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Product Specification

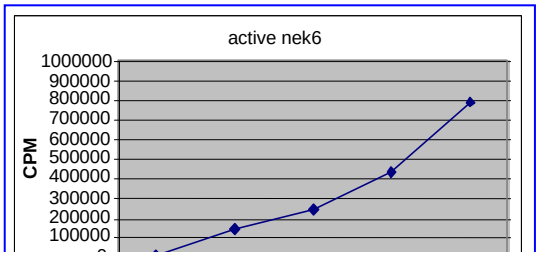
Nek6, active
(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7716
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 153 nmol/min/mg

Quality Control Analysis
Activity assessment
Nek6 protein (100 ng/µl concentration) was diluted to 20ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the myelin basic protein (MPB) in the following assay condition:

10 µl Diluted Nek6 protein
5 µl MBP (5 mg/ml stock)
5 µl water
5 µl [^{32}P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [^{32}P] ATP, were incubated at 30° C and the reaction started by the addition of [^{32}P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.



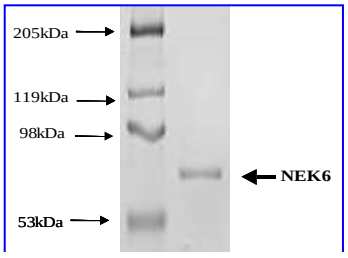


Fig. 1 NEK6 activity assay

Fig. 2 NEK6 protein gel

Purity assessment
1 µg of Nek6 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the Nek6 product, and the band was at ~63 kDa (Fig. 2).
Product Description

Recombinant full length human Nek6 containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells. The gene accession number is NM_014397.
This material is sold for research purposes only.

Specific Activity
153 nmol phosphate incorporated into MBP per minute per mg protein at 30° C for 15 minutes using a final concentration of 50 µM ATP (0.83 µCi/assay).

Formulation
Recombinant protein in storage buffer (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).

Storage and Stability
Store product frozen at or below -70° C. Stable for 1 year at -70° C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific 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 (never in mitosis, gene A) 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 suggesting that Nek6 is required for metaphase-anaphase transition. Nercc1/Nek9 binds to Nek6 and is likely to be responsible for the activation of Nek6 during mitosis representing a new signaling pathway that regulates mitotic progression (3). Northern blot analysis shows that Nek6 transcript is ubiquitously expressed with the highest expression found in the heart and skeletal muscle. Nek6 effectively phosphorylates histones H1 and H3, but not casein suggesting that, unlike other mammalian NIMA-related kinases, Nek6 is a mitotic histone kinase which regulates chromatin condensation in mammalian cells. In addition, Nek6 phosphorylates p70 S6 kinase at Thr412 and other sites and activates the p70 S6 kinase in vitro and in vivo, in a manner synergistic with PDK1 (4). Kinase-inactive Nek6 interferes with insulin activation of p70 S6 kinase implicating Nek6 as a possible physiologic regulator of the p70 S6 kinase.

References
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2. Yin MJ, Shao L, Voehringer D, Jallal B. *The serine/threonine kinase Nek6 is required for cell cycle progression through mitosis.* J Biol Chem. 2003 Dec 26;278(52):52454-60.
3. Roig J, Mikhailov A, Belham C, Avruch J. *Nercc1, a mammalian NIMA-family kinase, binds the Ran GTPase and regulates mitotic progression.* Genes Dev. 2002 Jul 1;16(13):1640-58.
4. Belham C, Comb MJ, Avruch J. Identification of the NIMA family kinases NEK6/7 as regulators of the p70 ribosomal S6 kinase.
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