Certificate of Analysis
NLK, 100 µg
Nemo-Like Kinase, GST-tagged

Part Number: PR7525A
Lot Number: 1652136L
Immediate Storage: -80°C
Shipping Conditions: dry ice

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Description:
Recombinant human full length protein, GST-tagged, expressed in insect cells. Activated *in vitro* via autophosphorylation.

Specific Activity:
9 nmoles of phosphate transferred to myelin basic protein (MBP) per minute per mg of total protein at 30°C. Activity determined at a final protein concentration of 8.33 µg/mL.

Concentration:
0.46 mg/mL total protein as measured using the Bradford protein assay with BSA as a standard.

Calculated **5,440 nM**.

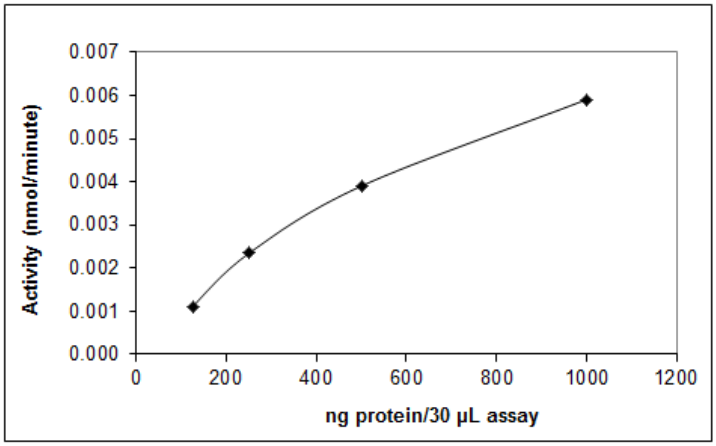
Aliases:
FLJ21033, DKFZp761G1211

Storage and Handling:
For maximum recovery please spin prior to use. Unless noted below, aliquots of the 5 ug, 10ug and 20ug sizes of kinase are not recommended as materials can be used in original packaging until exhausted. For larger sizes, the number of freeze/thaws may be reduced by preparing aliquots, aliquots below 20 µL are not recommended. **Please never store a kinase diluted.** If properly stored at −80°C, this product is guaranteed for 6 months from date of purchase.

Storage Buffer:
50 mM Tris (pH 7.5), 150 mM NaCl, 0.5 mM EDTA, 0.02% Triton® X–100, 2 mM DTT and 50% Glycerol.

QUALITY ASSURANCE

NLK Activity Graph



Dilution Buffer:
20 mM Tris (pH 7.5), 0.02% Triton® X−100, 0.1 mg/mL BSA, 2 mM DTT, 0.5 mM Na_3VO_4 and 10% Glycerol.

Assay Conditions:
NLK was pre-diluted in enzyme dilution buffer and assayed in 25 mM Tris (pH 7.5), 10 mM $MgCl_2$, 0.5 mM EGTA, 0.5 mM Na_3VO_4, 5 mM β−glycerophosphate, 2.5 mM DTT, 0.01% Triton® X-100, 100 µM ATP, 667 µg/mL myelin basic protein (MBP) and trace [^{32}P]-γ-ATP for 10 minutes at 30°C.

Gel Information for NLK

Page Description: The SDS-PAGE and/or Native PAGE were run on 4-20% Tris-Glycine Novex® gels (Catalog #: EC6025BOX).

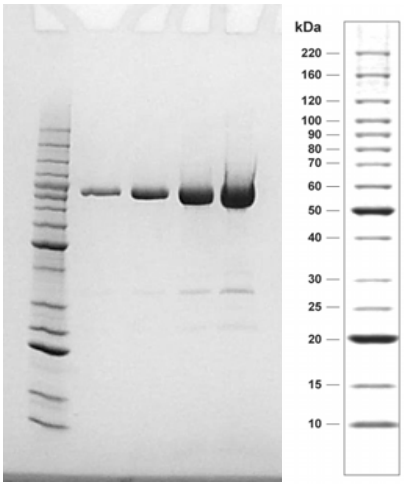
Lane 1: Invitrogen™ BenchMark™ Protein Ladder (Catalog #: 10747-012).

Lane 2: 1 µg NLK

Lane 3: 2 µg NLK

Lane 4: 5 µg NLK

Lane 5: 10 µg NLK



Purity:
90% as determined by a Coomassie® blue stained SDS-PAGE gel.

Molecular Weight:
84.5 kDa. Calculated from the protein sequence(s).

Mass Spectrometry:
NLK was subjected to proteolytic digest followed by mass spec analysis. The resulting MS/MS data verified NLK identity by comparison against the amino acid sequence(s) of the recombinant protein.

Protein sequence alignment with reference sequence(s)

GenBank Accession Number: NP_057315.1

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   1 MAPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL GST TAG
   1 MAPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL IVGN NLK
   1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- NP_057315.1

 101 DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA
 101 DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA
   1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 201 WPLQGWQATF GGGDHPPKSD LVPR
 201 WPLQGWQATF GGGDHPPKSD LVPRHNQTSL YKKAGTMAAY NGGTSAAAAG HHHHHHHHLP HLPPPHLHHH HHPQHHLHPG SAAAVHPVQQ HTSSAAAAAA
   1 ---------- ---------- ---------- ------MAAY NGGTSAAAAG HHHHHHHHLP HLPPPHLHHH HHPQHHLHPG SAAAVHPVQQ HTSSAAAAAA

 224
 301 AAAAAAAMLN PGQQQPYFPS PAPGQAPGPA AAAPAQVQAA AAATVKAHHH QHSHHPQQQL DIEPDRPIGY GAFGVVWSVT DPRDGKRVAL KKMPNVFQNL
  65 AAAAAAAMLN PGQQQPYFPS PAPGQAPGPA AAAPAQVQAA AAATVKAHHH QHSHHPQQQL DIEPDRPIGY GAFGVVWSVT DPRDGKRVAL KKMPNVFQNL

 224
 401 VSCKRVFREL KMLCFFKHDN VLSALDILQP PHIDYFEEIY VVTELMQSDL HKIIVSPQPL SSDHVKVFLY QILRGLKYLH SAGILHRDIK PGNLLVNSNC
 165 VSCKRVFREL KMLCFFKHDN VLSALDILQP PHIDYFEEIY VVTELMQSDL HKIIVSPQPL SSDHVKVFLY QILRGLKYLH SAGILHRDIK PGNLLVNSNC

 224
 501 VLKICDFGLA RVEELDESRH MTQEVVTQYY RAPEILMGSR HYSNAIDIWS VGCIFAELLG RRILFQAQSP IQQLDLITDL LGTPSLEAMR TACEGAKAHI
 265 VLKICDFGLA RVEELDESRH MTQEVVTQYY RAPEILMGSR HYSNAIDIWS VGCIFAELLG RRILFQAQSP IQQLDLITDL LGTPSLEAMR TACEGAKAHI

 224
 601 LRGPHKQPSL PVLYTLSSQA THEAVHLLCR MLVFDPSKRI SAKDALAHPY LDEGRLRYHT CMCKCCFSTS TGRVYTSDFE PVTNPKFDDT FEKNLSSVRQ
 365 LRGPHKQPSL PVLYTLSSQA THEAVHLLCR MLVFDPSKRI SAKDALAHPY LDEGRLRYHT CMCKCCFSTS TGRVYTSDFE PVTNPKFDDT FEKNLSSVRQ

 224
 701 VKEIIHQFIL EQQKGNRVPL CINPQSAAFK SFISSTVAQP SEMPPSPLVW E.
 465 VKEIIHQFIL EQQKGNRVPL CINPQSAAFK SFISSTVAQP SEMPPSPLVW E
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* highlighted residues denote differences from the reference protein sequence(s).

Tony Goossens, Engineer II, QA/QC Date: 27/Oct/2014