Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ PKC-eta

protein kinase C eta

Recombinant Human Active Protein Kinase

HGNC Symbol: PRKCH

Synonyms: nPKC-eta, PKC-L

Product No.: 0420-0000-1

Lot: 006

Description: Human PKC-eta, full length, amino acids M_1-P_{683} (as in NCBI/Protein entry NP_006246.2), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: PKC-eta Lot 006, was confirmed as PKC-eta by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 107,725 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

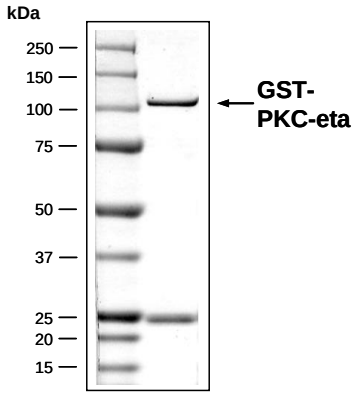
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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

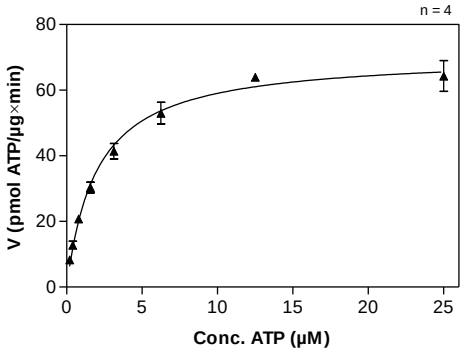
Biochemical Parameters:
Specific kinase activity (P_i transfer): 71 pmol/µg × min
ATP-K_M: 2 µM

PKC-eta Lot 006:
Coomassie stain



2.0 µg GST-PKC-eta

PKC-eta Lot 006:
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 $MnCl_2$
3 µM Na-orthovanadate
1.2 mM DTT
50 µg/ml $PEG_{20.000}$
ATP (variable)
Substrate: Histone H2B 30 µg/ml
Kinase: 0.5 µg/ml
• Filter binding assay
MSFC membrane (Millipore)

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GST-PKC-eta 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 RRRASVAAGI 240
241  LVPRGSPGLD GICSRNSMSS GTMKFNGYLR VRIGEAVGLQ PTRWSLRHSL FKKGHQLLDP 300
301  YLTVSVDQVR VGQTSTKQKT NKPTYNEEFC ANVTDGGHLE LAVFHETPLG YDHFVANCTL 360
361  QFQELLRTTG ASDTFEGWVD LEPEGKVFVV ITLTGSFTEA TLQRDRIFKH FTRKRQRAMR 420
421  RRVHQINGHK FMATYLRQPT YCSHCREFIW GVFGKQGYQC QVCTCVVHKR CHHLIVTACT 480
481  CQNNINKVDS KIAEQRFGIN IPHKFSIHNY KVPTFCDHCG SLLWGIMRQG LQCKICKMNV 540
541  HIRCQANVAP NCGVNAVELA KTLAGMGLQP GNISPTSKLV SRSTLRRQGK ESSKEGNGIG 600
600  VNSSNRLGID NFEFIRVLGK GSFGKVMLAR VKETGDLYAV KVLKKDVILQ DDDVECTMTE 660
661  KRILSLARNH PFLTQLFCCF QTPDRLFFVM EFVNGGDLMF HIQKSRRFDE ARARFYAAEI 720
721  ISALMFLHDK GIIYRDLKLD NVLLDHEGHC KLADFGMCKE GICNGVTTAT FCGTPDYIAP 780
781  EILQEMLYGP AVDWWAMGVL LYEMLCGHAP FEAENEDDLF EAILNDEVVY PTWLHEDATG 840
841  ILKSFMTKNP TMRLGSLTQG GEHAILRHPF FKEIDWAQLN HRQIEPPFRP RIKSREDVSN 900
901  FDPDFIKEEP VLTPIDEGHL PMINQDEFRN FSYVSPELQP                       960
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1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: PKC-eta

PKC-eta wt[1] Amino Acid Sequence

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  1  MSSGTMKFNG YLRVRIGEAV GLQPTRWSLR HSLFKKGHQL LDPYLTVSVD QVRVGQTSTK  60
 61  QKTNKPTYNE EFCANVTDGG HLELAVFHET PLGYDHFVAN CTLQFQELLR TTGASDTFEG 120
121  WVDLEPEGKV FVVITLTGSF TEATLQRDRI FKHFTRKRQR AMRRRVHQIN GHKFMATYLR 180
181  QPTYCSHCRE FIWGVFGKQG YQCQVCTCVV HKRCHHLIVT ACTCQNNINK VDSKIAEQRF 240
241  GINIPHKFSI HNYKVPTFCD HCGSLLWGIM RQGLQCKICK MNVHIRCQAN VAPNCGVNAV 300
301  ELAKTLAGMG LQPGNISPTS KLVSRSTLRR QGKESSKEGN GIGVNSSNRL GIDNFEFIRV 360
361  LGKGSFGKVM LARVKETGDL YAVKVLKKDV ILQDDDVECT MTEKRILSLA RNHPFLTQLF 420
421  CCFQTPDRLF FVMEFVNGGD LMFHIQKSRR FDEARARFYA AEIISALMFL HDKGIIYRDL 480
481  KLDNVLLDHE GHCKLADFGM CKEGICNGVT TATFCGTPDY IAPEILQEML YGPAVDWWAM 540
541  GVLLYEMLCG HAPFEAENED DLFEAILNDE VVYPTWLHED ATGILKSFMT KNPTMRLGSL 600
601  TQGGEHAILR HPFFKEIDWA QLNHRQIEPP FRPRIKSRED VSNFDPDFIK EEPVLTPIDE 660
661  GHLPMINQDE FRNFSYVSPE LQP
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blue: PKC-eta sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_006246.2

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