Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ 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

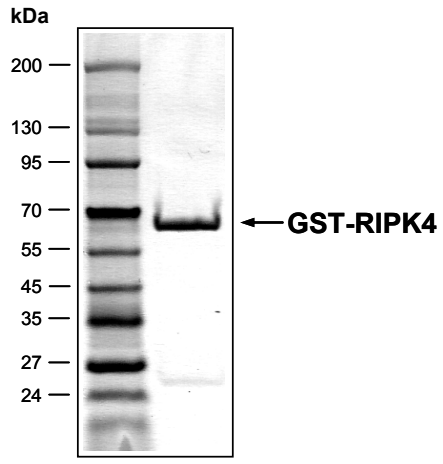
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

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)

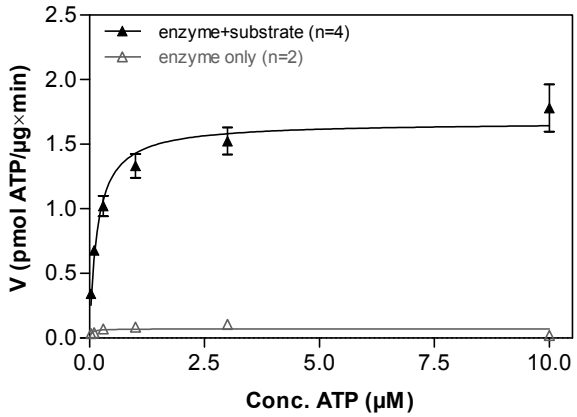
Biochemical Parameters:
Specific kinase activity (P_i transfer): 2 pmol/µg×min
ATP-K_M: 0.17 µM

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 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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ProQinase™ RIPK4

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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

blue: RIPK4 sequence expressed in fusionprotein

[1]NCBI/Protein accession number NP_065690.2

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