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Product Specification

NEK7, active
(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7717
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 221 nmol/min/mg

Quality Control Analysis
Activity assessment
NEK7 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 10 µl 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 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.

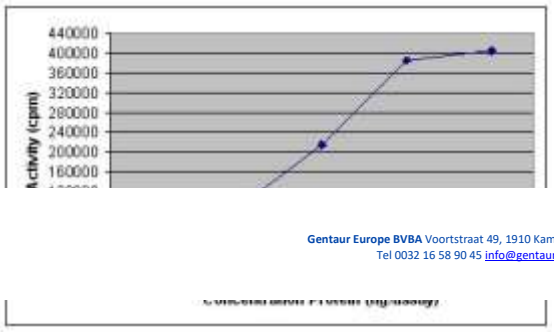

Fig.1 NEK7 activity assay

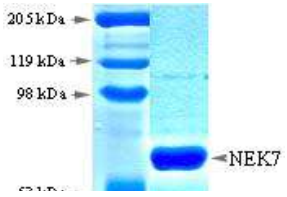

Fig. 2 NEK7 protein gel

Purity assessment

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)

Product Description
Recombinant full length human NEK7 containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.
The gene accession number is NM_133494.
This material is sold for research purposes only.

Specific Activity
221 nmol phosphate incorporated into β-casein 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
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 (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 (2). 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 (3). Nek7 kinase activity is rapidly and efficiently increased by serum deprivation, and may be regulated in a cell cycle-dependent manner.

References
1. Kandli M, Feige E, Chen A, Kilfin G, Motro B. 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. Feige E, Motro B. The related murine kinases, Nek6 and Nek7, display distinct patterns of expression. Mech Dev. 2002 Jan;110(1-2):219-23.
3. Belham C, Comb MJ, Avruch J. 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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