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Certificate of Analysis

NEK6, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-578, 14-578-K, 14-578M
Parent Lot # D7KN057U

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 NEK6 amino acids 2–end, expressed in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 92% by SDS-PAGE and Coomassie blue staining. MW = 40.5kDa.

Specific Activity (Parent lot# D7KN057U): 110U/mg, where one unit of NEK6, active activity is defined as 1nmol phosphate incorporated into 300µM (FLAKSFGSPNRAYKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.322mg/ml of enzyme in 50mM Tris/HCl pH8.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

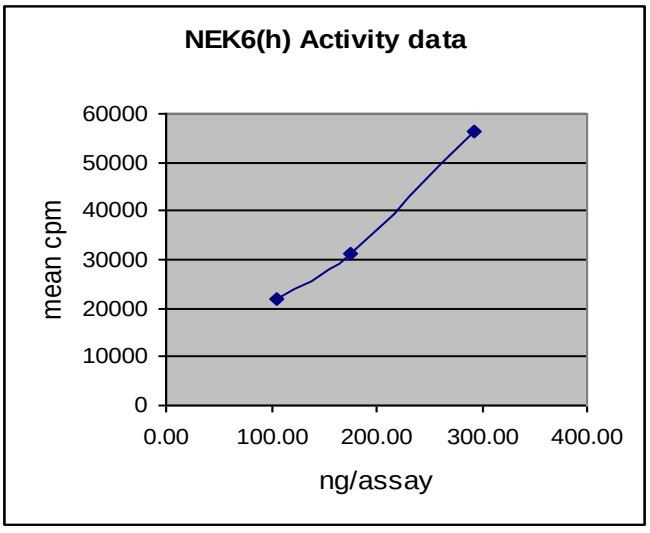
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

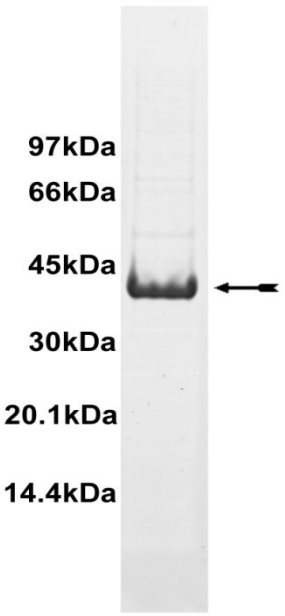
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 106–292ng 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 NEK6 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 NEK6, active.

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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. **NEK6, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 106–292ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.2mM 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 to wells.
2. Add 2.5µl of **(FLAKSFGSPNRAYKK)**.
3. Add **2.5µl (106–292ng) NEK6, 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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NEK6 Sequence Information

Protein Human NEK6

Tags *N*-Terminal 6His

Native sequence A41 of the recombinant protein is equivalent to A2 of human NEK6

Accession number GenBank NM_014397

Recombinant NEK6 amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMGIRN SKAYVDELTS AGQPGHMPHG GSSNNLCHTL
  61 GPVHPPDPQR HPNTLSFRCS LADFQIEKKI GRGQFSEVYK ATCLLDRKTV ALKKVQIFEM
 121 MDAKARQDCV KEIGLLKQLN HPNIIKYLDS FIEDNELNIV LELADAGDLS QMIKYFKKQK
 181 RLIPERTVWK YFVQLCSAVE HMHSRRVMHR DIKPANVFIT ATGVVKLGDL GLGRFFSSET
 241 TAAHSLVGTP YYMSPERIHE NGYNFKSDIW SLGCLLYEMA ALQSPFYGDK MNLFSLCQKI
 301 EQCDYPPLPG EHYSEKLREL VSMCICPDPH QRPDIGYVHQ VAKQMHIWMS ST
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Recombinant NEK6 nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatggg gatccggaat tcaaaggcct acgtcgacga gctcactagt
 121 gcaggacagc ccggccacat gccccatgga gggagttcca acaacctctg ccacaccctg
 181 gggcctgtgc atcctcctga cccacagagg catcccaaca cgctgtcttt tcgctgctcg
 241 ctggcggact tccagatcga aaagaagata ggccgaggac agttcagcga ggtgtacaag
 301 gccacctgcc tgctggacag gaagacagtg gctctgaaga aggtgcagat ctttgagatg
 361 atggacgcca aggcgaggca ggactgtgtc aaggagatcg gcctcttgaa gcaactgaac
 421 cacccaaata tcatcaagta tttggactcg tttatcgaag acaacgagct gaacattgtg
 481 ctggagttgg ctgacgcagg ggacctctcg cagatgatca agtactttaa gaagcagaag
 541 cggctcatcc cggagaggac agtatggaag tactttgtgc agctgtgcag cgccgtggag
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 661 gccacgggcg tcgtgaagct cggtgacctt ggtctgggcc gcttcttcag ctctgagacc
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 961 gtcagcatgt gcatctgccc tgaccccCAC cagagacctg acatcggata cgtgcaccag
1021 gtggccaagc agatgcacat ctggatgtcc agcacctga
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Reviewed and approved by site quality representative.