

ProQinase™ PKC-alpha

protein kinase C alpha

Recombinant Human Active Protein Kinase

HGNC Symbol: PRKCA

Synonyms: PKCA, PKC-A, PRKACA

Product No.: 0222-0000-1

Lot: 005

Description: Human PKC-alpha, full length, amino acids M₁-V₆₇₂ (as in [NCBI/Protein](#) entry NP_002728.1), N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: PKC-alpha Lot 005 was confirmed as PKC-alpha by specific Western Blotting

Theoretical MW_{Fusion Protein}: 109,972 Da

Expression host: Sf9 insect 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: 0.170 µg/µl

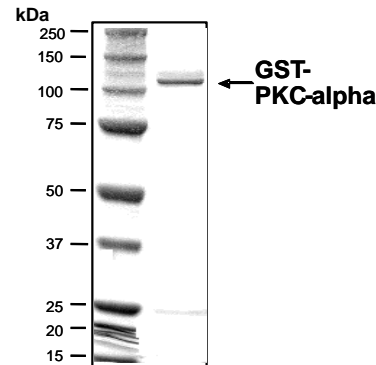
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 526 pmol/µg × min

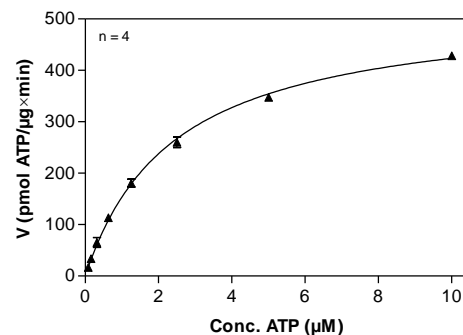
ATP-K_M: 2.4 µM

PKC-alpha Lot 005:
Coomassie stain



2.0 µg GST-PKC-alpha

PKC-alpha Lot 005:
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
 - 1 mM CaCl₂
 - 4 mM EDTA
 - 3 µM Na-orthovanadate
 - 5 µg/ml Phosphatidylserine
 - 1 µg/ml 1,2 Dioleoyl-glycerol
 - 1.2 mM DTT
 - 50 µg/ml PEG_{20,000}
 - ATP (variable)
 - Substrate: Histone H1, 20 µg/ml
 - Kinase: 1 µg/ml
- Filter binding assay
- MSFC membrane (Millipore)

Additional assay technology:

PKC-alpha Lot 005 was also successfully tested by Reaction Biology 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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ProQinase™ PKC-alpha

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GST-PKC-alpha Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQG WQATF	GGGDHPPKSD	PMGHHHHHG	RRRASVAAGI	240
241	LVPRGSPGLD	GIYARGIQAS	MGARGRQCDG	YLQNSPLSMA	DVFPNGNSTA	SQDVANRFAR	300
301	KGALRQKNVH	EVKDHKFIAR	FFKQPTFCSH	CTDFIWGFGK	QGFQCQVCCF	VVHKRCHEFV	360
361	TFSCPGADKG	PDTDDPRSKH	KFKIHTY GSP	TFCDHCGSL	YGLIHQGMKC	DTCDMNVHRQ	420
421	CVINVP SLCG	MDHTEKRGRI	YLKAEVADEK	LHVTVRDAKN	LIRMDPNGLS	DPYVKLKLIP	480
481	DPKNESKQKT	KTIRSTLN PQ	WNESFTFKLK	PSDKDRRLSV	EIWDWDRTR	NDFMGSLSFG	540
541	VSELMKMPAS	GWYKLLNQEE	GEYYNVP IPE	GDEEGN MELR	QKFEKAKLGP	AGNKVISPSE	600
601	DRKQPSNNLD	RVKLTDFN FL	MVLGKGSFGK	VMLADRKGTE	ELYAIKILKK	DVVIQDDVDE	660
661	CTMVEKRVLA	LLDKPPFLTQ	LHSCFQTVDR	LYFVMEYVNG	GDLMYHIQVQ	GKFKEPQAVF	720
721	YAAEISIGLF	FLHKGRIIYR	DLKLDNVMLD	SEGHIKIADF	GMCKEHMDG	VTRTRFCGTP	780
781	DYIAPEIIAY	QPYGKSV DWW	AYGVLLYEML	AGQPPFDGED	EDEL FQSIM	HNSVYPKSL	840
841	KEAVSICKGL	MTKHPAKRLG	CGPEGERDVR	EHAFFRRIDW	EKLENREIQP	PFKPKVCGKG	900
901	AENFDKFFTR	GQPVLTPPDQ	LVIANIDQSD	FEGFSYVNPQ	FVHPILQSAV	GKGEFQHTGG	960
961	RY						1020

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

PKC-alpha wt ¹ Amino Acid Sequence							
1	MADVFPNGDS	TASQDVANRF	ARKGALRQKN	VHEVKDHKFI	ARFFKQPTFC	SHCTDFIWGF	60
61	GKQGFQCQVC	CFVVHKRCHE	FVTFSCPGAD	KGPD TDDPRS	KHKFKIHTYG	SPTFCDHCGS	120
121	LLYGLIHQGM	KCDTCDMNVH	KQCVINVPSL	CGMDHTEKRG	RIYLYKAEVAD	EKLHVTVRDA	180
181	KNLI PMPNG	LSDPYVKLKL	IPDPKNESKQ	KTKTIRSTLN	PQWNESFTFK	LKPSDKDRRL	240
241	SVEIWDWDR	TRNDFMGSL	FGVSELMKMP	ASGWYKLLNQ	EEGEYYNVP I	PEGDEEGNME	300
301	LRQKFEKAKL	GPAGNKVISP	SEDRKQPSNN	LDRVKLTDFN	FLMVLGKGSF	GKVMLADRKG	360
361	TEELYAIKIL	KKDVVIQDD	VECTMVEKRV	LALLDKPPFL	TQLHSCFQTV	DRLYFVMEYV	420
421	NGGDLMYHIQ	QVGKFKEPQA	VFYAAEISIG	LFFLHKGRII	YRDLKLDNVM	LDSEGHIKIA	480
481	DFGMCKEHMM	DGVTTRTFCG	TPDYIAPEII	AYQPYGKSV	WWAYGVLLYE	MLAGQPPFDG	540
541	EDEDEL FQSI	MEHNSVYPKS	LSKEAVSICK	GLMTKHPAKR	LGCGPEGERD	VREHAFFRRI	600
601	DWEKLENREI	QPPFKPKVCG	KGAENFDKFF	TRGQPVLTPP	DQLVIANIDQ	SDFEGFSYVN	660
661	PQFVHPILQS	AV					720

blue: PKC-alpha sequence expressed in recombinant protein Red: variant in recombinant protein

¹NCBI/Protein accession number NP_002728.1

P185R: see NCBI/Nucleotide accession number AV759756.1 for reference