NEK2 (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPN03

Lot No. K002-2
5 µg 0.1 µg/µl

Background:

NEK 2 is closely related in its catalytic domain to the serine/threonine protein kinase NIMA of Aspergillus nidulans that is required for entry into mitosis and may function in parallel to the universal mitotic inducer p34cdc2. Like NIMA, the NEK2 protein is almost undetectable during G1 but accumulated progressively throughout S, reaching maximal levels in late G2 (1). NEK2 is shown to be expressed most abundantly in the testis of the adult tissues examined being localized to the nucleus (2).

Product Description:

Recombinant full-length human NEK2 was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_002497.

Gene Aliases:

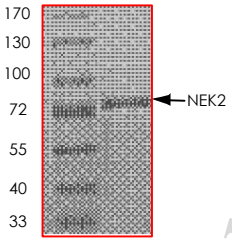
NLK1; HsPK21

Formulation:

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

Purity & Molecular Weight:

The purity was determined to be >90% by densitometry. Approx. MW 76kDa.



Storage:

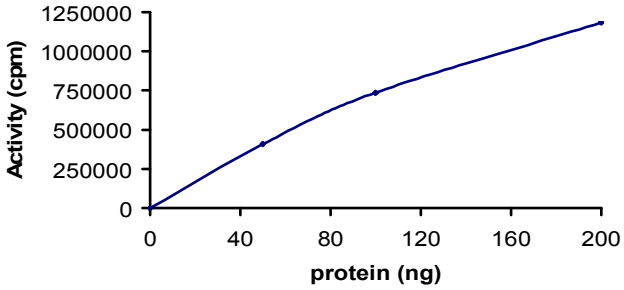
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

Unopened vial at –70 °C, 1 year from date of shipment.

Specific Activity:

The specific activity was determined to be 269 nmol/min/mg as per Activity Assay Protocol.



Activity Assay Protocol:

Assay activity of the kinase in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 10 µL of 1 mg/ml the Substrate Solution, 5 µL of diluted kinase and 5 µL of 250 µM ATP solution containing [gamma ^{32}P] ATP (0.167 µCi/µL). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 µL of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

Substrate Solution:

Myelin basic protein (MBP) diluted in distilled H_2O to a final concentration of 1 mg/ml.

5 X Kinase Assay Buffer:

25mM MOPS, 12.5mM β-glycerol-phosphate, 25mM $MgCl_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

1.Schultz, SJ. et al: A. Cell cycle-dependent expression of Nek2, a novel human protein kinase related to the NIMA mitotic regulator of Aspergillus nidulans. Cell Growth Differ. 1994 Jun;5(6):625-35.

2.Fry, AM. et al: Substrate specificity and cell cycle regulation of the Nek2 protein kinase, a potential human homolog of the mitotic regulator NIMA of Aspergillus nidulans. J Biol Chem. 1995 May 26; 270(21):12899-905

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