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Certificate of Analysis

NEK9, active
(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-936, 14-936-K, 14-936M

Parent Lot # D13HP007N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: *N*-terminal 6His-tagged, recombinant, human NEK9 amino acids 1-324 expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.
Purity 89.7% by SDS-PAGE and Coomassie blue staining. MW = 41kDa.

Specific Activity (Parent lot# D13HP007N): 6027U/mg, where one unit of NEK9, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml MBP per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.168mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 20mM β-glycerophosphate, 0.25mM sodium orthovanadate, 10mM NaF, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

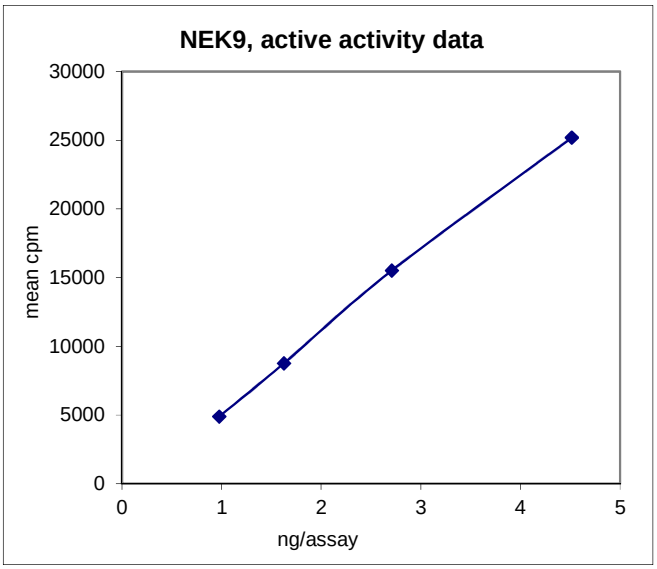
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

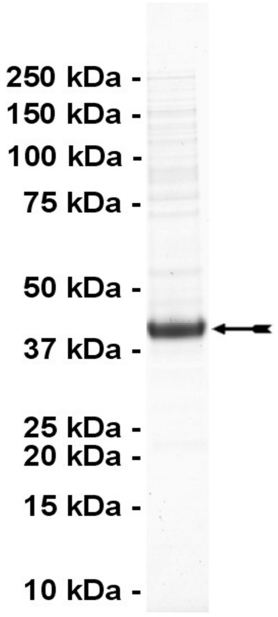
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 1–5ng of this lot of enzyme phosphorylated 0.33mg/ml MBP in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as NEK9 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of NEK9, active.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **MBP**: Use at a final assay concentration of 0.33mg/ml. Prepare a 3.33mg/ml stock and add 2.5µl of stock per assay point.
3. **Sodium Chloride**: Use at a final assay concentration of 100mM. Prepare a 3M stock in water. Use 0.83µl per assay point.
4. **Manganese Chloride**: Use at final assay concentration of 5mM. Prepare a 100mM stock in water. Use 1.25µl per assay point.
5. **Sodium orthovanadate**: Use at a final assay concentration of 1mM. Prepare a 100mM stock. Use 0.25µl per assay point.
6. **Sodium β-glycerophosphate:** Use at a final assay concentration of 5mM. Prepare a 1M stock and 0.125µl per assay point.
7. **NEK9, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1–5ng per assay point.
8. **[γ-^{33}P]ATP:** 2.5 x MgAc/[γ^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 0.83µl of 3M sodium chloride.
3. Add 1.25µl of 100mM manganese chloride.
4. Add 0.25µl 100mM sodium orthovanadate.
5. Add 0.125µl of 1M sodium β-glycerophosphate.
6. Add 2.5µl of MBP.
7. Add 2.54µl of dH_2O.
8. Add 2.5µl **(1–5ng) NEK9, active.**
9. Add 10µl of diluted [γ-^{33}P]ATP mixture.
10. Incubate for 10 minutes at 30°C.
11. Stop the reaction by adding 5µl of 3% phosphoric acid.
12. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat.**
13. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
14. Wash the filtermat once for 2 minutes with methanol.
15. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
16. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

NEK9 Sequence Information

Protein human NEK9

Tags *N*-terminal 6His

Native sequence M37 of the recombinant protein is equivalent to M1 of human NEK9

Accession number GenBank NM_033116

Recombinant NEK9 amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMSVL GEYERHCDSI NSDFGSESGG
  61 CGDSSPGPSA SQGPRAGGGA AEQEELHYIP IRVLGRGAFG EATLYRRTED DSLVVWKEVD
 121 LTRLSEKERR DALNEIVILA LLQHDNIIAY YNHFMDNTTL LIELEYCNGG NLYDKILRQK
 181 DKLFEEEMVV WYLFQIVSAV SCIHKAGILH RDIKTLNIFL TKANLIKLGD YGLAKKLNSE
 241 YSMAETLVGT PYYMSPELCQ GVKYNFKSDI WAVGCVIFEL LTLKRTFDAT NPLNLCVKIV
 301 QGIRAMEVDS SQYSLELIQM VHSCLDQDPE QRPTADELLD RPLLRKRRRE MEEKVTLLNA
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Recombinant NEK9 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatgga tccggaattc aaaggcctac gtcgacaaat gtcggtgctg
 121 ggcgagtacg agcgacactg cgattccatc aactcggact ttgggagcga gtccgggggt
 181 tgcggggact cgagtccggg gcctagcgcc agtcaggggc cgcgagccgg cggcggcgcg
 241 gcggagcagg aggaactgca ctacatcccc atccgcgtcc tgggccgcgg cgccttcggg
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1081 taa
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Reviewed and approved by site quality representative.