

Certificate of Analysis



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 M₁-R₅₉₁ (as in NCBI/Protein entry NP_005875.1), N-terminal GST-HIS₆ 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

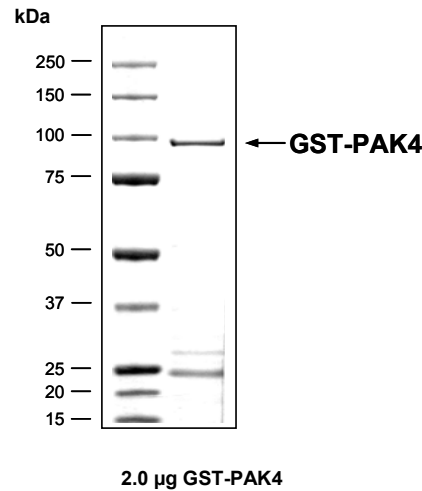
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

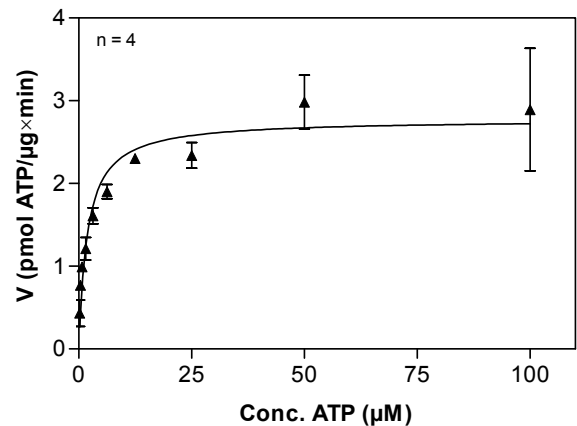
Specific kinase activity (P_i transfer): 3 pmol/µg×min

ATP-K_M: 1.9 µM

PAK4 Lot 004: Coomassie stain



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 MgCl₂
 - 3 mM MnCl₂
 - 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)

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PAK4 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHGG	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	SGPDVGTPOP	AGLASGAKLA	AGRPFNTPR	ADTDHPSRGA	480
481	QGEPHDVAPN	GPSAGGLAIP	QSSSSSRPP	TRARGAPSPG	VLGPHASEPQ	LAPPACTPAA	540
541	PAVPGPPGPR	SPQREPQRVS	HEQFRAALQL	VDPGDPRSY	LDNFIKIGEG	STGIVCIATV	600
601	RSSGKLVAVK	KMDLRKQQR	ELLFNEVVIM	RDYQHENVVE	MYNSYLVGDE	LWVMEFLEG	660
661	GALTDIVTHT	RMNEEQIAAV	CLAVLQALS	LHAQGVHRD	IKSDSILLTH	DGRVKLSDFG	720
721	FCAQVSKEVP	RRKSLVGTPT	WMAPELISRL	PYGPEVDIWS	LGIMVIEMVD	GEPPIYFNEPP	780
781	LKAMKMRDN	LPPRLKLNHK	VSPSLKGFLL	RLLVDPAPQR	ATAAELLKHP	FLAKAGPPAS	840
841	IVPLMRQNR	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	SGPDVGTPOP	AGLASGAKLA	AGRPFNTPR	ADTDHPSRGA	QGEPHDVAPN	GPSAGGLAIP	240
241	QSSSSSRPP	TRARGAPSPG	VLGPHASEPQ	LAPPACTPAA	PAVPGPPGPR	SPQREPQRVS	300
301	HEQFRAALQL	VDPGDPRSY	LDNFIKIGEG	STGIVCIATV	RSSGKLVAVK	KMDLRKQQR	360
361	ELLFNEVVIM	RDYQHENVVE	MYNSYLVGDE	LWVMEFLEG	GALTDIVTHT	RMNEEQIAAV	420
421	CLAVLQALS	LHAQGVHRD	IKSDSILLTH	DGRVKLSDFG	FCAQVSKEVP	RRKSLVGTPT	480
481	WMAPELISRL	PYGPEVDIWS	LGIMVIEMVD	GEPPIYFNEPP	LKAMKMRDN	LPPRLKLNHK	540
541	VSPSLKGFLL	RLLVDPAPQR	ATAAELLKHP	FLAKAGPPAS	IVPLMRQNR	R	600

blue: PAK4 sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_005875.1