Certificate of Analysis

PKC-zeta aa184-592 K281M (PKM-zeta)

Protein kinase C, zeta

Recombinant Human inactivated Protein Kinase

HGNC Symbol: PRKCZ

Synonyms: PKM-zeta, nPKC-zeta, PKC2

Product No.: 1241-0000-1

Lot: 001

Description: Human PKC-zeta, C-terminal fragment, amino acids M_{184}-V_{592} (as in NCBI/Protein entry NP_002735.3), fragment also termed PKM-zeta, mutationally inactivated by a $K_{281}M$ mutation, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Theoretical $MW_{Protein}$: 74,899 Da

Expression: Sf9 cells

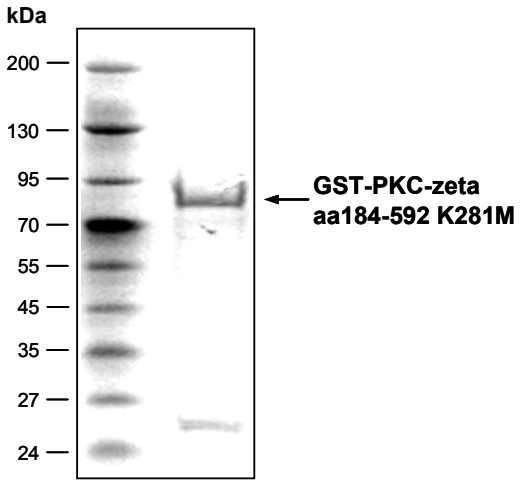
Purification: GST-Affinity Chromatography

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

Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!

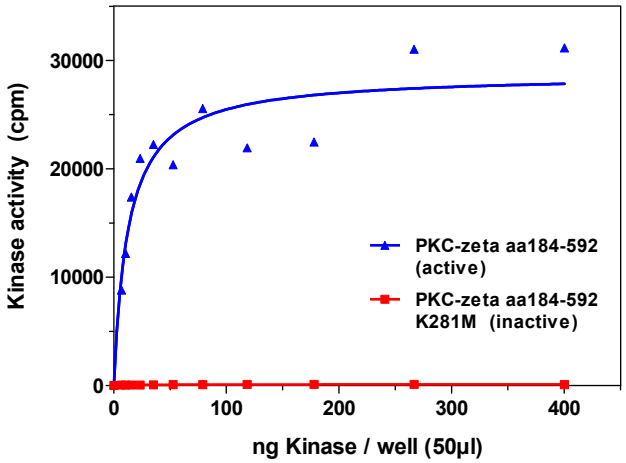
Protein concentration: 0.140 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Coomassie stain:



2.0 µg GST-PKC-zeta aa184-592 K281M

Kinase activity of PKC-zeta aa184-592 K281M (inactivated) vs PKC-zeta wt aa184-592:



Final assay concentrations:
- 60 mM HEPES-NaOH, pH 7.5
- 3 mM $MgCl_2$
- 3 mM $MnCl_2$
- 3 µM Na-orthovanadate
- 1.2 mM DTT
- 50 µg / ml $PEG_{20.000}$
- 1 µM ATP
- Substrate: PKC-derived peptide (R_{11}-RFARKGSLRQKNV) 10 µg/ml
- Recombinant PKC-zeta aa184-592 K281M or PKC-zeta wt aa184-592 (variable concentrations)

Assay: ^{33}PanQinase® Assay

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PKC-zeta aa184-592 K281M (PKM-zeta)

Product No.: 1241-0000-1

PKC-zeta aa184-592 K281M Recombinant Fusion Protein Amino Acid Sequence

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  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 MVVKKELVHD 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
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1-218: GST **Red: HIS6-tag** **Green: 3C** **blue:PKC-zeta fragment** M: K281M mutation

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
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blue: PKC-zeta sequence expressed in fusionprotein **Red**: K281M point mutation

[1]NCBI/Protein accession number NP_002735.3