

Catalogue #	Aliquot Size
<b>N05-10G-05</b>	<b>5 µg</b>
<b>N05-10G-10</b>	<b>10 µg</b>
<b>N05-10G-20</b>	<b>20 µg</b>

## NEK3, Active

Full-length recombinant protein expressed in Sf9 cells

**Catalog # N05-10G**

Lot # I352-2

### Product Description

Recombinant full-length human NEK3 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM\\_002498](#).

### Gene Aliases

HSPK36, MGC29949

### Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

### Storage and Stability

Store product at  $-70^{\circ}\text{C}$ . 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.

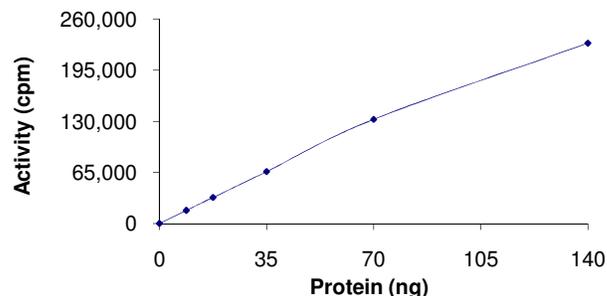
### Scientific Background

NEK3 is a member of the NEK family of protein kinases that share high amino acid homology with NIMA (never in mitosis gene a). NEK3 mRNA is detected in all the proliferating cell lines with the amount not changing during the cell cycle (1). During Prolactin receptor signaling, VAV2 is phosphorylated and activated by NEK3. Overexpression of NEK3 in Chinese hamster ovary cells increases cytoskeletal re-organization in response to Prolactin while downregulation of NEK3 expression by siRNA block these effects (2). Prolactin also stimulates interaction between NEK3 and paxillin leading to increased paxillin phosphorylation. Analysis of breast tissue microarrays show a significant up-regulation of NEK3 expression in malignant versus normal specimens.

### References

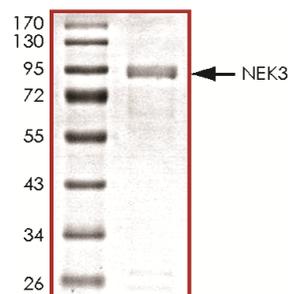
- Kimura, M. et al: Molecular cloning and characterization of the human NIMA-related protein kinase 3 gene (NEK3). *Cytogenet Cell Genet.* 2001;95(3-4):177-82.
- Miller, S.L. et al: Nek3 kinase regulates prolactin-mediated cytoskeletal reorganization and motility of breast cancer cells. *Oncogene.* 2007 Jul 12;26(32):4668-78.

### Specific Activity



The specific activity of NEK3 was determined to be **112 nmol /min/mg** as per activity assay protocol.

### Purity



The purity was determined to be **>90%** by densitometry. Approx. MW **86kDa**.

## NEK3, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	N05-10G
Specific Activity	112 nmol/min/mg
Specific Lot Number	I352-2
Purity	>90%
Concentration	0.1µg/µl
Stability	1yr At $-70^{\circ}\text{C}$ from date of shipment
Storage & Shipping	Store product at $-70^{\circ}\text{C}$ . 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. Product shipped on dry ice.

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# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: N05-10G)

Active NEK3 (0.1µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active NEK3 for optimal results).

### Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

### Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [<sup>33</sup>P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

### 10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at -20°C.

### Substrate (Catalog #: M42-51N)

Myelin basic protein (MBP) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active NEK3, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
  - Component 1.** 10µl of diluted Active NEK3 (Catalog #N05-10G)
  - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
  - Component 3.** 5µl distilled H<sub>2</sub>O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5µl [<sup>33</sup>P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H<sub>2</sub>O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

### Calculation of [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [<sup>33</sup>P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

### Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

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