NEK7 (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPN09

Lot No. A272-1
5 µg 0.1 µg/µl

Background:

NEK7 is a member of the NIMA family of serine/threonine kinases. In contrast to the other documented NIMA-related kinases, NEK7 harbor its catalytic domain in the C-terminus of the protein. Immunofluorescence studies suggest that NEK7 is cytoplasmic and located on chromosome 1 (1). The major protein kinase that is active on the p70 S6 kinase hydrophobic regulatory site (FXXFS/TF/Y) Thr412, was purified from rat liver and identified as NEK7 (2). NEK7 kinase activity is rapidly and efficiently increased by serum deprivation, and may be regulated in a cell cycle-dependent manner.

Product Description:

Recombinant full-length human NEK7 was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_133494.

Gene Aliases:

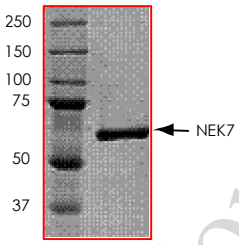
None

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 63kDa.



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:

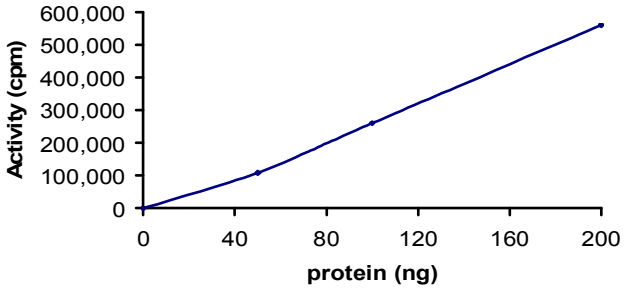
Unopened vial at –70 °C, 1 year from date of shipment.

For Reference Purpose Only!

For Research Use Only, Not for use in diagnostic procedures

Specific Activity:

The specific activity was determined to be 221 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:

Casein substrate 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.Kandli, M. et al: Isolation and characterization of two evolutionarily conserved murine kinases (Nek6 and nek7) related to the fungal mitotic regulator, NIMA. Genomics. 2000 Sep 1;68(2):187-96.

2.Belham, C. et al: Identification of the NIMA family kinases NEK6/7 as regulators of the p70 ribosomal S6 kinase. Curr Biol. 2001 Aug 7;11(15):1155-67

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