

Certificate of Analysis

NEK11, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-700, 14-700-K, 14-700M

Parent Lot # D18DP015N

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 full length, NEK11, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 77% by SDS-PAGE and Coomassie blue staining. MW = 59.3kDa.

Specific Activity (Parent lot# D18DP015N): 275U/mg, where one unit of NEK11, 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: 1.72mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamide, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

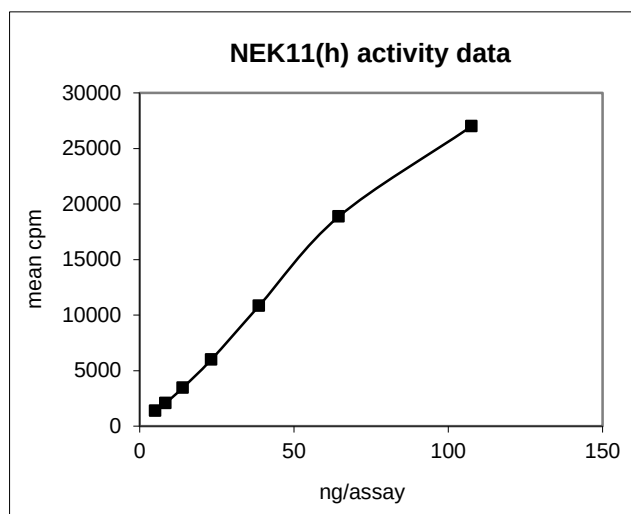
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

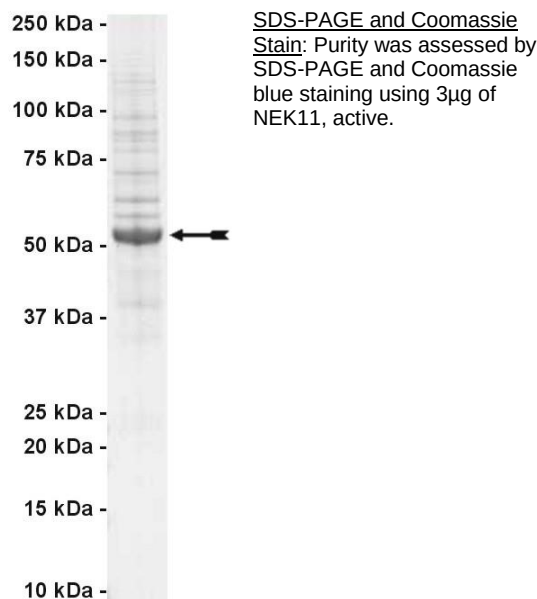
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 5.02–107.50ng of this lot of enzyme phosphorylated 0.33mg/ml myelin basic protein (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 NEK11 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. Make up a 3.33mg/ml stock. Use 2.5µl of stock per assay point.
- 3. NEK11, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 5.02–107.50ng 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 **myelin basic protein (MBP)**.
3. Add **2.5µl (5.02–107.50ng) NEK11, 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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NEK11 Sequence Information

Protein	Human NEK11
Tags	N-Terminal 6His
Native sequence	M31 of recombinant sequence is equivalent to M1 of human NEK11
Accession number	GenBank BC028587

Recombinant NEK11 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MLKFQEAAKC VSGSTAISTY PKTLIARRYV
61 LQKQLGSGSF GTVYLVSDKK AKRGEELKVL KEISVGELNP NETVQANLEA QLLSKLDHPA
121 IVKFHASFVE QDNFCIITEY CEGRDLDKDI QEYKQAGKIF PENQIIIEWFI QLLLGVDDYMH
181 ERRILHRDLK SKNVFLKNNL LKIGDFGVSR LLMGSCDLAT TLTGTPHYMS PEALKHQGYD
241 TKSDIWSLAC ILYEMCCMNH AFAGSNFLSI VLKIVEGDTL SLPERYPKEL NAIMESMLNK
301 NPSLRPSAIE ILKIPYLDEQ LQNLRCRYSE MTLEDKNLDC QKEAAHIINA MQKRIHLQTL
361 RALSEVQKMT PRERMRLRKL QAADEKARKL KKIVEEKYEE NSKRMQELRS RNFQQLSVDV
421 LHEKTHLKGM EEKEEQPEGR LSCSPQDEDE ERWQGREEES DEPTLENLPE SQPIPSMDLH
481 ELESIVEDAT SDLGYHGDCN LISLDEYWNK EK

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Recombinant NEK11 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc atgctgaaat tccaagaggc agctaagtgt
121 gtgagtggat caacagccat ttccacttat ccaaagacct tgattgcaag aagatacgtg
181 cttcaacaaa aacttggcag tggaagtgtt ggaactgtct atctggtttc agacaagaaa
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1501 ctaatttcac tagacgaata ctggaaaaat gaaaaataa

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