

Product Information

NEK5, active, GST-tagged, human PRECISIO® Kinase recombinant, expressed in Sf9 cells

Catalog Number **SRP5328**

Storage Temperature –70 °C

Synonym: MGC75495

Product Description

NEK5 or NIMA (never in mitosis gene a)-related kinase 5 is a serine/threonine kinase that is involved in cell cycle regulation. NEK5 has been shown to interact with NEK6, required for the *ibo1/nek6* ectopic outgrowth phenotype in epidermal cells.¹ NEK6 interacts and co-localizes with NEK5 in a transient expression assay. The *ibo1-3* mutation markedly increases the interaction between NEK6 and NEK5. NEK6 forms heterodimers with NEK5 to regulate cortical microtubule organization possibly through the phosphorylation of β-tubulins. The *NEK5* gene is conserved in chimpanzee, cow, mouse, rat, and chicken.

Recombinant human NEK5 (1-508) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM_199289. It is supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~84 kDa

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot:
≥70% (SDS-PAGE, densitometry)

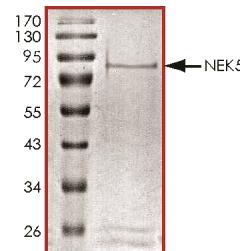
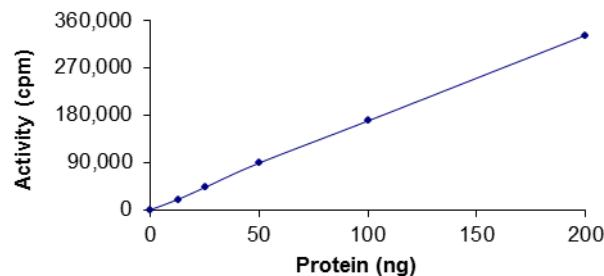


Figure 2.
Specific Activity of Typical Lot:
80–120 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl₂, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/μL BSA solution.

Kinase Solution – Dilute the active NEK5 (0.05 µg/µL) with Kinase Dilution Buffer to the desired concentration.

Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active NEK5 kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 mL of Kinase Assay Buffer. Store in 200 µL aliquots at –20 °C.

γ -³³P-ATP Assay Cocktail (250 µM) – Combine 5.75 mL of Kinase Assay Buffer, 150 µL of 10 mM ATP Stock Solution, 100 µL of γ -³³P-ATP (1 mCi/100 µL). Store in 1 mL aliquots at –20 °C.

Substrate Solution – Myelin basic protein (MBP) diluted in distilled water to a final concentration of 1 mg/mL.

1% phosphoric acid solution – Dilute 10 mL of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay

This assay involves the use of the ³³P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active NEK5, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ -³³P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 µL:
10 µL of Kinase Solution
5 µL of Substrate Solution
5 µL of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5 µL of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 µL of the γ -³³P-ATP Assay Cocktail, bringing the final reaction volume to 25 µL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 µL of the reaction mixture onto an individually pre-cut strip of phosphocellulose P81 paper.

6. Air dry the pre-cut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ -³³P-ATP counts introduced into the reaction. Spot 5 µL of the γ -³³P-ATP Assay Cocktail on a pre-cut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of } 5 \mu\text{L of } \gamma\text{-}^{33}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

cpm – value from control (step 7)
nmole – 1.25 nmole (5 µL of 250 µM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta \text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)
 Δ cpm = cpm of the sample – cpm of the blank (step 3)
25 = total reaction volume
20 = spot volume
T = reaction time (minutes)
E = amount of enzyme (mg)

References

1. Motose, H. et al., NIMA-related kinases 6, 4, and 5 interact with each other to regulate microtubule organization during epidermal cell expansion in *Arabidopsis thaliana*. *Plant J.*, **67**(6), 993-1005 (2011).

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