Certificate of Analysis

REACTION BIOLOGY

ProQinase™ NIK

mitogen-activated protein kinase kinase kinase 14

Recombinant Human Active Protein Kinase

HGNC Symbol: MAP3K14

Synonyms: FTDCR1B, HS, HSNIK

Product No.: 0344-0000-1

Lot: 041

Description: Human NIK, full length, amino acids M_1-P_{947} (as in NCBI/Protein entry NP_003945.2), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: NIK Lot 041, was confirmed as NIK by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 133,437 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

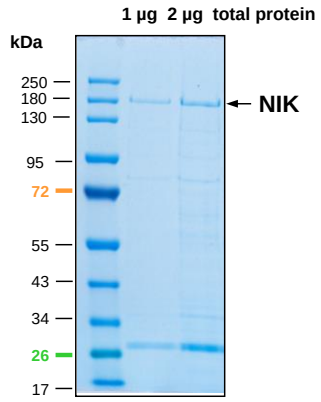
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

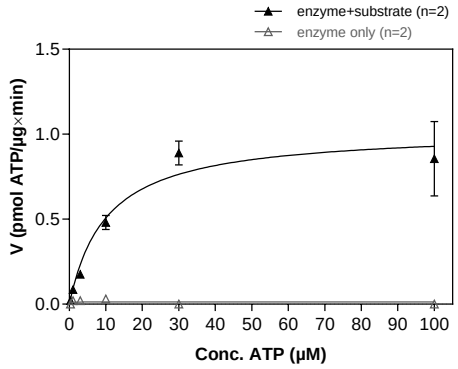
Protein concentration: 0.116 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

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

NIK Lot 041:
Coomassie stain



NIK Lot 041:
Determination of V_{max} and K_M value for ATP



- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: CDC25C-derived peptide 160 µg/ml
  Kinase: 2 µg/ml
- Filter binding assay

MSPH membrane (Millipore)

Recombinant Proteins

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Sequence information

GST-NIK Recombinant Fusion Protein Amino Acid Sequence

```
   1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID   60
  61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV  120
 121  DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK  180
 181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI  240
 241  LVPRGSPGLD GICSRMAVME MACPGAPGSA VGQQKELPKA KEKTPPLGKK QSSVYKLEAV  300
 301  EKSPVFCGKW EILNDVITKG TAKEGSEAGP AAISIIAQAE CENSQEFSPT FSERIFIAGS  360
 361  KQYSQSESLD QIPNNVAHAT EGKMARVCWK GKRRSKARKK RKKKSSKSLA HAGVALAKPL  420
 421  PRTPEQESCT IPVQEDESPL GAPYVRNTPQ FTKPLKEPGL GQLCFKQLGE GLRPALPRSE  480
 481  LHKLISPLQC LNHVWKLHHP QDGGPLPLPT HPFPYSRLPH PFPFHPLQPW KPHPLESFLG  540
 541  KLACVDSQKP LPDPHLSKLA CVDSPKPLPG PHLEPSCLSR GAHEKFSVEE YLVHALQGSV  600
 601  SSGQAHSLTS LAKTWAARGS RSREPSPKTE DNEGVLLTEK LKPVDYEYRE EVHWATHQLR  660
 661  LGRGSFGEVH RMEDKQTGFQ CAVKKVRLEV FRAEELMACA GLTSPRIVPL YGAVREGPWV  720
 721  NIFMELLEGG SLGQLVKEQG CLPEDRALYY LGQALEGLEY LHSRRILHGD VKADNVLLSS  780
 781  DGSHAALCDF GHAVCLQPDG LGKSLLTGDY IPGTETHMAP EVVLGRSCDA KVDVWSSCCM  840
 841  MLHMLNGCHP WTQFFRGPLC LKIASEPPPV REIPPSCAPL TAQAIQEGLR KEPIHRVSAA  900
 901  ELGGKVNRAL QQVGGLKSPW RGEYKEPRHP PPNQANYHQT LHAQPRELSP RAPGPRPAEE  960
 961  TTGRAPKLQP PLPPEPPEPN KSPPLTLSKE ESGMWEPLPL SSLEPAPARN PSSPERKATV 1020
1021  PEQELQQLEI ELFLNSLSQP FSLEEQEQIL SCLSIDSLSL SDDSEKNPSK ASQSSRDTLS 1080
1081  SGVHSWSSQA EARSSSWNMV LARGRPTDTP SYFNGVKVQI QSLNGEHLHI REFHRVKVGD 1140
1141  IATGISSQIP AAAFSLVTKD GQPVRYDMEV PDSGIDLQCT LAPDGSFAWS WRVKHGQLEN 1200
1201  RP                                                               1260
```

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

NIK wt[1] Amino Acid Sequence

```
   1  MAVMEMACPG APGSAVGQQK ELPKAKEKTP PLGKKQSSVY KLEAVEKSPV FCGKWEILND   60
  61  VITKGTAKEG SEAGPAAISI IAQAECENSQ EFSPTFSERI FIAGSKQYSQ SESLDQIPNN  120
 121  VAHATEGKMA RVCWKGKRRS KARKKRKKKS SKSLAHAGVA LAKPLPRTPE QESCTIPVQE  180
 181  DESPLGAPYV RNTPQFTKPL KEPGLGQLCF KQLGEGLRPA LPRSELHKLI SPLQCLNHVW  240
 241  KLHHPQDGGP LPLPTHPFPY SRLPHPFPFH PLQPWKPHPL ESFLGKLACV DSQKPLPDPH  300
 301  LSKLACVDSP KPLPGPHLEP SCLSRGAHEK FSVEEYLVHA LQGSVSSGQA HSLTSLAKTW  360
 361  AARGSRSREP SPKTEDNEGV LLTEKLKPVD YEYREEVHWA THQLRLGRGS FGEVHRMEDK  420
 421  QTGFQCAVKK VRLEVFRAEE LMACAGLTSP RIVPLYGAVR EGPWVNIFME LLEGGSLGQL  480
 481  VKEQGCLPED RALYYLGQAL EGLEYLHSRR ILHGDVKADN VLLSSDGSHA ALCDFGHAVC  540
 541  LQPDGLGKSL LTGDYIPGTE THMAPEVVLG RSCDAKVDVW SSCCMMLHML NGCHPWTQFF  600
 601  RGPLCLKIAS EPPPVREIPP SCAPLTAQAI QEGLRKEPIH RVSAAELGGK VNRALQQVGG  660
 661  LKSPWRGEYK EPRHPPPNQA NYHQTLHAQP RELSPRAPGP RPAEETTGRA PKLQPPLPPE  720
 721  PPEPNKSPPL TLSKEESGMW EPLPLSSLEP APARNPSSPE RKATVPEQEL QQLEIELFLN  780
 781  SLSQPFSLEE QEQILSCLSI DSLSLSDDSE KNPSKASQSS RDTLSSGVHS WSSQAEARSS  840
 841  SWNMVLARGR PTDTPSYFNG VKVQIQSLNG EHLHIREFHR VKVGDIATGI SSQIPAAAFS  900
 901  LVTKDGQPVR YDMEVPDSGI DLQCTLAPDG SFAWSWRVKH GQLENRP               960
```

blue: NIK sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_003945.2

Recombinant Proteins

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