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Certificate of Analysis

NEK7, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-565, 14-565-K, 14-565M
Parent Lot # 1606071

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 NEK7, amino acids 2–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 95.2% by SDS-PAGE and Coomassie blue staining. MW = 37.9kDa.

Specific Activity (Parent lot# 1606071): 16U/mg, where one unit of NEK7 activity is defined as 1nmol phosphate incorporated into 300µM (FLAKSFGSPNRAYKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.213mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

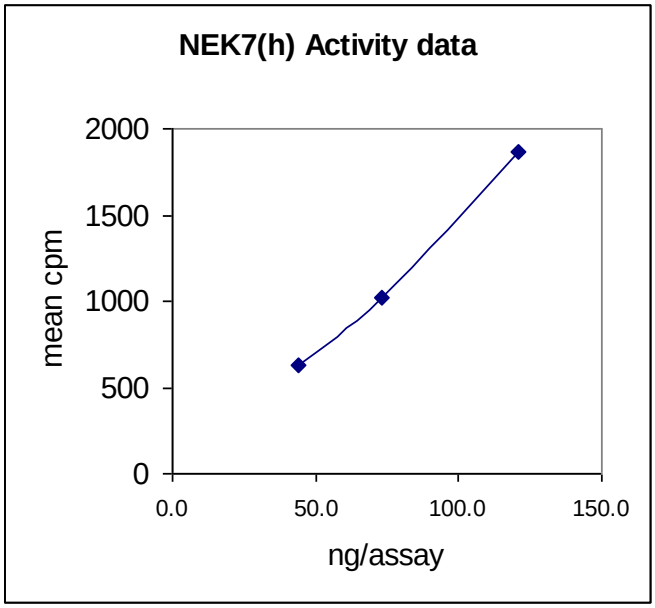
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

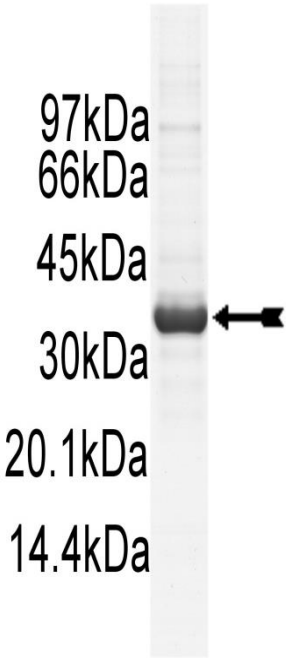
Quality Control Testing

Kinase Assay: 43.9–120.9ng of this lot of enzyme phosphorylated 300µM (FLAKSFGSPNRAYKK) 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 NEK7 with the translated native sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of NEK7, 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. **(FLAKSFGSPNRAYKK):** Use at a final assay concentration of 300µM. Prepare a 3mM stock and add 2.5µl of stock per assay point.
3. **NEK7, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 43.9–120.9ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[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 2.5µl of **(FLAKSFGSPNRAYKK)**.
3. Add **2.5µl (43.9–120.9ng) NEK7, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]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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NEK7 Sequence Information

Protein human NEK7

Tags N-terminal 6His

Native sequence D29 of the recombinant protein is equivalent to D2 of human NEK7

Accession number GenBank AB062450

Recombinant NEK7 amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMGSDE QSQGMQGPPV PQFQPQKALR PDMGYNTLAN
 61 FRIEKKIGRG QFSEVYRAAC LLDGVPVALK KVQIFDLMDA KARADCIKEI DLLKQLNHPN
121 VIKYYASFIE DNELNIVLEL ADAGDLSRMI KHFKKQKRLI PERTVWKYFV QLCSALEHMH
181 SRRVMHRDIK PANVFITATG VVKLGDLGLG RFFSSKTTAA HSLVGTPYYM SPERIHENGY
241 NFKSDIWSLG CLLYEMAALQ SPFYGDKMNL YSLCKKIEQC DYPPLPSDHY SEELRQLVNM
301 CINPDPEKRP DVTYVYDVAK RMHACTASS
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Recombinant NEK7 nucleotide sequence:

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  1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
 61 tattttcagg gcgccatggg atccgatgag caatcacaag gaatgcaagg gccacctgtt
121 cctcagttcc aaccacagaa ggccttacga ccggatatgg gctataatac attagccaac
181 tttcgaatag aaaagaaaat tggtcgcgga caatttagtg aagtttatag agcagcctgt
241 ctcttggatg gagtaccagt agctttaaaa aaagtgcaga tatttgattt aatggatgcc
301 aaagcacgtg ctgattgcat caaagaaata gatcttctta agcaactcaa ccatccaaat
361 gtaataaaat attatgcatc attcattgaa gataatgaac taaacatagt tttggaacta
421 gcagatgctg gcgacctatc cagaatgatc aagcatttta agaagcaaaa gaggctaatt
481 cctgaaagaa ctgtttggaa gtattttgtt cagctttgca gtgcattgga acacatgcat
541 tctcgaagag tcatgcatag agatataaaa ccagctaatg tgttcattac agccactggg
601 gtggtaaaac ttggagatct tgggcttggc cggtttttca gctcaaaaac cacagctgca
661 cattctttag ttggtacgcc ttattacatg tctccagaga gaatacatga aaatggatac
721 aacttcaaat ctgacatctg gtctcttggc tgtctactat atgagatggc tgcattacaa
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901 tgcatcaacc cagatccaga gaagcgacca gacgtcacct atgtttatga cgtagcaaag
961 aggatgcatg catgcactgc aagcagctaa
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Reviewed and approved by site quality representative.