

NEK6 (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPN08

Lot No. A102-1
5 µg 0.1 µg/µl

Background:

NEK6 is a serine/threonine kinase that is a member of the Nek family of protein kinases that share an amino-terminal catalytic domain related to NIMA family (1). Nek6 is a nuclear and cytoplasmic kinase that is required for mitotic progression of human cells. Nek6 is phosphorylated and activated during M phase of the cell cycle (2). Inhibition of Nek6 function by either overexpression of an inactive Nek6 mutant or elimination of endogenous Nek6 by siRNA arrests cells in M phase and triggers apoptosis.

Product Description:

Recombinant full-length human NEK6 was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_014397.

Gene Aliases:

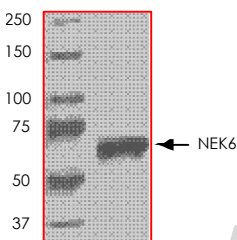
SID6-1512

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:

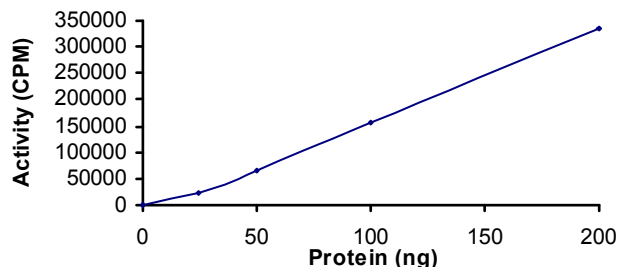
Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

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

**Specific Activity:**

The specific activity was determined to be 153 nmol/min/mg as per Activity Assay Protocol.

**Activity Assay Protocol:**

Assay activity of the kinase in a 25 μ L reaction consisting of 5 μ L of 5 X Kinase Assay Buffer, 10 μ L of 1 mg/ml the Substrate Solution, 5 μ L of diluted kinase and 5 μ L of 250 μ M ATP solution containing [γ - 32 P] ATP (0.167 μ Ci/ μ L). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 μ L 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 H₂O to a final concentration of 1 mg/ml.

5 X Kinase Assay Buffer:

25mM MOPS, 12.5mM β -glycerol-phosphate, 25mM MgCl₂, 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.Yin, MJ. Et al: The serine/threonine kinase Nek6 is required for cell cycle progression through mitosis. J Biol Chem. 2003 Dec 26;278(52):52454-60.

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