

Catalogue #	Aliquot Size
N11-11G -05	5 µg
N11-11G -10	10 µg
N11-11G -20	20 µg

NEK9, Active

Human recombinant protein expressed in Sf9 cells

Catalog # N11-11G

Lot # W354-3

Product Description

Recombinant human NEK9 (1-979; deleted fragment of 346-731) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_033116](#).

Gene Aliases

NEK8, NERCC, NERCC1, MGC16714, MGC138306, DKFZp434D0935

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°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.

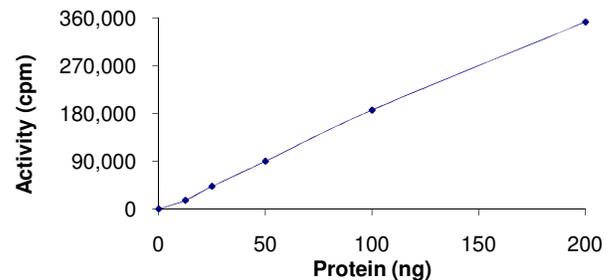
Scientific Background

NEK9 is a member of the NEK family and has high homology to NEK1, NEK3 and NEK4. NEK9 is activated during mitosis and binds specifically to RAN GTPase and is a substrate for CDC2 phosphorylation (1). NEK9 plays a role in the control of mitotic progression and is regulated by CDC2 and RAN GTPase. Overexpression of both active and inactive variants of NEK9 is toxic to cells and inhibits cell division causing abnormal nuclear morphologies. NEK9 can catalyze the phosphorylation of recombinant NEK6 and NEK7 in vitro leading to its activation. This suggests that NEK9 may be responsible for activation of NEK6 and NEK7 during mitosis (2).

References

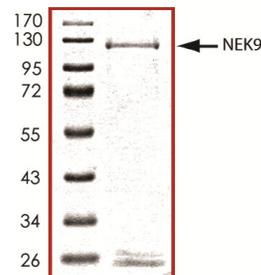
1. Roig J, et al: Nercc1, a mammalian NIMA-family kinase, binds the Ran GTPase and regulates mitotic progression. *Genes Dev.* 16: 1640-1658, 2002.
2. Belham C, et al: A mitotic cascade of NIMA family kinases: Nercc1/Nek9 activates the Nek6 and Nek7 kinases. *J. Biol. Chem.* 278: 34897-34909, 2003.

Specific Activity



The specific activity of NEK9 was determined to be **121 nmol /min/mg** as per activity assay protocol.

Purity



The purity of NEK9 was determined to be **>75%** by densitometry, approx. MW **115kDa**.

NEK9, Active

Human recombinant protein expressed in Sf9 cells

Catalog Number	N11-11G
Specific Activity	121 nmol/min/mg
Specific Lot Number	W354-3
Purity	>75%
Concentration	0.1 µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°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 #: N11-11G)

Active NEK9 (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 NEK9 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 50ng/µl BSA solution.

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

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following radioactive components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-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) substrate diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active NEK9, 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 NEK9 (Catalog #N11-11G)
 - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
 - Component 3.** 5µl distilled H₂O (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 H₂O.
- Step 5.** Initiate the reaction by the addition of 5 µl [³³P]-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 20 µl 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 H₂O) 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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

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

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

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

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