

Certificate of Analysis

NEK3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-694, 14-694-K, 14-694M

Parent Lot # D7EN058U

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, full length, human NEK3, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 84% by SDS-PAGE and Coomassie blue staining. MW = 62.2kDa.

Specific Activity (Parent lot# D7EN058U): 138U/mg, where one unit of NEK3, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.31mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

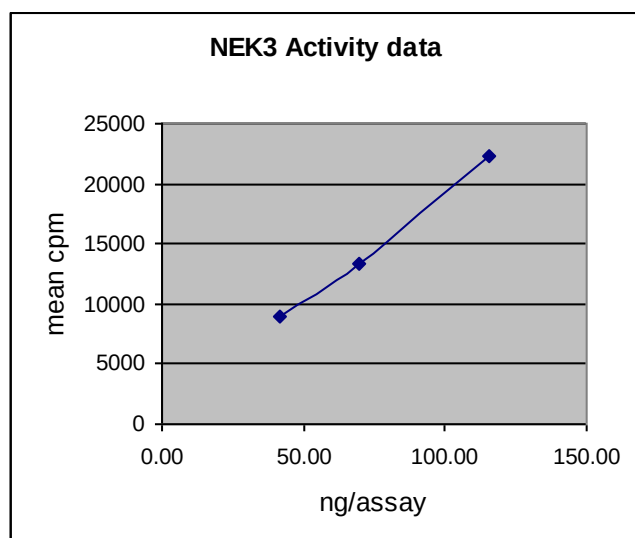
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

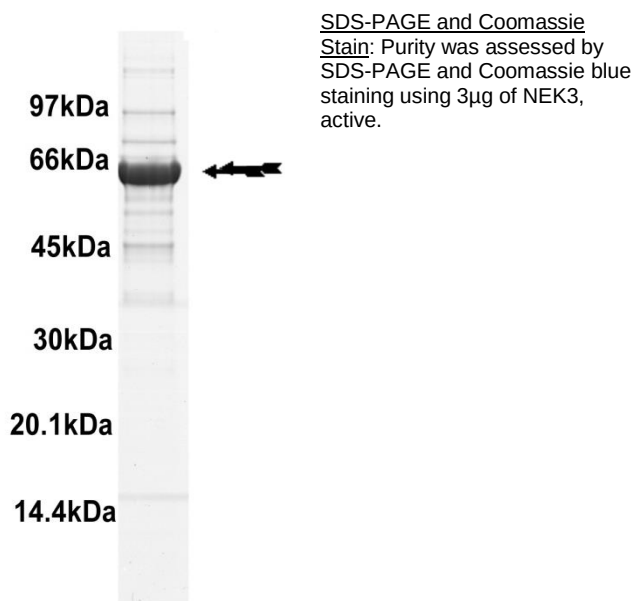
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 42–116ng 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 NEK3 with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **Myelin Basic Protein (MBP):** Use at a final concentration of 0.33mg/ml. Make a 3.33mg/ml stock. Add 2.5µl of stock per assay point.
3. **NEK3, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 42–116ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]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 2.5µl of 3.33mg/ml **MBP**.
3. Add **2.5µl (42–116ng) NEK3, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. 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.

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NEK3 Sequence Information

<u>Protein</u>	Human NEK3
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M37 of recombinant protein is equivalent to M1 of human NEK3
<u>Accession number</u>	GenBank NM_002498

Recombinant NEK3 amino acid sequence:

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1 MSYYHHHHH DYDIPTTENL YFQGAMDPEF KGLRRQMDDY MVLRMIGEGS FGRALLVQHE
61 SSNQMFAMKE IRLPKSFSNT QNSRKEAVLL AKMKHPNIVA FKESFEAEGH LYIVMEYCDG
121 GDLMQKIKQQ KGKLFPEDMI LNWFTQMCLG VNHIHKKRVL HRDIKSKNIF LTQNGKVKLG
181 DFGSARLLSN PMAFACTYVG TPYYVPPEIW ENLPYNNKSD IWSLGCILYE LCTLKHPFQA
241 NSWKNLILKV CQGCISPLPS HYSYELQFLV KQMFKRNP SH RPSATTLLSR GIVARLVQKC
301 LPPEIIMEYG EEVLEEIKNS KHNTPRKKTN PSRIRIALGN EASTVQEEEQ DRKGSHTDLE
361 SINENLVESA LRRVNREEKG NKSVHLRKAS SPNLHRRQWE KNVPNTALTA LENASILTSS
421 LTAEDDRGGS VIKYSKNTR KQWLKETPDT LLNILKNADL SLAFQTYTIY RPGSEGFLKG
481 PLSEETEASD SVDGGHDSVI LDPERLEPGL DEEDTDFEEE DDNPDWVSEL KKRAGWQGLC
541 DR

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Recombinant NEK3 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc aaaggcctac gtcgacaaat ggatgactac
121 atggctctga gaatgattgg ggagggctcc ttcggcagag ctcttttggg tcagcatgaa
181 agcagtaatc agatgtttgc catgaaagaa ataaggcttc ccaagtcttt ctctaataca
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301 ttcaaagaat catttgaagc tgaaggacac ttgtatatgg tgatggaata ctgtgatgga
361 ggggatctaa tgcaaaagat taaacagcag aaaggaaagt tatttcctga agacatgata
421 cttaattggg ttacccaaat gtgccttgga gtaaatacaca ttcacaagaa acgtgtgcta
481 cacagagata tcaagtccaa gaatatcttc ctactcaga atggaaaagt gaaattggga
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1321 aagcagtggc tcaaagagac ccctgacact ttgttgaaca tccttaagaa tgctgatctc
1381 agcttggtct tcaaacata cacaatatat agaccaggtt cagaagggtt cttgaaaggc
1441 ccctgtctg aagaacaga agcatcggac agtgttgatg gaggtcacga ttctgtcatt
1501 ttggatccag agcgacttga gcctgggcta gatgaggagg acacggactt tgaggaggaa

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1561 gatgacaacc ccgactgggt gtcagagctg aagaagcgag ctggatggca aggcctgtgc
1621 gacagataa

Reviewed and approved by site quality representative.

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