# Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

# ProQinase™ NEK7

**NIMA related kinase 7**

**Recombinant Human Active Protein Kinase**

**HGNC Symbol:** NEK7

**Synonyms:** n/a

**Product No.:** 0669-0000-1

**Lot:** 002

**Description:** Human NEK7, amino acids $M_1$-$S_{302}$ (as in NCBI/Protein entry NP_598001.1), N-terminal GST-$HIS_6$ fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

**Product identity:** NEK7 Lot 002, was confirmed as NEK7 by mass spectroscopy LC-ESI-MS/MS

**Theoretical $MW_{Fusion\ Protein}$:** 65,270 Da

**Expression host:** Sf9 insect cells

**Purification:** GST-Affinity Chromatography

**Activation:** This kinase was not activated by special procedures

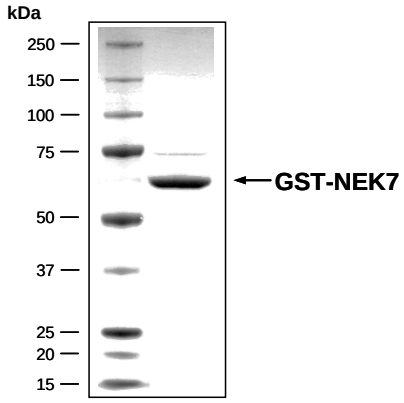
**Storage buffer:** 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

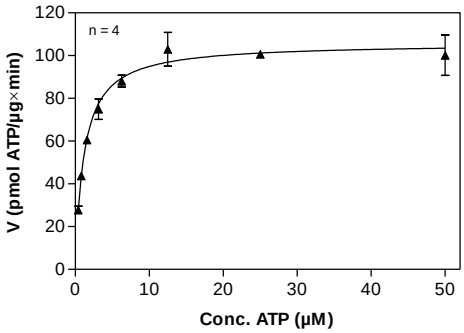
**Biochemical Parameters:**
Specific kinase activity ($P_i$ transfer): 106 pmol/µg × min
ATP-$K_M$: 1.1 µM

**NEK7 Lot 002:**
**Coomassie stain**




**2.0 µg GST-NEK7**

**NEK7 Lot 002:**
**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: Casein 100 µg/ml
  Kinase: 1 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

**Additional assay technology:**
NEK7 Lot 002 was also successfully tested by Reaction Biology 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

# ProQinase™ NEK7

**Product No.:** 0669-0000-1

**GST-NEK7 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 GIYARDSLEV LFQGPLAMDE QSQGMQGPPV PQFQPQKALR PDMGYNTLAN | 300 |
| 301 | FRIEKKIGRG QFSEVYRAAC LLDGVPVALK KVQIFDLMDA KARADCIKEI DLLKQLNHPN | 360 |
| 361 | VIKYYASFIE DNELNIVLEL ADAGDLSRMI KHFKKQKRLI PERTVWKYFV QLCSALEHMH | 420 |
| 421 | SRRVMHRDIK PANVFITATG VVKLGDLGLG RFFSSKTTAA HSLVGTPYYM SPERIHENGY | 480 |
| 481 | NFKSDIWSLG CLLYEMAALQ SPFYGDKMNL YSLCKKIEQC DYPPLPSDHY SEELRQLVNM | 540 |
| 541 | CINPDPEKRP DVTYVYDVAK RMHACTASS | 600 |

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **Green**: 3C cleavage site **blue**: NEK7

**NEK7 wt[1] Amino Acid Sequence**

| | | |
|---|---|---|
| 1 | MDEQSQGMQG PPVPQFQPQK ALRPDMGYNT LANFRIEKKI GRGQFSEVYR AACLLDGVPV | 60 |
| 61 | ALKKVQIFDL MDAKARADCI KEIDLLKQLN HPNVIKYYAS FIEDNELNIV LELADAGDLS | 120 |
| 121 | RMIKHFKKQK RLIPERTVWK YFVQLCSALE HMHSRRVMHR DIKPANVFIT ATGVVKLGDL | 180 |
| 181 | GLGRFFSSKT TAAHSLVGTP YYMSPERIHE NGYNFKSDIW SLGCLLYEMA ALQSPFYGDK | 240 |
| 241 | MNLYSLCKKI EQCDYPPLPS DHYSEELRQL VNMCINPDPE KRPDVTYVYD VAKRMHACTA | 300 |
| 301 | SS | 360 |

**blue**: NEK7 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_598001.1

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