

Product Specification

Nek2, active

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

Catalog #: 7719-5
 Lot #: _____
 Aliquot size: 5 µg protein in 50 µl
 Specific activity: 183 nmol/min/mg

Quality Control Analysis

Activity assessment

Nek2 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 MgCl₂, 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 Nek2 protein
- 5 µl MBP (5 mg/ml stock)
- 5 ul water
- 5 µl [³²P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30⁰C and the reaction started by the addition of [³²P] 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.

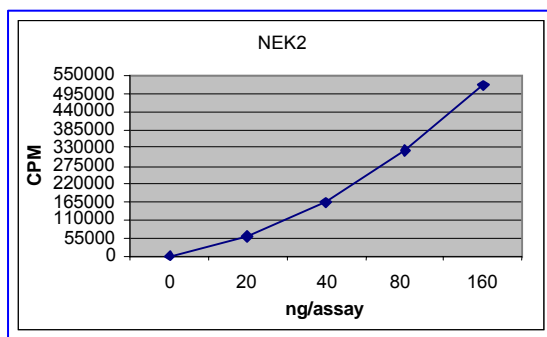


Fig. 1 NEK2 activity assay

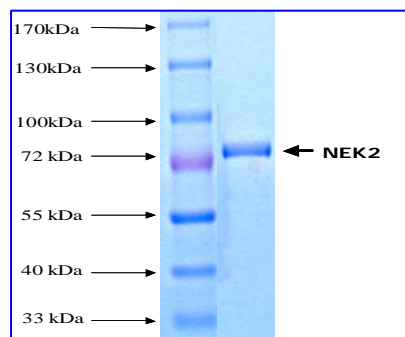


Fig. 2 NEK2 protein gel

Purity assessment

0.5 µg of Nek2 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the NEK2 product, and the band was at ~76 kDa (Fig. 2).

Product Description

Recombinant full length human Nek2 containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.

The gene accession number is NM_002497.

This material is sold for research purposes only.

Specific Activity

183 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

Nek 2 is closely related in its catalytic domain to the serine/threonine protein kinase NIMA of *Aspergillus nidulans* that is required for entry into mitosis and may function in parallel to the universal mitotic inducer p34cdc2. Like NIMA, the Nek2 protein is almost undetectable during G1 but accumulated progressively throughout S, reaching maximal levels in late G2 (1). These observations demonstrate that Nek2 resembles *Aspergillus* NIMA, not only in its catalytic domain, but also in its cell cycle-dependent expression. Recombinant Nek2 is active as a serine/threonine-specific protein kinase and may undergo autophosphorylation. Both human Nek2 and fungal NIMA phosphorylate a similar, albeit not identical, set of proteins and synthetic peptides, and beta-casein is a suitable substrate for assaying Nek2 in vitro (2). Nek2 is shown to be expressed most abundantly in the testis of the adult tissues examined (3). Its expression in the testis is restricted to the germ cells, with highest levels detected in spermatocytes at pachytene and diplotene stages. Immunohistochemical analysis revealed that Nek2 localized to nuclei, exhibiting a non-uniform distribution within the nucleus

References

1. Schultz SJ, Fry AM, Sutterlin C, Ried T, Nigg EA. Cell cycle-dependent expression of Nek2, a novel human protein kinase related to the NIMA mitotic regulator of *Aspergillus nidulans*. *Cell Growth Differ.* 1994 Jun;5(6):625-35.
2. Fry AM, Schultz SJ, Bartek J, Nigg EA. Substrate specificity and cell cycle regulation of the Nek2 protein kinase, a potential human homolog of the mitotic regulator NIMA of *Aspergillus nidulans*. *J Biol Chem.* 1995 May 26; 270(21):12899-905.
3. Rhee K, Wolgemuth DJ. The NIMA-related kinase 2, Nek2, is expressed in specific stages of the meiotic cell cycle and associates with meiotic chromosomes. *Development.* 1997 Jun; 124(11):2167-77.