

Certificate of Analysis



PRK1

Protein-kinase C-related kinase 1

Recombinant Human Active Protein Kinase

HGNC Symbol: PKN1

Synonyms: PRKCL1, DBK, PAK1, PKN

Product No.: 0207-0000-1

Lot: 004

Description: Human PRK1, full length, amino acids M₁-C₉₄₂ (as in NCBI/Protein entry NP_002732.1), N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: PRK1 Lot 004, was confirmed as PRK1 by specific Western Blotting using PRK1 antibody

Theoretical MW_{Fusion Protein}: 136,910 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, 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!

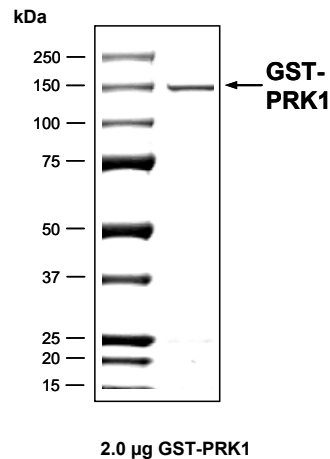
Protein concentration: 0.164 µg/µl (Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

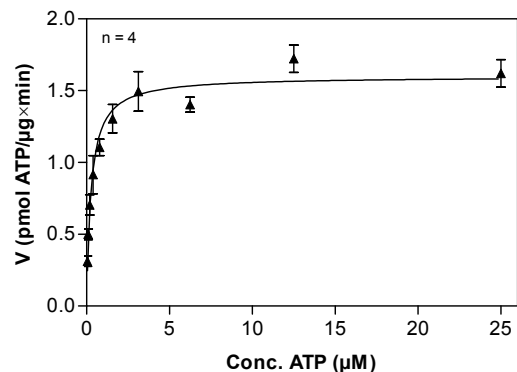
Specific kinase activity (Pi transfer): 1.6 pmol/µg×min

ATP-K_M: 0.3 µM

**PRK1 Lot 004:
Coomassie stain**



**PRK1 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: Histone H2B, 40 µg / ml
 - PRK1: 4.0 µg / ml
- Filter binding assay
 - MSFC membrane (Millipore)

Additional assay technology: PRK1 Lot 004

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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PRK1

Product No.: 0207-0000-0

PRK1 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGQWQATF	GGGDHPPKSD	PMGHHHHHGG	RRRASVAAGI	240
241	LVPRGSPGLD	GIYARGIQAS	MGARGRQCDG	YLQNSPLMAS	DAVQSEPRSW	SLLEQLGLAG	300
301	ADLAAPGVQQ	QLELERERLR	REIRKELKLK	EGAENLRRAT	TDLGRSLGPV	ELLLRGSSRR	360
361	LDLLHQQLQE	LHAHVLPDP	AATHDGPQSP	GAGGPTCSAT	NLSRVAGLEK	QLAIELKVQK	420
421	GAENMIQYTS	NGSTKDRKLL	LTAQQMLQDS	CTKIDIIIRMQ	LRRALQAGQL	ENQAAPDDTQ	480
481	GSPDLGAVEL	RIEELRHHFR	VEHAHAEGAK	NVLRLLSAAK	APDRKAVSEA	QEKLTESNQK	540
541	LGLLREALER	RLGELPADHP	KGRLLREELA	AASSAAFSTR	LAGPFPATHY	STLCKPAPLT	600
601	GTLEVRVVC	RDLPETIPWN	PTPSMGGPGT	PDSRPPFLSR	PARGLYSRSG	SLSGRSSLKA	660
661	EAENTSEVST	VKLDNTVVG	QTSWKPCGPN	AWDQSFTLEL	ERARELELAV	FWRDQRGLCA	720
721	LKFLLEDFL	DNERHEVQLD	MEPQGCLVAE	VTFRNPVIER	IPRLRRQKKI	FSKQQGKAFQ	780
781	RARQMNIDVA	TWVRLRLRI	PNATGTGTFS	PGASPGSEAR	TTGDISVEKL	NLGTDSDDSSP	840
841	QKSSRDPPSS	PSSLSSPIQE	STAPELPSET	QETPGPALCS	PLRKSPLTLE	DFKFLAVLGR	900
901	GHFGKVLLE	FRPSGELFAI	KALKKGDIVA	RDEVESLMCE	KRILAAVTS	A GHPFLVNLFG	960
961	CFQTPHEVCF	VMEYSAGGDL	MLHIHSDVFS	EPRAIFYSAC	VALGLQFLHE	HKIVYRDLKL	1020
1021	DNLLLDTEGY	VKIADFGCLK	EGMGYDRTS	TFCGTPEFLA	PEVLTDTSYT	RAVDWWGLGV	1080
1081	LLYELVGES	FPTGDDEEV	FDSIVNDEV	YPRFLSAEAI	GIMRLLLRN	PERRLGSSER	1140
1141	DAEDVKKQPF	FRTLGWAEAL	ARRLPPFPVP	TLSGRTDVSN	FDEEFTGEAP	TLSPPRDARP	1200
1201	LTAAEQAAFL	DFDFVAGGCK	GEFQHTGGRY				1260

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: PRK1 **boxed**: variation from RefSeq

PRK1 wt ¹ amino acid sequence							
1	MASDAVQSEP	RSWSLLEQLG	LAGADLAAPG	VQQQLELERE	RLRREIRKEL	KLKEGAENLR	60
61	RATTDLGRSL	GPVELLLRGS	SRRLDLLHQQ	LQELHAHVVL	PDPAATHDGP	QSPGAGGPTC	120
121	SATNLSRVAG	LEKQLAIELK	VKQGAENMIQ	TYSNGSTKDR	KLLLTAQQML	QDSKTKIDII	180
181	RMQLRRALQA	DQLENQAAPD	DTQGSPDLGA	VELRIEELRH	HFRVEHAVAE	GAKNVLRLLS	240
241	AAKAPDRKAV	SEAQEKLTES	NQKLGLLREA	LERRLGELPA	DHPKGRLLRE	ELAAASSAAF	300
301	STRLAGPFPA	THYSTLCKPA	PLTGTLEVRV	VGCRDLPETI	PWNPTPSMGG	PGTPDSRPPF	360
361	LSRPARGLYS	RSGSLSGRSS	LKAEAEENTSE	VSTVLKLDNT	VVGQTSWKPC	GPNAWDQSFT	420
421	LELERARELE	LAVFWRDQRG	LCALKFLKLE	DFLDNERHEV	QLDMEPQGCL	VAEVTFRNPV	480
481	IERIPRLRRQ	KKIFSKQQGK	AFQARQMN	DVATWVRLLR	RLIPNATGTG	TFSPGASPGS	540
541	EARTTGDISV	EKLNLTGDS	SSPQKSSRDP	PSSPSSLSSP	IQESTAPELP	SETQETPGPA	600
601	LCSPLRKSPL	TLEDFKFLAV	LGRGHFGKVL	LSEFRPSGEL	FAIKALKKGD	IVARDEVESL	660
661	MCEKRILAAV	TSAGHPFLVN	LFGCFQTEPH	VCFVMEYSAG	GDLMLHIHSD	VFSEPRAIIFY	720
721	SACVVLGLQF	LHEHKIVYRD	LKLDNLLD	EGYVKIADFG	LCKEKGMYGD	RTSTFCGTPE	780
781	FLAPEVLTDT	SYTRAVDWWG	LGVLLEYMLV	GESPPFGDDE	EEVFDSIVND	EVRYPRFLSA	840
841	EAIGIMRLL	RRNPERRLGS	SERDAEDVKK	QPFRTLGWE	ALLARLPPP	FVPTLSGRTD	900
901	VSNFDEEFTG	EAPTLSPPRD	ARPLTAAEQ	AFLDFDFVAG	GC		960

blue: PRK1 sequence expressed in fusionprotein **Red**: variant in fusionprotein

¹NCBI/Protein accession number NP_002732.1