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Recombinant full length human NEK7 kinase containing N-terminal GST tag

Catalog No.	CRN101A CRN101B	**Quantity:**	5 µg 20 µg

Description: Recombinant full length human NEK7 containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.

Nek7 is a member of the NIMA (never in mitosis, gene A) 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. During early embryogenesis Nek7 is expressed in the site of decidual reaction while later in embryogenesis, it is almost exclusively restricted to the nervous system in the dorsal thalamus. 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. Nek7 kinase activity is rapidly and efficiently increased by serum deprivation, and may be regulated in a cell cycle-dependent manner.

Concentration: 0.1 mg/ml

Gene Accession No.: NM_133494

Source: Sf9 insect cells

Formulation: Recombinant protein in storage buffer (50 mM Tris-HCl + 150 mM NaCl + 0.25 mM DTT + 0.1 mM EGTA + 0.1 mM EDTA + 0.1 mM PMSF + 25% glycerol; pH 7.5).

Purity: 3 µg of NEK7 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the NEK7 product, and the band was at ~63 kDa (Fig. 2).

Specific Activity: 221 nmol/min/mg: 221 nmol phosphate incorporated into β-casein per minute per mg protein minutes using a final concentration of 50 µM ATP (0.83 µCi/assay). See QA/QC section for details.

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

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QA/QC:

NEK7 protein (100 ng/µl concentration) was diluted to 20 ng/µl with assay dilution buffer (4 mM MOPS + 2.5 mM β-glycerophosphate + 1 mM EGTA + 0.4 mM EDTA + 4 mM $MgCl_2$ + 0.05 mM DTT; pH 7.2), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate β-casein in the following assay condition:

- 10 µl diluted NEK7 protein
- 10 µl β-casein (1 mg/ml stock)
- 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 30ºC 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 NEK7 activity assay

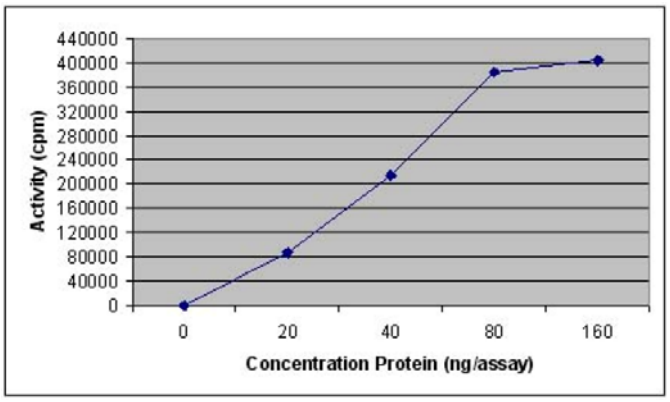
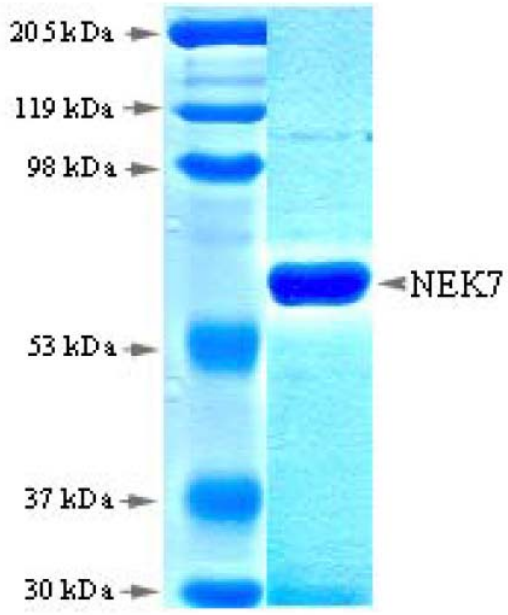


Fig. 2 NEK7 protein gel



Please note: always centrifuge vials before opening.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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