

Certificate of Analysis

PKC-mu

protein kinase D1

Recombinant Human Active Protein Kinase

HGNC Symbol: PRKD1

Synonyms: PRKCM; PKC-MU; PKCM; PKD

Product No.: 0115-0000-1

Lot: 004

Description: Human PKC-mu, full length, amino acids M₁-L₉₁₂ (as in NCBI/Protein entry NP_002733.1), N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: PKC-mu Lot 004, was confirmed as PKC-mu by mass spectroscopy LC-ESI-MS/MS (Protagen AG, Germany)

Theoretical MW_{Fusion Protein}: 139,489 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, 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: 0.120 µg/µl

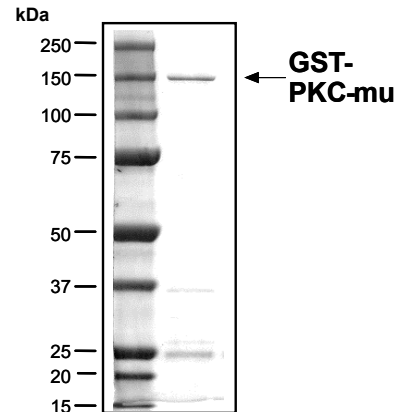
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 4.4 pmol/µg×min

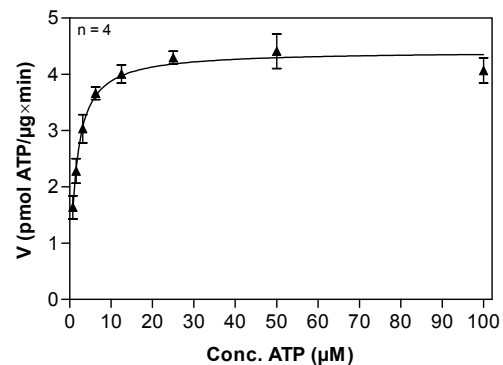
ATP-K_M: 1.4 µM

**PKC-mu Lot 004:
Coomassie stain**



2.0 µg GST-PKC-mu

**PKC-mu 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), 10 µg/ml
 - Kinase: 4 µg / ml
- Filter binding assay
 - MSFC membrane (Millipore)

Additional assay technology: PKC-mu 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

PKC-mu

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PKC-mu Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPPYYID	60
61	GDVKLTQ SMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHHG	RRRASVAAGI	240
241	LVPRGSGPLD	GIYARGIPFS	PPRPSRPP	PPRPPLPIL	IPLPSPSPGT	FPESFYFSPG	300
301	LSEKEAPGSA	AAKLSCCRAA	SPRPPLPGPA	PRRAMSAPPV	LRPPSPLLPV	AAARAAAAAA	360
361	LVPGSGPGPA	PFLAPVAAPV	GGISFHLQIG	LSREPVLLLQ	DSSGDYSLAH	VREMACSIVD	420
421	QKFPECGFYG	MYDKILLFRH	DPTSENIQL	VKAASDIQEG	DLIEVLSRS	ATFEDFQIRP	480
481	HALFVHSYRA	PAFCDHCGEM	LWGLVRQGLK	CEGCGLNYHK	RCAFKIPNNC	SGVRRRRLSN	540
541	VSLTGVSTIR	TSSAELSTSA	PDEPLLQKSP	SEFIGREKR	SNSQSYIGRP	IHLDKILMSK	600
601	VKVPHTFVIH	SYTRPTVCQY	CKKLLKGLFR	QGLQCKDCRF	NCHKRCAPKV	PNNCLGEVTI	660
661	NGDLLSPGAE	SDVVMEEGSD	DNDSERNSGL	MDDMEEAMVQ	DAEMAMAECQ	NDSGEMQDPD	720
721	PDHEDANRTI	SPSTSNNIPL	MRVQSVKHT	KRKSSTVMKE	GWMVHYTSKD	TLRKRHYWRL	780
781	DSKCITLQFN	DTGSRYYKEI	PLSEILSLEP	VKTSALIPNG	ANPHCFEITT	ANVVYVGEN	840
841	VVNPSPPSPN	NSVLTSGVGA	DVARMWEIAI	QHALMPVIK	GSSVGTGTNL	HRDISVSISV	900
901	SNCIQENVD	ISTVYQIFPD	EVLGSGQFGI	VYGGKHKRTG	RDVAIKIIDK	LRFPKQESQ	960
961	LRNEVAILQN	LHHPGVNLE	CMFETPERVF	VVMEKLHGDM	LEMILSSEKG	RLPEHITKFL	1020
1021	ITQILVALRH	LHFKNIVHCD	LKPENVLLAS	ADFPQVKLC	DFGFARIIGE	KSFRRSVVGT	1080
1081	PAYLAPEVLR	NKGYNRS LDM	WSVGVIIYS	LSGTFPFNED	EDIHQIQNA	AFMYPNPWK	1140
1141	EISLAEIDL I	NNLLQVKMRK	RSVDKTL SH	PWLQDYQ T WL	DLRELECKIG	ERYITHESDD	1200
1201	LRWEKYAGEQ	RLQYPTHLIN	PSASHDTP E	TEETEMKALG	ERSVIL		1260

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: PKC-mu **boxed**: A₂₀R

PKC-mu wt ¹ Amino Acid Sequence							
1	MSAPPVLRPP	SPLL PVA AAA	AAAAAALVPG	SGPGPAPFLA	PVAAPVGGIS	FHLQIGLSRE	60
61	PVLLLQDSSG	DYSLAHVREM	ACSIVDQKFP	ECGFYGM YDK	ILLFRHDPTS	ENILQLVKAA	120
121	SDIQEGDLIE	VVLSRSATFE	DFQIRPHALF	VHSYRAPAFC	DHCGEMLWGL	VRQGLKCEGC	180
181	GLNYHKRCAF	KIPNNCSGVR	RRRLSNVSLT	GVSTIRTSSA	ELSTSAPDEP	LLQKSPSESF	240
241	IGREKRSNSQ	SYIGRPIHLD	KILMSKV KVP	HTFVIHSYTR	PTVCQYCKKL	LKGLFRQGLQ	300
301	CKDCRFNCHK	RCAPKVPNNC	LGEVTINGDL	LSPGAESD VV	MEEGSDDNDS	ERN SGLMDDM	360
361	EEAMVQDAEM	AMAECQND SG	EMQDPDPDHE	DANRTISPST	SNNIPLMRVV	QSVKHTKRKS	420
421	STVMKEGWMV	HYTSKDTLRK	RHYWRLD SKC	ITLFQNDTGS	RYYKEIPLSE	ILSLEPVKTS	480
481	ALIPNGANPH	CFEITTANV V	YVGENV VNP	SSPSPNNSVL	TSGVGADVAR	MWEIAIQHAL	540
541	MPVIPKGSSV	GTGTNLHRDI	SVSISVSNCO	IQENVDISTV	YQIFPDEV LG	SGQFGIVYGG	600
601	KHRKTGRDVA	IKIIDKLRFP	TKQESQLRNE	VAILQNLHHP	GVVNLECMFE	TPERVFV VME	660
661	KLHGDMLEMI	LSSEKGR LPE	HITKFLITQI	LVALRHLHFK	NIVHCDLKPE	NVLLASADPF	720
721	PQVKLCDFGF	ARIIGEK SFR	RSVVGTPAYL	APEVLRNKG Y	NRS LDMWSVG	VIIYVLSLGT	780
781	FPFNEDEDIH	DQIQNAAFMY	PPNPWKEISH	E AIDLINLL	QVKMRKRYSV	DKTL SHPWLQ	840
841	DYQTWLDLRE	LECKIGERYI	THESDDL RWE	KYAGEQRLQY	PTHLINPSAS	HS DTPETEET	900
901	EMKALGERVS	IL					960

blue: PKC-mu sequence expressed in fusion protein **Red**: variant in fusion protein

¹NCBI/Protein accession number NP_002733.1
A₂₀R: SNP variation see EST BF310986.1