Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ WNK1

WNK lysine deficient protein kinase 1

Recombinant Human Active Protein Kinase

HGNC Symbol: WNK1

Synonyms: HSAN2, HSN2, hWNK1, KDP, p65, PRKWNK1, PSK

Product No.: 1111-0000-1

Lot: 002

Description: Human WNK1, internal fragment, amino acids L_{171}-E_{529} (as in NCBI/Protein entry NP_061852.3), N-terminal 4xFLAG, C-terminal HIS_8 fusion protein, expressed in Sf9 insect cells

Product identity: WNK1 Lot 002, was confirmed as WNK1 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 47,013 Da

Expression host: Sf9 insect cells

Purification: Immobilized Metal Affinity Chromatography

Activation: This kinase was not activated by special procedures

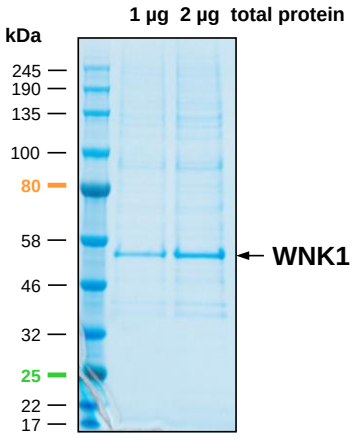
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 20 % glycerol

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!

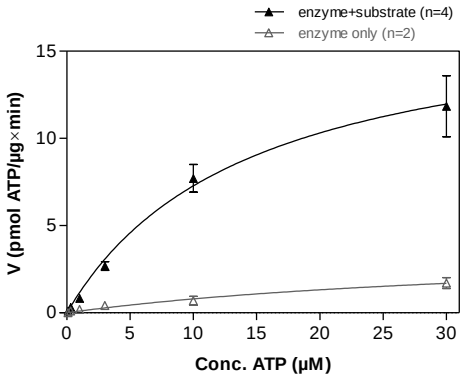
Protein concentration: 0.553 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 18 pmol/µg × min
ATP-K_M: 14 µM

WNK1 Lot 002:
Coomassie stain



WNK1 Lot 002:
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: Bio-RS-Peptide 80 µg/ml
  Kinase: 4 µg/ml
- Filter binding assay
  MSIP membrane (Millipore)

Additional assay technology:
WNK1 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

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HIS-WNK1 Recombinant Fusion Protein Amino Acid Sequence

```
   1  MDYKDDDDKD YKDDDDKDYK DDDDKDYKDD DDKSGGGSLV GSKEEPPPAR SGSGGGSAKE  60
  61  PQEERSQQQD DIEELETKAV GMSNDGRFLK FDIEIGRGSF KTVYKGLDTE TTVEVAWCEL 120
 121  QDRKLTKSER QRFKEEAEML KGLQHPNIVR FYDSWESTVK GKKCIVLVTE LMTSGTLKTY 180
 181  LKRFKVMKIK VLRSWCRQIL KGLQFLHTRT PPIIHRDLKC DNIFITGPTG SVKIGDLGLA 240
 241  TLKRASFAKS VIGTPEFMAP EMYEEKYDES VDVYAFGMCM LEMATSEYPY SECQNAAQIY 300
 301  RRVTSGVKPA SFDKVAIPEV KEIIEGCIRQ NKDERYSIKD LLNHAFFQEE TGVRVELAEE 360
 361  DDGEKIAIKL WLRIEDIKKL KGKYKDNEAI EFSFDLEKLL EHHHHHHHH            420
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Pink: 4xFLAG-tag blue: WNK1 fragment Red: HIS8-tag

WNK1 wt[1] Amino Acid Sequence

```
1     MSGGAAEKQS STPGSLFLSP PAPAPKNGSS SDSSVGEKLG AAAADAVTGR TEEYRRRRHT  60
61    MDKDSRGAAA TTTTTEHRFF RRSVICDSNA TALELPGLPL SLPQPSIPAA VPQSAPPEPH 120
121   REETVTATAT SQVAQQPPAA AAPGEQAVAG PAPSTVPSST SKDRPVSQPS LVGSKEEPPP 180
181   ARSGSGGGSA KEPQEERSQQ QDDIEELETK AVGMSNDGRF LKFDIEIGRG SFKTVYKGLD 240
241   TETTVEVAWC ELQDRKLTKS ERQRFKEEAE MLKGLQHPNI VRFYDSWEST VKGKKCIVLV 300
301   TELMTSGTLK TYLKRFKVMK IKVLRSWCRQ ILKGLQFLHT RTPPIIHRDL KCDNIFITGP 360
361   TGSVKIGDLG LATLKRASFA KSVIGTPEFM APEMYEEKYD ESVDVYAFGM CMLEMATSEY 420
421   PYSECQNAAQ IYRRVTSGVK PASFDKVAIP EVKEIIEGCI RQNKDERYSI KDLLNHAFFQ 480
481   EETGVRVELA EEDDGEKIAI KLWLRIEDIK KLKGKYKDNE AIEFSFDLER DVPEDVAQEM 540
541   VESGYVCEGD HKTMAKAIKD RVSLIKRKRE QRQLVREEQE KKKQEESSLK QQVEQSSASQ 600
601   TGIKQLPSAS TGIPTASTTS ASVSTQVEPE EPEADQHQQL QYQQPSISVL SDGTVDSGQG 660
661   SSVFTESRVS SQQTVSYGSQ HEQAHSTGTV PGHIPSTVQA QSQPHGVYPP SSVAQGQSQG 720
721   QPSSSSLTGV SSSQPIQHPQ QQQGIQQTAP PQQTVQYSLS QTSTSSEATT AQPVSQPQAP 780
781   QVLPQVSAGK QLPVSQPVPT IQGEPQIPVA TQPSVVPVHS GAHFLPVGQP LPTPLLPQYP 840
841   VSQIPISTPH VSTAQTGFSS LPITMAAGIT QPLLTLASSA TTAAIPGVST VVPSQLPTLL 900
901   QPVTQLPSQV HPQLLQPAVQ SMGIPANLGQ AAEVPLSSGD VLYQGFPPRL PPQYPGDSNI 960
961   APSSNVASVC IHSTVLSPPM PTEVLATPGY FPTVVQPYVE SNLLVPMGGV GGQVQVSQPG 1020
1021  GSLAQAPTTS SQQAVLESTQ GVSQVAPAEP VAVAQTQATQ PTTLASSVDS AHSDVASGMS 1080
1081  DGNENVPSSS GRHEGRTTKR HYRKSVRSRS RHEKTSRPKL RILNVSNKGD RVVECQLETH 1140
1141  NRKMVTFKFD LDGDNPEEIA TIMVNNDFIL AIERESFVDQ VREIIEKADE MLSEDVSVEP 1200
1201  EGDQGLESLQ GKDDYGFSGS QKLEGEFKQP IPASSMPQQI GIPTSSLTQV VHSAGRRFIV 1260
1261  SPVPESRLRE SKVFPSEITD TVAASTAQSP GMNLSHSASS LSLQQAFSEL RRAQMTEGPN 1320
1321  TAPPNFSHTG PTFPVVPPFL SSIAGVPTTA AATAPVPATS SPPNDISTSV IQSEVTVPTE 1380
1381  EGIAGVATST GVVTSGGLPI PPVSESPVLS SVVSSITIPA VVSISTTSPS LQVPTSTSEI 1440
1441  VVSSTALYPS VTVSATSASA GGSTATPGPK PPAVVSQQAA GSTTVGATLT SVSTTTSFPS 1500
1501  TASQLCIQLS SSTSTPTLAE TVVVSAHSLD KTSHSSTTGL AFSLSAPSSS SSPGAGVSSY 1560
1561  ISQPGGLHPL VIPSVIASTP ILPQAAGPTS TPLLPQVPSI PPLVQPVANV PAVQQTLIHS 1620
1621  QPQPALLPNQ PHTHCPEVDS DTQPKAPGID DIKTLEEKLR SLFSEHSSSG AQHASVSLET 1680
1681  SLVIESTVTP GIPTTAVAPS KLLTSTTSTC LPPTNLPLGT VALPVTPVVT PGQVSTPVST 1740
1741  TTSGVKPGTA PSKPPLTKAP VLPVGTELPA GTLPSEQLPP FPGPSLTQSQ QPLEDLDAQL 1800
1801  RRTLSPEMIT VTSAVGPVSM AAPTAITEAG TQPQKGVSQV KEGPVLATSS GAGVFKMGRF 1860
1861  QVSVAADGAQ KEGKNKSEDA KSVHFESSTS ESSVLSSSSP ESTLVKPEPN GITIPGISSD 1920
1921  VPESAHKTTA SEAKSDTGQP TKVGRFQVTT TANKVGRFSV SKTEDKITDT KKEGPVASPP 1980
1981  FMDLEQAVLP AVIPKKEKPE LSEPSHLNGP SSDPEAAFLS RDVDDGSGSP HSPHQLSSKS 2040
2041  LPSQNLSQSL SNSFNSSYMS SDNESDIEDE DLKLELRRLR DKHLKEIQDL QSRQKHEIES 2100
2101  LYTKLGKVPP AVIIPPAAPL SGRRRRPTKS KGSKSSRSSS LGNKSPQLSG NLSGQSAASV 2160
2161  LHPQQTLHPP GNIPESGQNQ LLQPLKPSPS SDNLYSAFTS DGAISVPSLS APGQGTSSTN 2220
2221  TVGATVNSQA AQAQPPAMTS SRKGTFTDDL HKLVDNWARD AMNLSGRRGS KGHMNYEGPG 2280
2281  MARKFSAPGQ LCISMTSNLG GSAPISAASA TSLGHFTKSM CPPQQYGFPA TPFGAQWSGT 2340
2341  GGPAPQPLGQ FQPVGTASLQ NFNISNLQKS ISNPPGSNLR TT                    2400
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blue: WNK1 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_061852.3

Recombinant Proteins