Certificate of Analysis

ProQinase
TARGETING CANCER

VRK2

Vaccinia related kinase 2

Recombinant Human Active Protein Kinase

HGNC Symbol: VRK2

Synonyms: n.a.

Product No.: 1520-0000-1

Lot: 002

Description: Human VRK2, N-terminal fragment, amino acids M_1-T_{483} (as in NCBI/Protein entry NP_006287.2), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: VRK2 Lot 002, was confirmed as VRK2 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 83,227 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

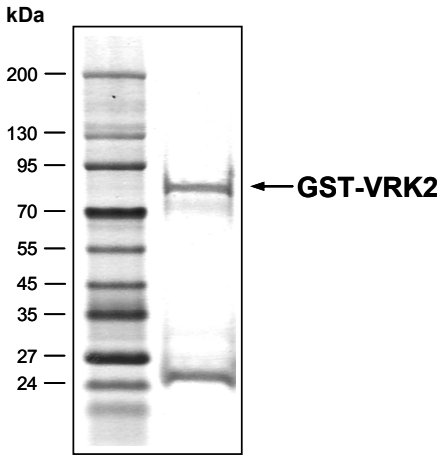
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

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

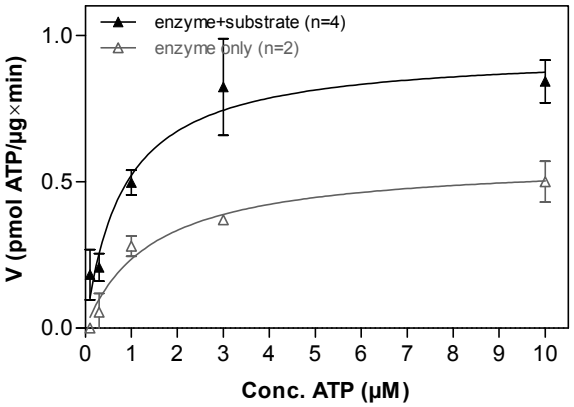
Biochemical Parameters:
Specific kinase activity (P_i transfer): 1.0 pmol/µg×min
ATP-K_M: 0.8 µM

VRK2 Lot 002:
Coomassie stain



2.0 µg GST-VRK2

VRK2 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, 20 µg / ml
  VRK2: 2.0 µg / ml
- Filter binding assay
  MSFC membrane (Millipore)

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VRK2 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  PMPPKRNEKY KLPIPFPEGK VLDDMEGNQW VLGKKIGSGG FGLIYLAFPT NKPEKDARHV 300
301  VKVEYQENGP LFSELKFYQR VAKKDCIKKW IERKQLDYLG IPLFYGSGLT EFKGRSYRFM 360
361  VMERLGIDLQ KISGQNGTFK KSTVLQLGIR MLDVLEYIHE NEYVHGDIKA ANLLLGYKNP 420
421  DQVYLADYGL SYRYCPNGNH KQYQENPRKG HNGTIEFTSL DAHKGVALSR RSDVEILGYC 480
481  MLRWLCGKLP WEQNLKDPVA VQTAKTNLLD ELPQSVLKWA PSGSSCCEIA QFLVCAHSLA 540
541  YDEKPNYQAL KKILNPHGIP LGPLDFSTKG QSINVHTPNS QKVDSQKAAT KQVNKAHNRL 600
601  IEKKVHSERS AESCATWKVQ KEEKLIGLMN NEAAQESTRR RQKYQESQEP LNEVNSFPQK 660
661  ISYTQFPNSF YEPHQDFTSP DIFKKSRSPS WYKYTSTVST GITDLESSTG LWPTISQFTL 720
721  SEET                                                              780
```

1-218: GST **Red: HIS6-tag** **Green:** 3C cleavage site **blue**:**VRK2 fragment**

VRK2 wt[1] Amino Acid Sequence

```
1    MPPKRNEKYK LPIPFPEGKV LDDMEGNQWV LGKKIGSGGF GLIYLAFPTN KPEKDARHVV  60
61   KVEYQENGPL FSELKFYQRV AKKDCIKKWI ERKQLDYLGI PLFYGSGLTE FKGRSYRFMV 120
121  MERLGIDLQK ISGQNGTFKK STVLQLGIRM LDVLEYIHEN EYVHGDIKAA NLLLGYKNPD 180
181  QVYLADYGLS YRYCPNGNHK QYQENPRKGH NGTIEFTSLD AHKGVALSRR SDVEILGYCM 240
241  LRWLCGKLPW EQNLKDPVAV QTAKTNLLDE LPQSVLKWAP SGSSCCEIAQ FLVCAHSLAY 300
301  DEKPNYQALK KILNPHGIPL GPLDFSTKGQ SINVHTPNSQ KVDSQKAATK QVNKAHNRLI 360
361  EKKVHSERSA ESCATWKVQK EEKLIGLMNN EAAQESTRRR QKYQESQEPL NEVNSFPQKI 420
421  SYTQFPNSFY EPHQDFTSPD IFKKSRSPSW YKYTSTVSTG ITDLESSTGL WPTISQFTLS 480
481  EETNADVYYY RIIIPVLLML VFLALFFL                                    540
```

blue: VRK2 sequence expressed in fusionprotein

[1]NCBI/Protein accession number NP_006287.2

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