

Certificate of Analysis

NLK

nemo like kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: NLK

Synonyms: LAK1

Product No.: 0253-0000-1

Lot: 002

Description: Human NLK, amino acids M₁-E₅₁₅ (as in NCBI/Protein entry NP_057315.3), N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: NLK Lot 002, was confirmed as NLK by mass spectroscopy LC-ESI-MS/MS (Protagen AG, Germany)

Theoretical MW_{Fusion Protein}: 91,818 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

Storage buffer: 50 mM TRIS-HCL pH 8.0, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

Storage temperature: -80°C

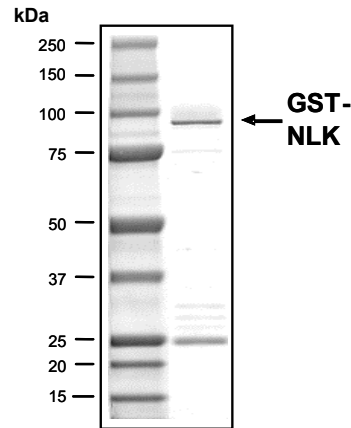
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.106 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

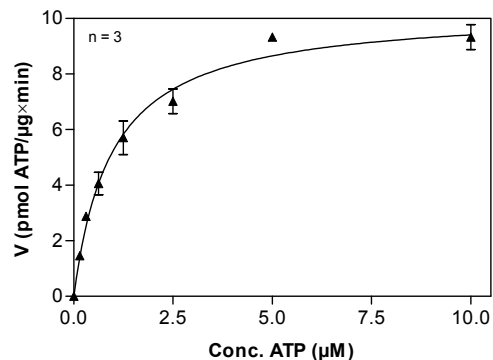
Specific kinase activity (P_i transfer): 10 pmol/µg×min
ATP-K_M: 1 µM

**NLK Lot 002:
Coomassie stain**



2.0 µg GST-NLK

**NLK Lot 002:
Determination of V_{max} and K_M value for ATP**



Determination of K_M value & Specific activity:

- Assay conditions:
 - 60 mM HEPES-NaOH, pH 7.5
 - 3 mM MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg / ml PEG_{20,000}
 - ATP (variable)
 - Substrate: RB-CTF 50 µg/ml
 - Kinase: 1.0 µg / ml
- Filter binding assay
 - MSFC membrane (Millipore)

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NLK Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG HHHHHH HG	RRRASVAAGI	240
241	LVPRGS PGLD	GIYARGIQAS	MGARGRQCDG	YLQNSPLMAA	YNGGTSAAAA	GHHHHHHHHL	300
301	PHLPPPHLHH	HHHPQHHLHP	GSAAAVHPVQ	QHTSSAAAAA	AAAAAAAAML	NPGQQQPYFP	360
361	SPAPGQAPGP	AAAAPAQVQA	AAAATVKAHH	HQSHHPQQQ	LDIEPDRPIG	YGAFGVVWSV	420
421	TDPRDGKRVA	LKKMPNVFQN	LVSCKRVFRE	LKMLCFFKHD	NVLSALDILQ	PPHIDYFEEI	480
481	YVTELMQSD	LHKIIVSPQP	LSSDHVKVFL	YQILRGLKYL	HSAGILHRDI	KPGNLLVNSN	540
541	CVLKICDFGL	ARVEELDES	HMTQEVVTOY	YRAPEILMGS	RHYSNAIDIW	SVGCIFAELL	600
601	GRRILFQAQS	PIQQLDLITD	LLGTPSLEAM	RTACEGAKAH	ILRGPHKQPS	LPVLYTLSSQ	660
661	ATHEAVHLLC	RMLVFDPSKR	ISAKDALAHP	YLDEGRRLYH	TCMCKCCFST	STGRVYTSDF	720
721	EPVTNPKFDD	TFEKNLSSVR	QVKEIIHQFI	LEQQKGNRVP	LCINPQSAAF	KSFISSTVAQ	780
781	PSEMPPSPLV	WEQGRIPAHW	RPLLVDPSSV	PPGDLYRLC			840

1-218: GST **Red:** HIS6-tag **Pink:** Thrombin cleavage site **blue:** NLK

NLK wt ¹ Amino Acid Sequence							
1	MAAYNGG TSA	AAAGHHHHHH	HHLPHLPPP	LHHHHHPQH	LHPGSAAAVH	PVQQHTSSAA	60
61	AAAAAAAAA	AMLNPGQQP	YFPSPAPQA	PGPAAAAPAQ	VQAAAAATVK	AHHHQHSHHP	120
121	QQQLDIEPDR	PIGYGAFGV	WSVTDPRDGK	RVALKKMPNV	FQNLVSKRV	FRELKMLCFF	180
181	KHDNVLSALD	ILQPPHIDYF	EIYVVTTELM	QSDLHKIIVS	PQPLSSDHVK	VFLYQILRGL	240
241	KYLHSAGILH	RDIKPGNLLV	NSNCVLKICD	FGLARVEELD	ESRHMTQEVV	TQYYRAPEIL	300
301	MGSRHYSNAI	DIWSVGCIFA	ELLGRRILFQ	AQSPIQQLDL	ITDLLGTPSL	EAMRTACEGA	360
361	KAHILRGPHK	QPSLPVLYTL	SSQATHEAVH	LLCRMLVFDP	SKRISAKDAL	AHPYLDEGRL	420
421	RYHTCMCKCC	FSTSTGRVYT	SDFEPVTNPK	FDDTFEKNLS	SVRQVKEIIH	QFILEQQKGN	480
481	RVPLCINPQS	AAFKSFISST	VAQPSEMPPS	PLVWE			540

blue: NLK sequence expressed in fusion protein

¹NCBI/Protein accession number NP_057315.3