Certificate of Analysis

ProQinase
TARGETING CANCER

RIPK2

receptor-interacting serine-threonine kinase 2

Recombinant Human Active Protein Kinase

HGNC Symbol: RIPK2

Synonyms: CARD3; CARDIAK; CCK; GIG30; RICK; RIP-2; RIP2

Product No.: 0676-0000-1

Lot: 003

Description: Human RIPK2, N-terminal fragment, amino acids M_1-E_{299} (as in NCBI/Protein entry NP_003812.1), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: RIPK2, Lot 003, was confirmed as RIPK2 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 63,815 Da

Expression: Baculovirus infected Sf9 cells/E.coli

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

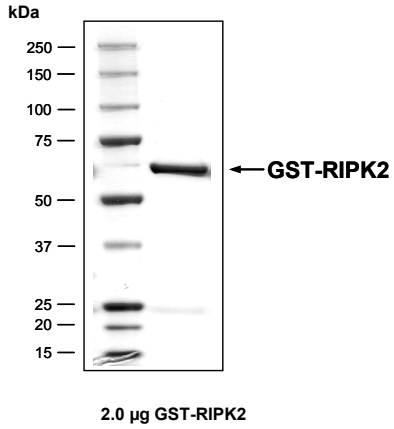
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20% glycerol

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

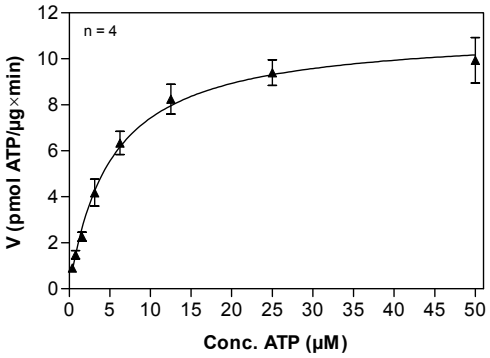
Biochemical Parameters:
Specific kinase activity (P_i transfer): 11 pmol/µg×min
ATP-K_M: 5.1 µM

RIPK2 Lot 003:
Coomassie stain



2.0 µg GST-RIPK2

RIPK2 Lot 003:
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: RBER-CHKtide 50 µg/ml
  Kinase: 1.0 µg/ml
- Filter binding assay
  MSFC membrane (Millipore)

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RIPK2 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 GIYARGMNGE AICSALPTIP YHKLADLRYL SRGASGTVSS ARHADWRVQV 300
301 AVKHLHIHTP LLDSERKDVL REAEILHKAR FSYILPILGI CNEPEFLGIV TEYMPNGSLN 360
361 ELLHRKTEYP DVAWPLRFRI LHEIALGVNY LHNMTPPLLH HDLKTQNILL DNEFHVKIAD 420
421 FGLSKWRMMS LSQSRSSKSA PEGGTIIYMP PENYEPGQKS RASIKHDIYS YAVITWEVLS 480
481 RKQPFEDVTN PLQIMYSVSQ GHRPVINEES LPYDIPHRAR MISLIESGWA QNPDERPSFL 540
541 KCLIELEPVL RTFEE                                                  600
```

1-218: GST **Red:** HIS6-tag **Pink:** Thrombin cleavage site **blue**:RIPK2 fragment

RIPK2 wt[1] Amino Acid Sequence

```
  1 MNGEAICSAL PTIPYHKLAD LRYLSRGASG TVSSARHADW RVQVAVKHLH IHTPLLDSER  60
 61 KDVLREAEIL HKARFSYILP ILGICNEPEF LGIVTEYMPN GSLNELLHRK TEYPDVAWPL 120
121 RFRILHEIAL GVNYLHNMTP PLLHHDLKTQ NILLDNEFHV KIADFGLSKW RMMSLSQSRS 180
181 SKSAPEGGTI IYMPPENYEP GQKSRASIKH DIYSYAVITW EVLSRKQPFE DVTNPLQIMY 240
241 SVSQGHRPVI NEESLPYDIP HRARMISLIE SGWAQNPDER PSFLKCLIEL EPVLRTFEEI 300
301 TFLEAVIQLK KTKLQSVSSA IHLCDKKKME LSLNIPVNHG PQEESCGSSQ LHENSGSPET 360
361 SRSLPAPQDN DFLSRKAQDC YFMKLHHCPG NHSWDSTISG SQRAAFCDHK TTPCSSAIIN 420
421 PLSTAGNSER LQPGIAQQWI QSKREDIVNQ MTEACLNQSL DALLSRDLIM KEDYELVSTK 480
481 PTRTSKVRQL LDTTDIQGEE FAKVIVQKLK DNKQMGLQPY PEILVVSRSP SLNLLQNKSM 540
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blue: RIPK2 sequence expressed in fusion protein

[1]NCBI/Protein accession number NP_003812.1