Certificate of Analysis

REACTION BIOLOGY

ProQinase™ PAK4

p21 protein (Cdc42/Rac)-activated kinase 4

Recombinant Human Active Protein Kinase

HGNC Symbol: PAK4

Synonyms: n/a

Product No.: 0365-0000-1

Lot: 004

Description: Human PAK4, full length, amino acids M_1-R_{591} (as in NCBI/Protein entry NP_005875.1), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: PAK4 Lot 004, was confirmed as PAK4 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 93,968 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

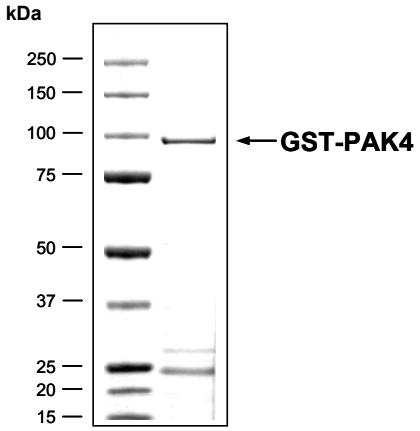
Storage buffer: 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20% glycerol

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

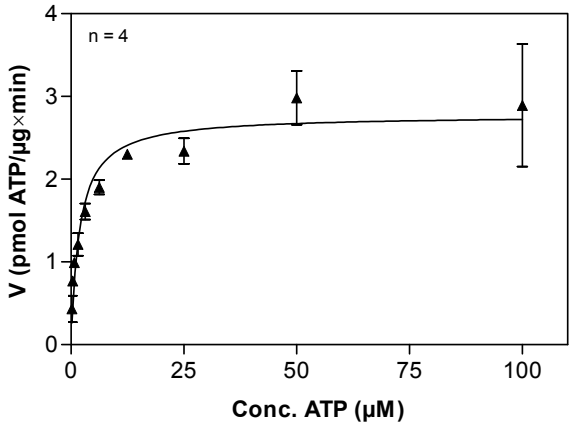
Biochemical Parameters:
Specific kinase activity (P_i transfer): 3 pmol/µg×min
ATP-K_M: 1.9 µM

PAK4 Lot 004:
Coomassie stain



2.0 µg GST-PAK4

PAK4 Lot 004:
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: tetra(LRRWSLG), 50 µg/ml
  PAK4: 4.0 µg/ml
- Filter binding assay
  MSPH membrane (Millipore)

Recombinant Proteins

ProQinase™ PAK4

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PAK4 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 GIYARGIQAS MFGKRKKRVE ISAPSNFEHR VHTGFDQHEQ KFTGLPRQWQ	300
301	SLIEESARRP KPLVDPACIT SIQPGAPKTI VRGSKGAKDG ALTLLLDEFE NMSVTRSNSL	360
361	RRDSPPPPAR ARQENGMPEE PATTARGGPG KAGSRGRFAG HSEAGGGSGD RRRAGPEKRP	420
421	KSSREGSGGP QESSRDKRPL SGPDVGTPQP AGLASGAKLA AGRPFNTYPR ADTDHPSRGA	480
481	QGEPHDVAPN GPSAGGLAIP QSSSSSSRPP TRARGAPSPG VLGPHASEPQ LAPPACTPAA	540
541	PAVPGPPGPR SPQREPQRVS HEQFRAALQL VVDPGDPRSY LDNFIKIGEG STGIVCIATV	600
601	RSSGKLVAVK KMDLRKQQRR ELLFNEVVIM RDYQHENVVE MYNSYLVGDE LWVVMEFLEG	660
661	GALTDIVTHT RMNEEQIAAV CLAVLQALSV LHAQGVIHRD IKSDSILLTH DGRVKLSDFG	720
721	FCAQVSKEVP RRKSLVGTPY WMAPELISRL PYGPEVDIWS LGIMVIEMVD GEPPYFNEPP	780
781	LKAMKMIRDN LPPRLKNLHK VSPSLKGFLD RLLVRDPAQR ATAAELLKHP FLAKAGPPAS	840
841	IVPLMRQNRT R	900

1-218: GST **Red:** HIS6-tag **Pink:** Thrombin cleavage site **blue**: PAK4

PAK4 wt[1] Amino Acid Sequence

1	MFGKRKKRVE ISAPSNFEHR VHTGFDQHEQ KFTGLPRQWQ SLIEESARRP KPLVDPACIT	60
61	SIQPGAPKTI VRGSKGAKDG ALTLLLDEFE NMSVTRSNSL RRDSPPPPAR ARQENGMPEE	120
121	PATTARGGPG KAGSRGRFAG HSEAGGGSGD RRRAGPEKRP KSSREGSGGP QESSRDKRPL	180
181	SGPDVGTPQP AGLASGAKLA AGRPFNTYPR ADTDHPSRGA QGEPHDVAPN GPSAGGLAIP	240
241	QSSSSSSRPP TRARGAPSPG VLGPHASEPQ LAPPACTPAA PAVPGPPGPR SPQREPQRVS	300
301	HEQFRAALQL VVDPGDPRSY LDNFIKIGEG STGIVCIATV RSSGKLVAVK KMDLRKQQRR	360
361	ELLFNEVVIM RDYQHENVVE MYNSYLVGDE LWVVMEFLEG GALTDIVTHT RMNEEQIAAV	420
421	CLAVLQALSV LHAQGVIHRD IKSDSILLTH DGRVKLSDFG FCAQVSKEVP RRKSLVGTPY	480
481	WMAPELISRL PYGPEVDIWS LGIMVIEMVD GEPPYFNEPP LKAMKMIRDN LPPRLKNLHK	540
541	VSPSLKGFLD RLLVRDPAQR ATAAELLKHP FLAKAGPPAS IVPLMRQNRT R	600

blue: PAK4 sequence expressed in fusionprotein

[1]NCBI/Protein accession number NP_005875.1

Recombinant Proteins