Certificate of Analysis

REACTION BIOLOGY

ProQinase™ NEK3

NIMA (never in mitosis gene a)-related kinase 3

Recombinant Human Active Protein Kinase

HGNC Symbol: NEK3

Synonyms: HSPK36, HSPK 36, MGC29949

Product No.: 1624-0000-1

Lot: 003

Description: Human NEK3, full length, amino acids M_1-R_{506} (as in NCBI/Protein entry NP_689933.1), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: NEK3 Lot 003, was confirmed as NEK3 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 85,954 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

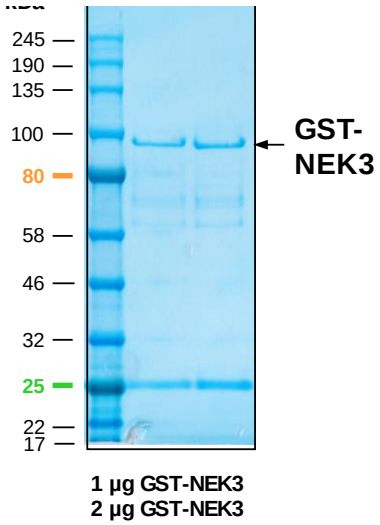
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

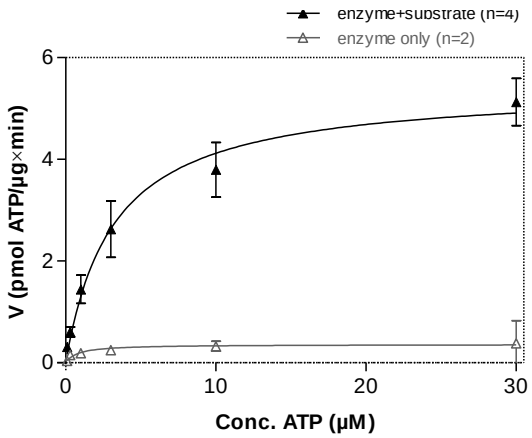
Protein concentration: 0.263 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 5.4 pmol/µg × min
ATP-K_M: 3.2 µM

NEK3 Lot 003:
Coomassie stain



NEK3 Lot 003:
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 $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: CDC25C-derived peptide 80 µg/ml
  Kinase: 2 µg/ml
- Filter binding assay
  MSPH membrane (Millipore)

Additional assay technology: NEK3 Lot 003
was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

Promega

Recombinant Proteins

ProQinase™ NEK3

Product No.: 1624-0000-1

GST-NEK3 Recombinant Fusion Protein Amino Acid Sequence

```
   1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
  61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
 121  DFLSKLPEML KMFKDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
 181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG 240
 241  PLAMDDYMVL RMIGEGSFGR ALLVQLESSN QMFAMKEIRL PKSFSNTQNS RKEAVLLAKM 300
 301  KHPNIVAFKE SFEAEGHLYI VMEYCDGGDL MQKIKQQKGK LFPEDMILNW FTQMCLGVNH 360
 361  IHKKRVLHRD IKSKNIFLTQ NGKVKLGDFG SARLLSNPMA FACTYVGTPY YVPPEIWENL 420
 421  PYNNKSDIWS LGCILYELCT LKHPFQANSW KNLILKVCQG CISPLPSHYS YELQFLVKQM 480
 481  FKRNPSHRPS ATTLLSRGIV ARLVQKCLPP EIIMEYGEEV LEEIKNSKHN TPRKKTNPSR 540
 541  IRIALGNEAS TVQEEEQDRK GSHTDLESIN ENLVESALRR VNREEKGNKS VHLRKASSPN 600
 601  LHRRQWEKNV PNTALTALEN ASILTSSLTA EDDRGGSVIK YSKNTTRKQW LKETPDTLLN 660
 661  ILKNADLSLA FQTYTIYRPG SEGFLKGPLS EETEASDSVD GGHDSVILDP ERLEPGLDEE 720
 721  DTDFEEEDDN PDWVSELKKR AGWQGLCDR                                   780
```

1-218: GST **Red:** HIS6-tag **Green:** 3C cleavage site **blue**:NEK3 **boxed**:variation from RefSeq

NEK3 wt[1] Amino Acid Sequence

```
1    MDDYMVLRMI GEGSFGRALL VQHESSNQMF AMKEIRLPKS FSNTQNSRKE AVLLAKMKHP  60
61   NIVAFKESFE AEGHLYIVME YCDGGDLMQK IKQQKGKLFP EDMILNWFTQ MCLGVNHIHK 120
121  KRVLHRDIKS KNIFLTQNGK VKLGDFGSAR LLSNPMAFAC TYVGTPYYVP PEIWENLPYN 180
181  NKSDIWSLGC ILYELCTLKH PFQANSWKNL ILKVCQGCIS PLPSHYSYEL QFLVKQMFKR 240
241  NPSHRPSATT LLSRGIVARL VQKCLPPEII MEYGEEVLEE IKNSKHNTPR KKTNPSRIRI 300
301  ALGNEASTVQ EEEQDRKGSH TDLESINENL VESALRRVNR EEKGNKSVHL RKASSPNLHR 360
361  RQWEKNVPNT ALTALENASI LTSSLTAEDD RGGSVIKYSK NTTRKQWLKE TPDTLLNILK 420
421  NADLSLAFQT YTIYRPGSEG FLKGPLSEET EASDSVDGGH DSVILDPERL EPGLDEEDTD 480
481  FEEEDDNPDW VSELKKRAGW QGLCDR                                      540
```

blue: NEK3 sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_689933.1
H23L: SNP variation see NCBI/dbSNP ID: rs17482764

Recombinant Proteins