

## ProQinase™ NEK1

NIMA related kinase 1

Recombinant Human Active Protein Kinase

HGNC Symbol: NEK1

Synonyms: NY-REN-55

Product No.: 0844-0000-1

Lot: 004

**Description:** Human NEK1, N-terminal fragment, amino acids M<sub>1</sub>-K<sub>505</sub> (as in [NCBI/Protein](#) entry NP\_036356.1), N-terminal GST-HIS<sub>6</sub> fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

**Product identity:** NEK1 Lot 004, was confirmed as NEK1 by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW**<sub>Fusion Protein</sub>: 86,316 Da

**Expression host:** Sf9 insect cells

**Purification:** GST-Affinity Chromatography

**Activation:** in vitro auto activation

**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:** 0.253 µg/µl

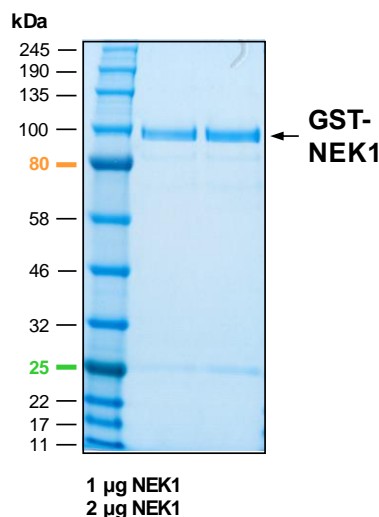
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

**Biochemical Parameters:**

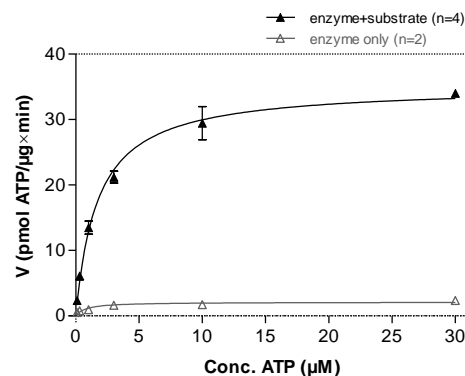
Specific kinase activity (P<sub>i</sub> transfer): 35 pmol/µg × min

ATP-K<sub>M</sub>: 1.7 µM

### NEK1 Lot 004: Coomassie stain



### NEK1 Lot 004: 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: RBER-GSK3 40 µg/ml
  - Kinase: 0.5 µg/ml
- Filter binding assay
- MSFC membrane (Millipore)

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GST-NEK1 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG <b>HHHHHG</b>	RDS <b>LEVL</b> <b>FQ</b>	240
241	<b>PLAMVMEKYV</b>	<b>RLQKIGEGSF</b>	<b>GKAILVKSTE</b>	<b>DGRQYVIKEI</b>	<b>NISMSSKER</b>	<b>EESRREVAVL</b>	300
301	<b>ANMKHPNIVQ</b>	<b>YRESFEENG</b>	<b>LYIVMDYCEG</b>	<b>GDLFKRINAQ</b>	<b>KGVL</b> <b>FQEDQI</b>	<b>LDW</b> <b>FVQICLA</b>	360
361	<b>LKHVHDRKIL</b>	<b>HRDIKSONIF</b>	<b>LTKDGTVQLG</b>	<b>DFGIARVLS</b>	<b>TVELARTCIG</b>	<b>TPY</b> <b>LSPEIC</b>	420
421	<b>ENKPYNNKSD</b>	<b>IWALGCVLYE</b>	<b>LCTLKHAFEA</b>	<b>GSMKNLVLKI</b>	<b>ISGSFPPVSL</b>	<b>HYSYDLRSLV</b>	480
481	<b>SQLFKRNPRD</b>	<b>RPSVNSILEK</b>	<b>GFI</b> <b>AKRIEK</b>	<b>LSPQLIAEEF</b>	<b>CLKT</b> <b>FSKFGS</b>	<b>QPI</b> <b>PAKRPAS</b>	540
541	<b>GQNSISVMPA</b>	<b>QKITKPAKY</b>	<b>GIPLAYKYG</b>	<b>DKKLHEKPL</b>	<b>QKHQ</b> <b>QAHTP</b>	<b>EKR</b> <b>VNTGEER</b>	600
600	<b>KKISEEAARK</b>	<b>RRLEFIEKEK</b>	<b>KQKQIISLM</b>	<b>KAEQMKRQEK</b>	<b>ERLERINRAR</b>	<b>EQG</b> <b>WRNVLSA</b>	660
661	<b>GGSGEVKAPF</b>	<b>LGS</b> <b>GTIAPS</b>	<b>SFSSRGYEH</b>	<b>YHAIFDQMQQ</b>	<b>QRAEDNEAKW</b>	<b>KREI</b> <b>YGRGLP</b>	720
721	<b>ERGILPGVVRP</b>	<b>GF</b> <b>PYGAAGHH</b>	<b>HFPDADDIRK</b>				780

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: NEK1 fragment

NEK1 wt <sup>1</sup> Amino Acid Sequence							
1	<b>MEKYVRLQKI</b>	<b>GE</b> <b>SGFGKAIL</b>	<b>VK</b> <b>STEDGRQY</b>	<b>VI</b> <b>KEINISRM</b>	<b>SS</b> <b>KEREESRR</b>	<b>EV</b> <b>AVLANMKH</b>	60
61	<b>PNIVQYRESF</b>	<b>EENG</b> <b>SLYIVM</b>	<b>DY</b> <b>CEGGDLFK</b>	<b>RINAQ</b> <b>GVLF</b>	<b>QEDQ</b> <b>ILDWFV</b>	<b>QICL</b> <b>LAKHVV</b>	120
121	<b>DRKILHRDIK</b>	<b>SNIF</b> <b>LTKDG</b>	<b>TV</b> <b>QLGDFGIA</b>	<b>RVLN</b> <b>STVELA</b>	<b>RTCIG</b> <b>TPYYL</b>	<b>SPEI</b> <b>CENKPY</b>	180
181	<b>NNKSDI</b> <b>WALG</b>	<b>CV</b> <b>LYELCTLK</b>	<b>HAF</b> <b>EAGSMKN</b>	<b>LVLK</b> <b>IISGSF</b>	<b>PPV</b> <b>SLHYSYD</b>	<b>LRS</b> <b>LVSQLFK</b>	240
241	<b>RNPRDR</b> <b>PSVN</b>	<b>SILE</b> <b>KGFIAK</b>	<b>RIE</b> <b>KFLSPQL</b>	<b>IAE</b> <b>EFCLKTF</b>	<b>SK</b> <b>FGSQPIPA</b>	<b>KRP</b> <b>ASGQNSI</b>	300
301	<b>SVMPAQKITK</b>	<b>PA</b> <b>KYGIPLA</b>	<b>YK</b> <b>YGDKKLH</b>	<b>EKK</b> <b>PLQKHKQ</b>	<b>AH</b> <b>QTPEKRVN</b>	<b>TGE</b> <b>ERRKISE</b>	360
361	<b>EAARKR</b> <b>RLEF</b>	<b>IE</b> <b>KEKQKQDQ</b>	<b>II</b> <b>SLMKAEQM</b>	<b>KR</b> <b>QEKERLER</b>	<b>IN</b> <b>RAREQGW</b>	<b>NV</b> <b>LSAGSGE</b>	420
421	<b>VKAPFL</b> <b>GSGG</b>	<b>TI</b> <b>APSSFSSR</b>	<b>GQ</b> <b>YEHYHAIF</b>	<b>DQ</b> <b>MQQQRAED</b>	<b>NE</b> <b>AKWKREIY</b>	<b>GR</b> <b>GLPERGIL</b>	480
481	<b>PGVVRP</b> <b>GFPGY</b>	<b>AAG</b> <b>HHFPA</b>	<b>DDIRK</b> <b>TLKRL</b>	<b>KAV</b> <b>SKQANAN</b>	<b>RQ</b> <b>KQLAVER</b>	<b>AK</b> <b>QVEEFLQR</b>	540
541	<b>KREAMQ</b> <b>NKAR</b>	<b>AEG</b> <b>HMVYLAR</b>	<b>LR</b> <b>QIRLQNFN</b>	<b>ER</b> <b>QIKAKLR</b>	<b>GE</b> <b>KKEANHSE</b>	<b>GQ</b> <b>EGSSEADM</b>	600
600	<b>RRKKIE</b> <b>SLKA</b>	<b>HAN</b> <b>ARA</b> <b>AVLK</b>	<b>EQ</b> <b>LERKRKEA</b>	<b>YER</b> <b>EKKVWEE</b>	<b>HL</b> <b>VAKGVKSS</b>	<b>DV</b> <b>SPPLGQHE</b>	660
661	<b>TGGSP</b> <b>SKQQM</b>	<b>RS</b> <b>VISVTSAL</b>	<b>KE</b> <b>VGVDSSLT</b>	<b>DT</b> <b>RETSEEMQ</b>	<b>KT</b> <b>NNAISSKR</b>	<b>EIL</b> <b>RRLNENL</b>	720
721	<b>KAQED</b> <b>EKGKQ</b>	<b>NLS</b> <b>DTFEINV</b>	<b>HE</b> <b>DAKEHEKE</b>	<b>KSV</b> <b>SSDRKKW</b>	<b>EAG</b> <b>GQLVIPL</b>	<b>DEL</b> <b>TLDTFS</b>	780
781	<b>TTERHT</b> <b>VGEV</b>	<b>IK</b> <b>LGPNGSPR</b>	<b>RAW</b> <b>GKSP</b> <b>TDS</b>	<b>VL</b> <b>KILGEAEL</b>	<b>QL</b> <b>QTELLENT</b>	<b>TIR</b> <b>SEISPEG</b>	840
841	<b>EKYK</b> <b>PLITGE</b>	<b>KK</b> <b>VQCISHEI</b>	<b>NPS</b> <b>AIVDSPV</b>	<b>ET</b> <b>KSP</b> <b>EFSEA</b>	<b>SP</b> <b>QMSLKLEG</b>	<b>NLE</b> <b>EPDDLET</b>	900
901	<b>EILQ</b> <b>EPSGTN</b>	<b>KDE</b> <b>SLPCTIT</b>	<b>DV</b> <b>WISEEKET</b>	<b>KET</b> <b>Q</b> <b>SADRIT</b>	<b>IQ</b> <b>ENEVSE</b> <b>EDG</b>	<b>VS</b> <b>STVDQLSD</b>	960
961	<b>IHI</b> <b>EPGT</b> <b>NDS</b>	<b>QHS</b> <b>KCDVDKS</b>	<b>VQ</b> <b>PEPFFHKV</b>	<b>VH</b> <b>SEHLN</b> <b>LVP</b>	<b>QV</b> <b>QSVQCSPE</b>	<b>ES</b> <b>FAFRSHSH</b>	1020
1021	<b>LPP</b> <b>KNKNKNS</b>	<b>LL</b> <b>IGLSTGLF</b>	<b>DAN</b> <b>NP</b> <b>KMLRT</b>	<b>CS</b> <b>LPDLSKLF</b>	<b>RT</b> <b>LMDVPTVG</b>	<b>DVR</b> <b>QDNLEID</b>	1080
1081	<b>EIE</b> <b>DENIK</b> <b>EG</b>	<b>PS</b> <b>DSE</b> <b>DIVFE</b>	<b>ET</b> <b>DTDLQELQ</b>	<b>AS</b> <b>MEQLLREQ</b>	<b>PGE</b> <b>EYSEEEE</b>	<b>SV</b> <b>LKNSDVEP</b>	1140
1141	<b>TAN</b> <b>GT</b> <b>DVADE</b>	<b>DD</b> <b>NPSSESAL</b>	<b>NEE</b> <b>WHS</b> <b>DN</b> <b>SD</b>	<b>GE</b> <b>IA</b> <b>SECECD</b>	<b>SV</b> <b>FNHLEELR</b>	<b>LH</b> <b>LEQEMGFE</b>	1200
1201	<b>KFF</b> <b>EVY</b> <b>EKIK</b>	<b>AI</b> <b>HEDE</b> <b>DENI</b>	<b>EI</b> <b>CS</b> <b>KIVQNI</b>	<b>LG</b> <b>NEHQHLYA</b>	<b>KIL</b> <b>LH</b> <b>VMADG</b>	<b>AY</b> <b>QEDNDE</b>	1260

**blue**: NEK1 sequence expressed in recombinant protein

<sup>1</sup>[NCBI/Protein](#) accession number NP\_036356.1