

PKC-nu

Protein kinase D3

Recombinant Human Active Protein Kinase

HGNC Symbol: PRKD3

Synonyms: PRKD3; EPK2

Product No.: 0661-0000-1

Lot: 003

Description: Human PKC-nu, full length, amino acids A₃-P₈₉₀ (as in NCBI/Protein entry NP_005804.1), untagged, expressed in Sf9 insect cells

Product identity: PKC-nu Lot 003, was confirmed as PKC-nu by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 100,793 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography, followed by 3C mediated removal of the GST tag

Activation: This kinase was not activated by special procedures

Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 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!

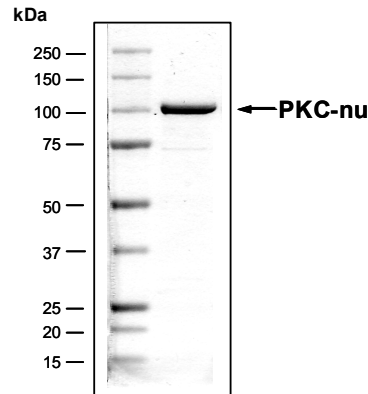
Protein concentration: 0.122 µg/µl

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

Biochemical Parameters:

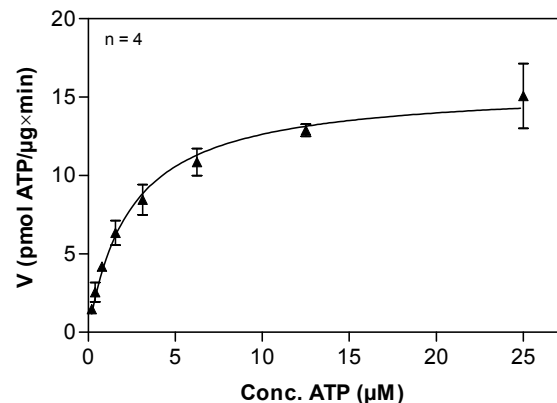
Specific kinase activity (P_i transfer): 16 pmol/µg×min
ATP-K_M: 2.4 µM

PKC-nu Lot 003: Coomassie stain



2.0 µg PKC-nu

PKC-nu Lot 003: 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), 20 µg/ml
 - PKC-nu: 1.0 µg/ml
- Filter binding assay
 - MSPH membrane (Millipore)

Additional assay technology: PKC-nu 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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PKC-nu Recombinant Protein Amino Acid Sequence								
1	GPLAMA	ANNS	PPSAQKSVLP	TAIPAVLPAA	SPCSSPKTGL	SARLSNGSFS	APSLTNSRGS	60
61	VHTVSFLLQI	GLTRESVTIE	AQELSLSAVK	DLVCSIVYQK	FPECGFFGMY	DKILLFRHDM		120
121	NSENIQLIT	SADEIHEGDL	VEVLSALAT	VEDFQIRPHT	LYVHSYKAPT	FCDYCGEMLW		180
181	GLVRQGLKCE	GCGLNYHKRC	AFKIPNNCSG	VRKRRLSNVS	LPGPGLSVPR	PLQPEYVALP		240
241	SEESHVHQP	SKRIPSWSGR	PIWMEKMVMC	RVKVPHTFAV	HSYTRPTICQ	YCKRLLKGLF		300
301	RQGMQCKDCK	FNCHKRCASK	VPRDCLGEVT	FNGEPSSLGT	DTDIPMDIDN	NDINSDSSRG		360
361	LDDTEEPSPP	EDKMFFLDPS	DLDERVEDEA	VKTISPSTSN	NIPLMRVVQS	IKHTKRKSST		420
421	MVKEGWMVHY	TSRDNLKRKH	YWRLLSKCLT	LFQNESGSKY	YKEIPLSEIL	RISSPRDFTN		480
481	ISQGSNPFCF	EIITDTMVYF	VGENNGDSSH	NPVLAATGVG	LDVAQSWEKA	IRQALMPVTP		540
541	QASVCTSPGQ	GKDHKDLSTS	ISVSNQIQE	NVDISTVYQI	FADEVLGSGQ	FGIVYGGKHR		600
600	KTGRDVAIKV	IDKMRFPKQ	ESQLRNEVAI	LQNLHHPGIV	NLECMFETPE	RVFVVMKELH		660
661	GDMLEMILSS	EKSRLPERIT	KFMVTQILVA	LRNLHFKNIV	HCDLKPENVL	LASAEFPQV		720
721	KLCDFGFARI	IGEKSFRRSV	VGTPAYLAPE	VLRKSGYNRS	LDMWSVGVI	YVSLSGTFPF		780
781	NEDEDINDQI	QNAAFMYPPN	PWREISGEAI	DLINLLQVK	MRKRYSDKS	LSHPWLQDYQ		840
841	TWLDLREFET	RIGERYITHE	SDDARWEIHA	YTHNLVYPKH	FIMAPNPDDM	EEDP		900

blue: PKC-nu fragment

PKC-nu wt ¹ Amino Acid Sequence								
1	MS	ANNSPPSA	QKSVLPTAIP	AVLPAASPCS	SPKTGLSARL	SNGSFSAPSL	TNSRGSVHTV	60
61	SFLLQIGLTR	ESVTIEAQEL	SLSAVKDLVC	SIVYQKFPEC	GFFGMYDKIL	LFRHDMNSEN		120
121	ILQLITSADE	IHEGDLVEV	LSALATVEDF	QIRPHTLYVH	SYKAPTFCDY	CGEMLWGLVR		180
181	QGLKCEGCGL	NYHKRCAFKI	PNNCSGVRKR	RLSNVSLPGP	GLSVPRPLQP	EYVALPSEES		240
241	HVHQEPSKRI	PSWSGRPIWM	EKMVMCRVKV	PHTFAVHSYT	RPTICQYCKR	LLKGLFRQGM		300
301	QCKDCKFNCH	KRCASKVPRD	CLGEVTFNGE	PSSLGTDTDI	PMDIDNNDIN	SDSSRGLDDT		360
361	EEPSPPEDKM	FFLDPSDLV	ERDEEAVKTI	SPSTSNNIPL	MRVVQSIKHT	KRKSSTMVKE		420
421	GWMVHYTSRD	NLRKRHYWRL	DSKCLTLFQN	ESGSKYYKEI	PLSEILRISS	PRDFTNISQG		480
481	SNPHCFEIIIT	DTMVYFVGEN	NGDSSHNPLV	AATGVGLDVA	QSWEKAIRQA	LMPVTPQASV		540
541	CTSPGQGDH	KDLSTSISVS	NCQIQENVDI	STVYQIFADE	VLGSGQFGIV	YGGKHKRTGR		600
600	DVAIKVIDKM	RFPTKQESQL	RNEVAIQLNL	HHPGIVNLEC	MFETPERVVFV	VMEKLGDMML		660
661	EMILSSEKSR	LPERITKFMV	TQILVALRNL	HFKNIVHCDL	KPENVLASA	EPFPQVKLCD		720
721	FGFARIIGEK	SFRRSVVGTP	AYLAPEVLR	KGYNRSLDMW	SVGVIIYVSL	SGTFPFNEDE		780
781	DINDQIQNAA	FMYPPNPWRE	ISGEAIDLIN	NLLQVKMRKR	YSVDKLSLHP	WLQDYQTWLD		840
841	LREFETRIGE	RYITHESDDA	RWEIHAYTHN	LVYPKHFIMA	PNPDDMEEDP			900

blue: PKC-nu sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_005804.1