

## Certificate of Analysis

### NEK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-545, 14-545-K, 14-545M

Parent Lot # D9BN029U

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 NEK2, expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>/NTA. Purity 65.6% by SDS-PAGE and Coomassie blue staining. MW = 52.8kDa.

**Specific Activity (Parent lot# D9BN029U):** 1120U/mg, where one unit of NEK2, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100µM.

**Formulation:** 0.583mg/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.

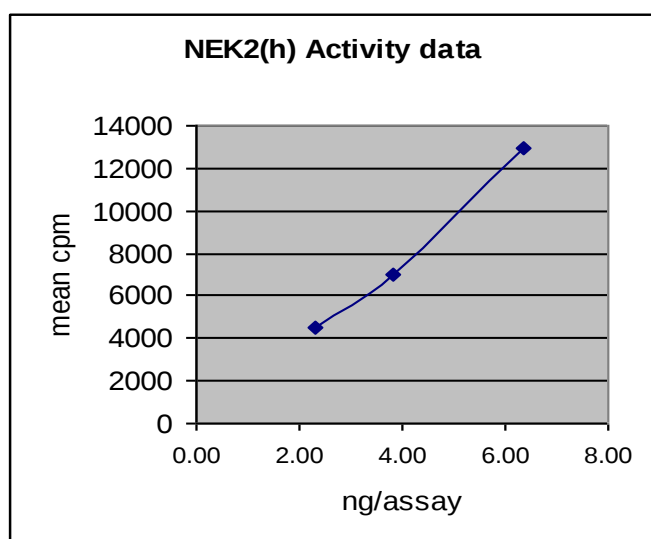
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

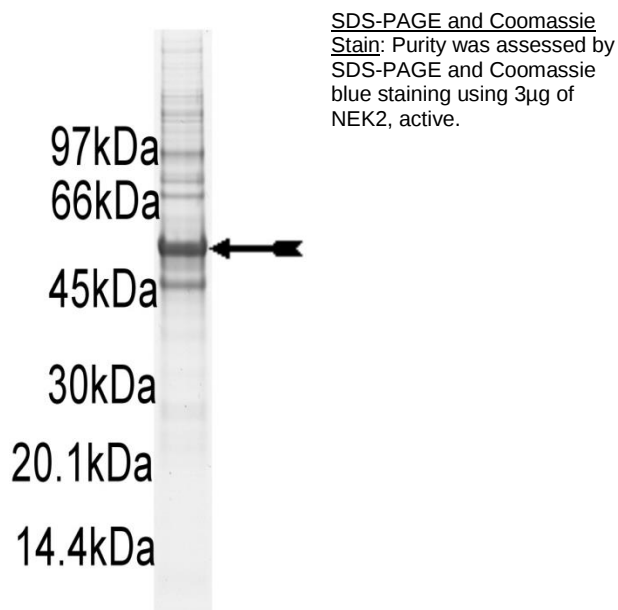
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 2.3–6.4ng 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 product identity NEK2 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 assay concentration of 0.33mg/ml. Prepare a 3.33mg/ml stock and add 2.5µl of stock per assay point.
3. **NEK2, active:** Dilute with 20mM MOPS/NaOH, 1mM EDTA, 0.01% Brij-35, 5 % glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2.3–6.4ng per assay point.
4. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>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 **myelin basic protein**.
3. Add **2.5µl (2.3–6.4ng) NEK2, active**.
4. Add 5µl of dH<sub>2</sub>O.
5. Add 10µl of diluted [ $\gamma$ -<sup>33</sup>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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## NEK2 Sequence Information

|                                |                                                                 |
|--------------------------------|-----------------------------------------------------------------|
| <b><u>Protein</u></b>          | Human NEK2                                                      |
| <b><u>Tags</u></b>             | N-terminal 6His                                                 |
| <b><u>Native sequence</u></b>  | M8 of the recombinant protein is equivalent to M1 of human NEK2 |
| <b><u>Accession number</u></b> | GenBank NM_002497                                               |

### Recombinant NEK2 amino acid sequence:

```

1  MHHHHHHMPS RAEDYEVLYT IGTGSYGRCQ KIRRKSDGKI LVWKELDYGS MTEAEKQMLV
61  SEVNLLRELK HPNIVRYRDR IIDRTNTTLY IVMEYCEGGD LASVITKGTK ERQYLDEEFV
121 LRVMTQTLTA LKECHRRSDG GHTVLHRDLK PANVFLDGKQ NVKLGDFGLA RILNHDTSFA
181 KTFVGTPPYM SPEQMNRMSY NEKSDIWSLG CLLYELCALM PPFTAFSQKE LAGKIREGKF
241 RRIPYRYSDE LNEIITRMLN LKDYHRPSVE EILENPLIAD LVADEQRRNL ERRGRQLGEP
301 EKSQDSSPVL SELKLKEIQL QERERALKAR EERLEQKEQE LCVRERLAED KLARAENLLK
361 NYSLLKERKF LSLASNPELL NLPSSVIKKK VHFSGESKEN IMRSENSESQ LTSKSKCKDL
421 KKRLHAAQLR AQALSDIEKN YQLKSRQILG MR

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### Recombinant NEK2 nucleotide sequence:

```

1  atgcaccatc accatcacca tatgccttcc cgggctgagg actatgaagt gttgtacacc
61  attggcacag gctcctacgg ccgctgccag aagatccgga ggaagagtga tggcaagata
121 ttagtttgga aagaacttga ctatggctcc atgacagaag ctgagaaaca gatgcttggt
181 tctgaagtga atttgcttcg tgaactgaaa catccaaaca tcgttcgtta ctatgatcgg
241 attattgacc ggaccaatac aacactgtac attgtaatgg aatattgtga aggaggggat
301 ctggctagtg taattacaaa gggaaccaag gaaaggcaat acttagatga agagtttggt
361 cttcgagtga tgactcagtt gactctggcc ctgaaggaaat gccacagacg aagtgatggg
421 ggtcataccg tattgcatcg ggatctgaaa ccagccaatg ttttcctgga tggcaagcaa
481 aacgtcaagc ttgggagact tgggctagct agaataattaa accacgacac gagttttgca
541 aaaacatttg ttggcacacc ttattacatg tctcctgaac aaatgaatcg catgtcctac
601 aatgagaaat cagatatctg gtcattgggc tgcttgctgt atgagttatg tgcattaatg
661 cctccattta cagcttttag ccagaaagaa ctcgctggga aaatcagaga aggcaaattc
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781 ttaaaggatt accatcgacc ttctgttgaa gaaattcttg agaacccttt aatagcagat
841 ttggttgtag acgagcaaag aagaaatctt gagagaagag ggcgacaatt aggagagcca
901 gaaaaatcgc aggattccag ccctgtattg agtgagctga aactgaagga aattcagtta
961 caggagcgag agcgagctct caaagcaaga gaagaaagat tggagcagaa agaacaggag
1021 ctttgtgttc gtgagagact agcagaggac aaactggcta gagcagaaaa tctgttgaag
1081 aactacagct tgctaaagga acggaagtgc ctgtctctgg caagtaatcc agaacttctt
1141 aatcttccat cctcagtaat taagaagaaa gttcatttca gtggggaaag taaagagaac
1201 atcatgagga gtgagaattc tgagagtcag ctcacatcta agtccaagtg caaggacctg
1261 aagaaaaggc ttcacgctgc ccagctgcgg gctcaagccc tgtcagatat tgagaaaaat
1321 taccaactga aaagcagaca gatcctgggc atgcgctag

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