

# 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<sub>1</sub>-R<sub>506</sub> (as in NCBI/Protein entry NP\_689933.1), N-terminal GST-HIS<sub>6</sub> 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**<sub>Fusion Protein</sub>: 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

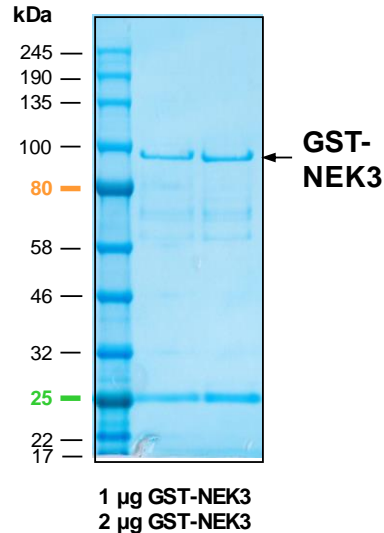
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

**Biochemical Parameters:**

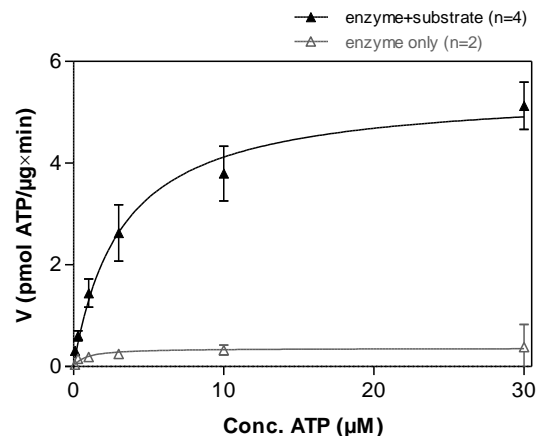
Specific kinase activity (P<sub>i</sub> transfer): 5.4 pmol/µg × min

ATP-K<sub>M</sub>: 3.2 µM

## NEK3 Lot 003: Coomassie stain



## NEK3 Lot 003: Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP



### Determination of K<sub>M</sub> value & Specific activity:

- Assay conditions:
  - 60 mM HEPES-NaOH, pH 7.5
  - 3 mM MgCl<sub>2</sub>
  - 3 mM MnCl<sub>2</sub>
  - 3 µM Na-orthovanadate
  - 1.2 mM DTT
  - 50 µg/ml PEG<sub>20,000</sub>
  - 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 ProQinase for the use with the ADP-Glo™ Kinase assay from Promega. ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details



## NEK3

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GST-NEK3 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFKDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG <b>HHHHHH</b> G	RDS <b>LEVL</b> FQG	240
241	<b>PLA</b> MDDY <b>MVL</b>	<b>RMIGEGS</b> FGR	<b>ALLVQ</b> LESSN	<b>QMFAM</b> KEIRL	<b>PKSFS</b> NTQNS	<b>RKEAV</b> LLAKM	300
301	<b>KHPNIVAF</b> KE	<b>SFEAEGH</b> LYI	<b>VMEYCD</b> GGDL	<b>MQKIK</b> QQK GK	<b>LPEDM</b> ILNW	<b>FTQM</b> CLGVNH	360
361	<b>IHKRVL</b> HRD	<b>IKSKNIF</b> L TQ	<b>NGKVK</b> L GDFG	<b>SARLL</b> SNPMA	<b>FACTY</b> VGTPY	<b>YVPEI</b> WENL	420
421	<b>PYNNK</b> SDIWS	<b>LGCILY</b> ELCT	<b>LKHPF</b> QANSW	<b>KNLIL</b> KVCQG	<b>CISPL</b> PSHYS	<b>YELQ</b> FLVKQM	480
481	<b>FKRNP</b> SHRPS	<b>ATLLS</b> RGIV	<b>ARLVQ</b> KCLPP	<b>EIIME</b> YGEEV	<b>LEEIK</b> NSKHN	<b>TPRK</b> KTNP SR	540
541	<b>IRIAL</b> GNEAS	<b>TVQEE</b> EQDRK	<b>GSHTD</b> LESIN	<b>ENLVES</b> ALRR	<b>VNREE</b> KGNKS	<b>VHLR</b> KASSPN	600
601	<b>LHRRQ</b> WEKNV	<b>PNTALT</b> ALEN	<b>ASILT</b> SSLTA	<b>EDDRG</b> GSVIK	<b>YSKNT</b> TRKQW	<b>LKETP</b> DTLLN	660
661	<b>ILKNAD</b> LSLA	<b>FQTYT</b> IYRPG	<b>SEGFL</b> KGPLS	<b>EETEAS</b> DSVD	<b>GGHDS</b> VILD P	<b>ERLEP</b> GLDEE	720
721	<b>DTDFEE</b> EDDN	<b>PDWVS</b> ELKKR	<b>AGWQ</b> GLCDR				780

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

NEK3 wt <sup>1</sup> Amino Acid Sequence							
1	<b>MDDY</b> MVLRMI	<b>GEGS</b> FGRALL	<b>VQ</b> HES <b>S</b> NQMF	<b>AMKEI</b> RLPKS	<b>FSNTQ</b> NSRKE	<b>AVLLA</b> KMKHP	60
61	<b>NIVAF</b> KESFE	<b>AEGLY</b> IIVME	<b>YCDG</b> GLMQK	<b>IKQO</b> KGLFP	<b>EDMIL</b> NWFTQ	<b>MCLG</b> VNHIHK	120
121	<b>KRVL</b> HRDIKS	<b>KNIFL</b> TQNGK	<b>VKLG</b> DGFSAR	<b>LLSNP</b> MAFAC	<b>TYVGT</b> PYYVP	<b>PEIWE</b> NLPYN	180
181	<b>NKSDI</b> WSLGC	<b>ILYEL</b> CTLKH	<b>PFQAN</b> SWKNL	<b>ILKVC</b> QGCIS	<b>PLPSH</b> YSYEL	<b>QFLV</b> KQMFKR	240
241	<b>NPSHR</b> PSATT	<b>LLSRG</b> IVARL	<b>VQKCL</b> PPEII	<b>MEYGE</b> EVLEE	<b>IKNSK</b> HNTPR	<b>KKTNP</b> SRIRI	300
301	<b>ALGNE</b> ASTVQ	<b>EEEQ</b> DRKGS	<b>TDLES</b> INENL	<b>VESAL</b> RRVNR	<b>EEKGN</b> KS VHL	<b>RKASS</b> PNLHR	360
361	<b>RQWEK</b> NPNT	<b>ALTAL</b> ENASI	<b>LTSSL</b> TAEDD	<b>RGGSV</b> IKYSK	<b>NTTRK</b> QWLKE	<b>TPD</b> TLLNILK	420
421	<b>NADLS</b> LAFQT	<b>YTIYR</b> PGSEG	<b>FLKG</b> PLSEET	<b>EASDS</b> V DGGH	<b>DSVIL</b> DPERL	<b>EPGL</b> DEEDTD	480
481	<b>FEEED</b> NPDW	<b>VSEL</b> KKRAGW	<b>QGL</b> CDR				540

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

<sup>1</sup>NCBI/Protein accession number NP\_689933.1

H23L: SNP variation see NCBI/dbSNP ID: rs17482764