Certificate of Analysis

REACTION BIOLOGY

ProQinase™ NEK8

NIMA related kinase 8

Recombinant Human Active Protein Kinase

HGNC Symbol: NEK8

Synonyms: NPHP9

Product No.: 2015-0000-1

Lot: 001

Description: Human NEK8, N-terminal fragment, amino acids M_1-L_{265} (as in NCBI/Protein entry NP_835464.1), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: NEK8 Lot 001, has been verified by mass spectrometry LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 58198 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: in vitro auto activation

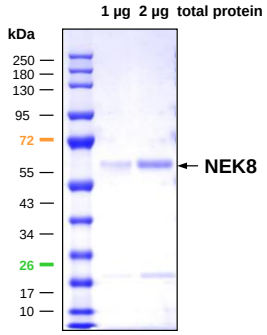
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

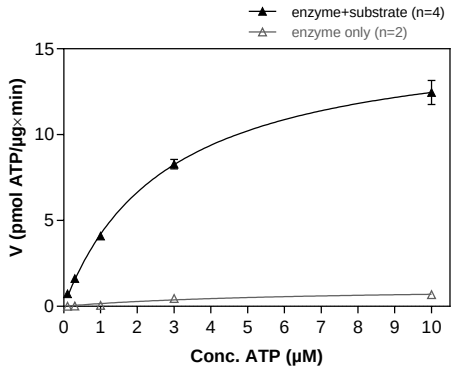
Protein concentration: 0.57 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 16 pmol/µg*min
ATP-K_M: 2.8 µM

NEK8 Lot 001:
Coomassie stain



NEK8 Lot 001:
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: Casein 40 µg/ml
  Kinase: 4 µg/ml

Assay technology:
Radiometric filter binding assay
MSFC membrane (96 well plate, Millipore)

Recombinant Proteins

Sequence information

GST-NEK8 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  PMEKYERIRV VGRGAFGIVH LCLRKADQKL VIIKQIPVEQ MTKEERQAAQ NECQVLKLLN 300
301  HPNVIEYYEN FLEDKALMIA MEYAPGGTLA EFIQKRCNSL LEEETILHFF VQILLALHHV 360
361  HTHLILHRDL KTQNILLDKH RMVVKIGDFG ISKILSSKSK AYTVVGTPCY ISPELCEGKP 420
421  YNQKSDIWAL GCVLYELASL KRAFEAANLP ALVLKIMSGT FAPISDRYSP ELRQLVLSLL 480
481  SLEPAQRPPL SHIMAQPLCI RALLNL                                      540
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1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: NEK8 fragment

NEK8 wt[1] Amino Acid Sequence

```
1    MEKYERIRVV GRGAFGIVHL CLRKADQKLV IIKQIPVEQM TKEERQAAQN ECQVLKLLNH  60
61   PNVIEYYENF LEDKALMIAM EYAPGGTLAE FIQKRCNSLL EEETILHFFV QILLALHHVH 120
121  THLILHRDLK TQNILLDKHR MVVKIGDFGI SKILSSKSKA YTVVGTPCYI SPELCEGKPY 180
181  NQKSDIWALG CVLYELASLK RAFEAANLPA LVLKIMSGTF APISDRYSPE LRQLVLSLLS 240
241  LEPAQRPPLS HIMAQPLCIR ALLNLHTDVG SVRMRRAEKS VAPSNTGSRT TSVRCRGIPR 300
301  GPVRPAIPPP LSSVYAWGGG LGTPLRLPML NTEVVQVAAG RTQKAGVTRS GRLILWEAPP 360
361  LGAGGGSLLP GAVEQPQPQF ISRFLEGQSG VTIKHVACGD FFTACLTDRG IIMTFGSGSN 420
421  GCLGHGSLTD ISQPTIVEAL LGYEMVQVAC GASHVLALST ERELFAWGRG DSGRLGLGTR 480
481  ESHSCPQQVP MPPGQEAQRV VCGIDSSMIL TVPGQALACG SNRFNKLGLD HLSLGEEPVP 540
541  HQQVEEALSF TLLGSAPLDQ EPLLSIDLGT AHSAAVTASG DCYTFGSNQH GQLGTNTRRG 600
601  SRAPCKVQGL EGIKMAMVAC GDAFTVAIGA ESEVYSWGKG ARGRLGRRDE DAGLPRPVQL 660
661  DETHPYTVTS VSCCHGNTLL AVRSVTDEPV PP                               720
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blue: NEK8 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_835464.1

Recombinant Proteins