

## Certificate of Analysis

### NLK, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-676, 14-676-K, 14-676M

Parent Lot # WAB0683

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 NLK, amino acids 121–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>-NTA agarose. Purity 67% by SDS-PAGE and Coomassie blue staining. MW = 48.7kDa.

**Specific Activity (Parent lot# WAB0683):** 17U/mg, where one unit of NLK, 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:** 0.54mg/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.

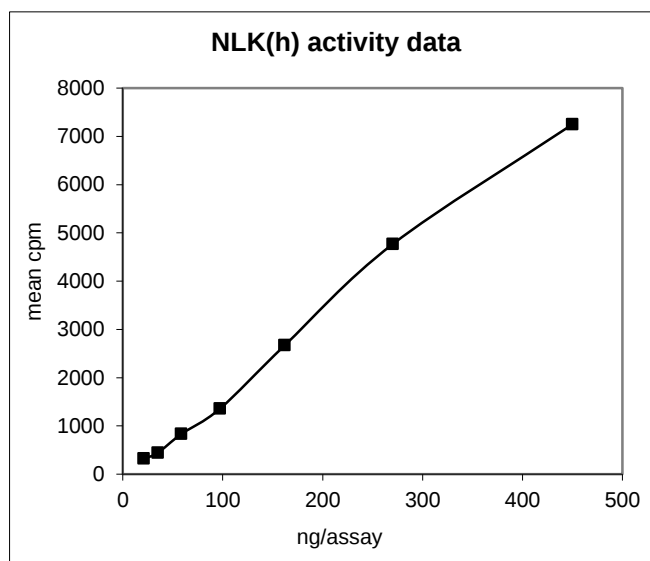
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

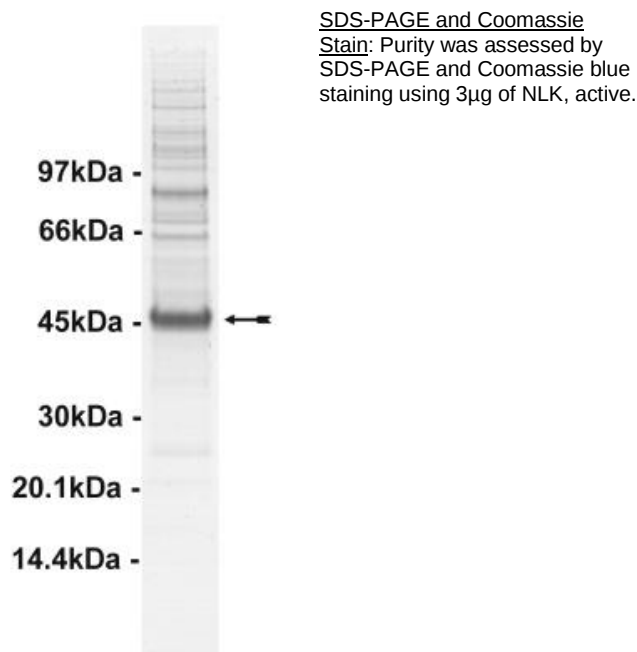
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 21–450ng 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 NLK 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 3.3mg/ml stock. Use 2.5µl of stock solution per assay point.
3. **NLK, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 21–450ng 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 (MBP)**.
3. Add **2.5µl (21–450ng) NLK, 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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## NLK Sequence Information

|                                |                                                                    |
|--------------------------------|--------------------------------------------------------------------|
| <b><u>Protein</u></b>          | Human NLK                                                          |
| <b><u>Tags</u></b>             | N-Terminal 6His                                                    |
| <b><u>Native sequence</u></b>  | Q31 of the recombinant sequence is equivalent to Q121 of human NLK |
| <b><u>Accession number</u></b> | GenBank NM_016231                                                  |

### Recombinant NLK amino acid sequence:

```

1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF QQQLDIEPDR PIGYGAFGVV WSVTDPRDGK
61 RVALKKMPNV FQNLVSCRV FRELKMLCFF KHDNVLSALD ILQPPHIDYF EEIYVVTELM
121 QSDLHKIIVS PQPLSSDHVK VFLYQILRGL KYLHSAGILH RDIKPGNLLV NSNCVLKICD
181 FGLARVEELD ESRHMTQEVV TQYYRAPEIL MGSRHYSNAI DIWSVGCIFA ELLGRRILFQ
241 AQSPIQQLDL ITDLLGTPSL EAMRTACEGA KAHILRGPHK QPSLPVLYTL SSQATHEAVH
301 LLCRMLVFPD SKRISAKDAL AHPYLDEGRL RYHTCMCKCC FSTSTGRVYT SDFEPVTNPK
361 FDDTFEKNLS SVRQVKEIIH QFILEQQKGN RVPLCINPQS AAFKSFISST VAQPSEMPPS
421 PLVWE

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### Recombinant NLK nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc cagcagcagc tggatattga gccggataga
121 cctattggat atggagcctt tgggtgtgtc tggtcagtaa cagatccaag agatggaaaag
181 agagtagcgc tcaaaaagat gcccaacgct tccagaatc tggctctctg caaaagggtc
241 ttccgggaat tgaagatggt gtgttttttt aagcatgata atgtactctc tgcccttgac
301 atactccaac ctccacacat tgactatttt gaagaaatat atgttgtcac agaattgatg
361 cagagtggac tacataaaat tatcgtctct cctcaaccac tcagctcaga tcatgtcaaa
421 gtttttcttt atcagatttt gcgaggtttg aaatatctcc attcagctgg cattttacat
481 cgagacatta agccagggaa tctccttgat aacagcaact gtgttctaaa gatttgtgat
541 ttggattgg ccagagtggg agaattagat gaatcccgtc atatgactca ggaagtgtgt
601 actcagtatt atcgggctcc agaaatcctg atgggcagcc gtcattacag caatgctatt
661 gacatctggt ctgtgggatg tatctttgca gaactactag gacgaagaat attgtttcag
721 gcacagagtc ccattcagca gttggatttg atcacggatc tgttgggcac accatcactg
781 gaagcaatga ggacagcttg tgaaggcgtc aaggcacata tactcagggg tcctcataaa
841 cagccatctc ttcctgtact ctataccctg tctagccagg ctacacatga agctgttcat
901 ctcttttgca ggatgttggc ctttgcattc tccaaaagaa tatccgctaa ggatgcctta
961 gcccccctt accatgatga agggcgacta cgatatcaca catgtatgtg taaatgttgc
1021 ttttccacct ccactggaag agtttatacc agtgactttg agcctgtcac caatccaaa
1081 tttgatgaca ctttcgagaa gaacctcagt tctgtccgac aggttaaaga aattattcat
1141 cagttcattt tggaaacgca gaaaggaaac agagtgcctc tctgcatcaa ccctcagtct
1201 gctgctttta agagctttat tagttccact gttgctcagc catctgagat gccccatct
1261 cctctggtgt gggagtga

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Reviewed and approved by site quality representative.

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