terminal Met and 6-His tag Accession # P36507.1
N-terminal Sequence Analysis	Protein identity confirmed by mass spectrometry
Predicted Molecular Mass	45 kDa

SPECIFICATIONS

SDS-PAGE	43-47 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 Active MEK2 Kinase His-tag (rhMEK2) (Catalog # 11825-ME)
- Substrate: 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 rhMEK2 to 40 µg/mL in Assay Buffer.
6. Load 25 µL of 40 µg/mL rhMEK2 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:

- rhMEK2: 1.0 µg
- ATP: 200 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. 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.

DATA

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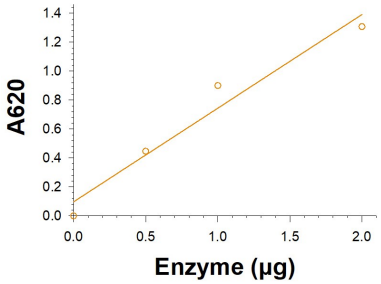
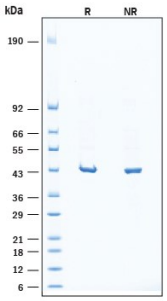
Enzyme Activity



Recombinant Human Active MEK2 Kinase His-tag Enzyme Activity. Recombinant Human Active MEK2 Kinase His-tag (Catalog # 11825-ME) is 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).

SDS-Page



Recombinant Human Active MEK2 Kinase His-tag SDS-PAGE. 2 µg/lane of Recombinant Human Active MEK2 Kinase His-tag (Catalog # 11825-ME) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 43-47 kDa, under reducing conditions.

BACKGROUND

MEK2 (MAPK/ERK kinase 2), also referred to as dual specificity mitogen-activated protein kinase kinase 2 (MAP2K2, MAPKK2) and ERK activator kinase 2 (ERK kinase 2), 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 hundreds of substrates within the RAS-MAPK-ERK pathway (1-3). Like, MEK1, MEK2 contains an N-terminus with an ERK binding domain, nuclear export sequence and a regulatory domain, a kinase domain with a P loop, ATP-binding site, activation loop, and proline rich domain, and a C-terminal region with a docking domain for binding to upstream kinases (3, 4). Although MEK1 and MEK2 are highly conserved and 86% identical in their catalytic domain, they diverge in their N-terminal and proline rich domains which contribute to distinct biological function through differing regulation (2, 3, 5, 6). Activation of both MEKs occurs through phosphorylation by upstream kinases, such as RAF kinases, which causes conformational changes in the kinase that allow it to phosphorylate ERK1/2 with high substrate specificity (3). Pathogenic variants in the MEK2 gene cause a gain of function in the RAS-MAPK pathway and result in a rare genetic condition called cardiofaciocutaneous syndrome type 4 (CFC4) (7). In addition, as a critical component of the MAP kinase signal transduction pathway, MEK2 participates in signaling cascades that transmit a variety of extra- and intracellular signals to mediate diverse biological functions such as cell growth, proliferation, survival, and differentiation (2, 3) frequently found to be dysregulated in cancers such as colorectal, pancreatic, endometrial, thyroid, and lung that involve BRAF and KRAS mutations (3). Significant research has been performed to discover targeted and combination small molecule and antibody inhibitors of MEK proteins as research tools to facilitate a better understanding of ERK biology and also to target MEK proteins for pharmacological intervention in cancer therapy and other diseases associated with MEK and MAP kinase pathway dysfunction (2, 3, 8-10).

References:

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