Certificate of Analysis

ProQinase
TARGETING CANCER

RIPK4

Receptor-interacting serine-threonine kinase 4

Recombinant Human Active Protein Kinase

HGNC Symbol: RIPK4

Synonyms: ANKK2, ANKRD3, DIK, PKK, RIP4

Product No.: 1518-0000-1

Lot: 007

Description: Human RIPK4, N-terminal fragment, amino acids M_1-D_{300} (as in NCBI/Protein entry NP_065690.2), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: RIPK4 Lot 007, was confirmed as RIPK4 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 63,283 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

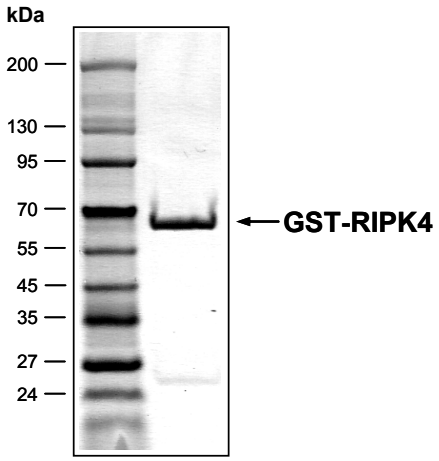
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

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

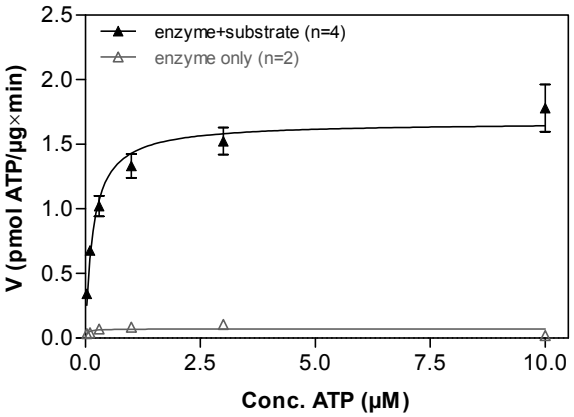
Biochemical Parameters:
Specific kinase activity (P_i transfer): 2 pmol/µg×min
ATP-K_M: 0.17 µM

RIPK4 Lot 007:
Coomassie stain



2.0 µg GST-RIPK4

RIPK4 Lot 007:
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
  RIPK4: 4.0 µg / ml
- Filter binding assay
  MSFC membrane (Millipore)

Additional assay technology: RIPK4 Lot 007
was also successfully tested by ProQinase 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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RIPK4

Product No.: 1518-0000-1

RIPK4 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  PLAMGARGRM EGDGGTPWAL ALLRTFDAGE FTGWEKVGSG GFGQVYKVRH VHWKTWLAIK  300
 301  CSPSLHVDDR ERMELLEEAK KMEMAKFRYI LPVYGICREP VGLVMEYMET GSLEKLLASE  360
 361  PLPWDLRFRI IHETAVGMNF LHCMAPPLLH LDLKPANILL DAHYHVKISD FGLAKCNGLS  420
 421  HSHDLSMDGL FGTIAYLPPE RIREKSRLFD TKHDVYSFAI VIWGVLTQKK PFADEKNILH  480
 481  IMVKVVKGHR PELPPVCRAR PRACSHLIRL MQRCWQGDPR VRPTFQEITS ETEDLCEKPD  540
 541  DEVKETAHD                                                         600
```

1-218: GST **Red: HIS6-tag** **Green:** 3C cleavage site **blue:RIPK4 fragment**

RIPK4 wt[1] Amino Acid Sequence

```
1    MEGDGGTPWA LALLRTFDAG EFTGWEKVGS GGFGQVYKVR HVHWKTWLAI KCSPSLHVDD   60
61   RERMELLEEA KKMEMAKFRY ILPVYGICRE PVGLVMEYME TGSLEKLLAS EPLPWDLRFR  120
121  IIHETAVGMN FLHCMAPPLL HLDLKPANIL LDAHYHVKIS DFGLAKCNGL SHSHDLSMDG  180
181  LFGTIAYLPP ERIREKSRLF DTKHDVYSFA IVIWGVLTQK KPFADEKNIL HIMVKVVKGH  240
241  RPELPPVCRA RPRACSHLIR LMQRCWQGDP RVRPTFQEIT SETEDLCEKP DDEVKETAHD  300
301  LDVKSPPEPR SEVVPARLKR ASAPTFDNDY SLSELLSQLD SGVSQAVEGP EELSRSSSES  360
361  KLPSSGSGKR LSGVSSVDSA FSSRGSLSLS FEREPSTSDL GTTDVQKKKL VDAIVSGDTS  420
421  KLMKILQPQD VDLALDSGAS LLHLAVEAGQ EECAKWLLLN NANPNLSNRR GSTPLHMAVE  480
481  RRVRGVVELL LARKISVNAK DEDQWTALHF AAQNGDESST RLLLEKNASV NEVDFEGRTP  540
541  MHVACQHGQE NIVRILLRRG VDVSLQGKDA WLPLHYAAWQ GHLPIVKLLA KQPGVSVNAQ  600
601  TLDGRTPLHL AAQRGHYRVA RILIDLCSDV NVCSLLAQTP LHVAAETGHT STARLLLHRG  660
661  AGKEAMTSDG YTALHLAARN GHLATVKLLV EEKADVLARG PLNQTALHLA AAHGHSEVVE  720
721  ELVSADVIDL FDEQGLSALH LAAQGRHAQT VETLLRHGAH INLQSLKFQG GHGPAATLLR  780
781  RSKT                                                              840
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blue: RIPK4 sequence expressed in fusionprotein

[1]NCBI/Protein accession number NP_065690.2

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