Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ PKC-zeta wt aa184-592 (PKM-zeta)

protein kinase C zeta

Recombinant Human Active Protein Kinase

HGNC Symbol: PRKCZ

Synonyms: PKM-zeta, nPKC-zeta, PKC2

Product No.: 1240-0000-1

Lot: 001

Description: Human PKC-zeta, C-terminal fragment, amino acids M_{184}-V_{592} (as in NCBI/Protein entry NP_002735.3), constitutive active fragment also denoted by PKM-zeta, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: PKC-zeta wt aa184-592 Lot 001, was confirmed as PKC-zeta by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 74,896 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

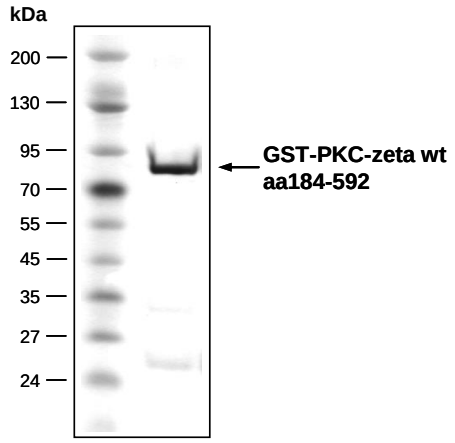
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

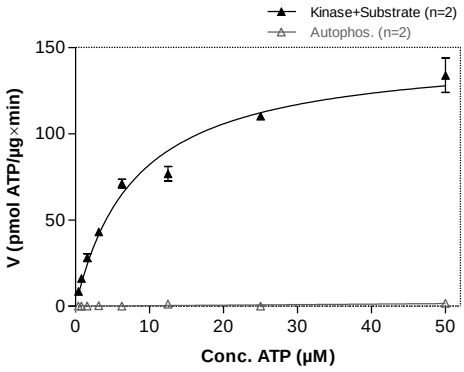
Biochemical Parameters:
Specific kinase activity (P_i transfer): 148 pmol/µg × min
ATP-K_M: 8.1 µM

PKC-zeta wt aa184-592 Lot 001: Coomassie stain



2.0 µg GST-PKC-zeta wt aa184-592

PKC-zeta wt aa184-592 Lot 001: 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 $MgCl_2$; 3 mM $MnCl_2$; 1 mM $CaCl_2$
  4 mM EDTA
  5 µg/ml Phosphatidylserine
  1 µg/ml 1.2 Dioleyl-glycerol
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg / ml $PEG_{20.000}$
  ATP (variable)
  Substrate: PKC-derived peptide 40 µg/ml
  PKC-zeta wt aa184-592: 1 µg/ml
- Filter binding assay

MSPH membrane (Millipore)

Additional assay technology:
PKC-zeta wt aa184-592 Lot001 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

ProQinase™ PKC-zeta wt aa184-592 (PKM-zeta)

Product No.: 1240-0000-1

GST-PKC-zeta wt aa184-592 Recombinant Fusion Protein Amino Acid Sequence

1	MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID	60
61	GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV	120
121	DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK	180
181	KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG	240
241	PLAMDSVMPS QEPPVDDKNE DADLPSEETD GIAYISSSRK HDSIKDDSED LKPVIDGMDG	300
301	IKISQGLGLQ DFDLIRVIGR GSYAKVLLVR LKKNDQIYAM KVVKKELVHD DEDIDWVQTE	360
361	KHVFEQASSN PFLVGLHSCF QTTSRLFLVI EYVNGGDLMF HMQRQRKLPE EHARFYAAEI	420
421	CIALNFLHER GIIYRDLKLD NVLLDADGHI KLTDYGMCKE GLGPGDTTST FCGTPNYIAP	480
481	EILRGEEYGF SVDWWALGVL MFEMMAGRSP FDIITDNPDM NTEDYLFQVI LEKPIRIPRF	540
541	LSVKASHVLK GFLNKDPKER LGCRPQTGFS DIKSHAFFRS IDWDLLEKKQ ALPPFQPQIT	600
601	DDYGLDNFDT QFTSEPVQLT PDDEDAIKRI DQSEFEGFEY INPLLLSTEE SV	660

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: PKC-zeta fragment

PKC-zeta wt[1] Amino Acid Sequence

1	MPSRTGPKME GSGGRVRLKA HYGGDIFITS VDAATTFEEL CEEVRDMCRL HQQHPLTLKW	60
61	VDSEGDPCTV SSQMELEEAF RLARQCRDEG LIIHVFPSTP EQPGLPCPGE DKSIYRRGAR	120
121	RWRKLYRANG HLFQAKRFNR RAYCGQCSER IWGLARQGYR CINCKLLVHK RCHGLVPLTC	180
181	RKHMDSVMPS QEPPVDDKNE DADLPSEETD GIAYISSSRK HDSIKDDSED LKPVIDGMDG	240
241	IKISQGLGLQ DFDLIRVIGR GSYAKVLLVR LKKNDQIYAM KVVKKELVHD DEDIDWVQTE	300
301	KHVFEQASSN PFLVGLHSCF QTTSRLFLVI EYVNGGDLMF HMQRQRKLPE EHARFYAAEI	360
361	CIALNFLHER GIIYRDLKLD NVLLDADGHI KLTDYGMCKE GLGPGDTTST FCGTPNYIAP	420
421	EILRGEEYGF SVDWWALGVL MFEMMAGRSP FDIITDNPDM NTEDYLFQVI LEKPIRIPRF	480
481	LSVKASHVLK GFLNKDPKER LGCRPQTGFS DIKSHAFFRS IDWDLLEKKQ ALPPFQPQIT	540
541	DDYGLDNFDT QFTSEPVQLT PDDEDAIKRI DQSEFEGFEY INPLLLSTEE SV	600

blue: PKC-zeta sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_002735.3

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