Store at –80°C

#7338

NEK2 Kinase

☑5 µg

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Description: Purified recombinant full-length human NEK2 kinase, supplied as a GST fusion protein.

Background: The NEK family of protein kinases is composed of 11 members in humans that share an amino-terminal catalytic domain related to NIMA, a serine/threonine kinase identified in *Aspergillus nidulans*. While NIMA is critical for cell cycle progression in fungus, the function of NEKs in mammalian cells is largely unknown. NEK1 was first identified by screening mouse cDNA expression libraries and was demonstrated to have dual kinase activity on both tyrosine and serine/threonine sites (1). NEK2 most closely resembles fungal NIMA in its primary structure and is believed to promote the splitting of duplicated centrosomes at the onset of mitosis (2,3). NEK3 is predominantly a cytoplasmic enzyme and its activity shows marginal variation throughout the cell cycle (4). NEK4 is ubiquitously expressed and its expression and subcellular location are not associated with cell cycle (5). NEK6/7 have been suggested to phosphorylate and activate p70 S6 kinase *in vitro* (6). Expression of an inactive NEK6 mutant arrests cells in M phase and interferes with chromosome segregation (7). NEK8 activity is not cell cycle regulated and may play a role in cell cycle independent microtubule dynamics (8). NEK9 is activated during mitosis and may participate in the activation of NEK6/7 during mitosis (9,10).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length human NEK2 (Met1-Arg445) (GenBank Accession No. NM_002497) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

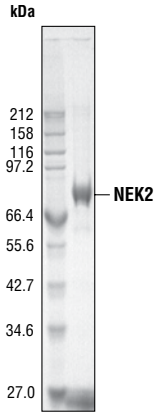

Figure 1. The purity of the NEK2 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the NEK2 fusion protein is 76 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. NEK2 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

(1) Letwin, K. et al. (1992) *EMBO J.* 11, 3521–3531.

(2) Fry, A.M. et al. (1995) *J. Biol. Chem.* 270, 12899–12905.

(3) Fry, A.M. (2002) *Oncogene* 21, 6184–6194.

(4) Tanaka, K. and Nigg, E.A. (1999) *J. Biol. Chem.* 274, 13491–13497.

(5) Hayashi, K. et al. (1999) *Biochem. Biophys. Res. Commun.* 264, 449–456.

(6) Belham, C. et al. (2001) *Curr. Biol.* 11, 1155–1167.

(7) Yin, M.J. et al. (2003) *J. Biol. Chem.* 278, 52454–52460.

(8) Holland, P.M. et al. (2002) *J. Biol. Chem.* 277, 16229–16240.

(9) Belham, C. et al. (2003) *J. Biol. Chem.* 278, 34897–34909.

(10) Roig, J. et al. (2002) *Genes Dev.* 16, 1640–1658.

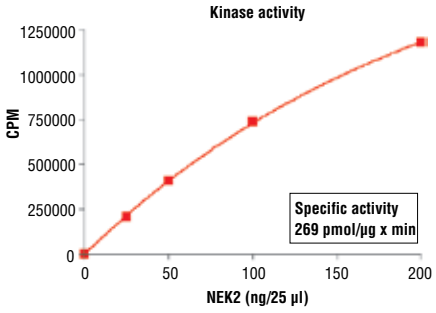

Figure 2. NEK2 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM $MgCl_2$, 0.05 mM DTT, 50 µM ATP, Substrate: MBP 400 ng/µL, and variable amounts of recombinant NEK2.

Storage: Enzyme is 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, 25% glycerol, 7 mM glutathione.
Store at –80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:
HTScan® NEK2 Kinase Assay Kit #7555

CREB (Ser133) Biotinylated Peptide #1331

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

Protocol for NEK2 Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. **Kinase Buffer (10X)**
   50 mM MOPS, pH 7.2
   25 mM β-glycerophosphate
   10 mM EGTA
   4 mM EDTA
   50 mM $MgCl_2$
   0.5 mM DTT
2. ATP (10 mM) #9804
3. ^{32}P-γATP
4. Myelin basic protein (1 µg/µl)

B Suggested Protocol

1. Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 µM ATP.
2. Dilute [^{32}p] ATP to 0.16 µCi/µl [^{32}p] ATP with 250 µM ATP solution.
3. Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
4. Dilute NEK2 protein to 20 ng/µl with 1X assay buffer followed by 2-fold serial dilutions.
5. To start the reaction combine 10 µl diluted NEK2 kinase solution, 10 µl MBP (1.0 µg/µl), and 5 µl 0.16 µCi/µl [^{32}P] ATP solution.

Final Assay Conditions
5 mM MOPS, pH 7.2
2.5 mM β-glycerophosphate
1 mM EGTA
0.4 mM EDTA
5 mM $MgCl_2$
0.05 mM DTT
400 ng/µl MBP

6. After 15 minutes terminate reaction by spotting 20 µl of the reaction mixture onto phosphocellulose P81 paper.
7. Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
8. Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
9. Count samples in a scintillation counter.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: drugdiscovery@cellsignal.com.