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Certificate of Analysis

PKCε, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-518, 14-518-K, 14-518M
Parent Lot # D8PN019U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: *N*-terminal 6His-tagged, recombinant PKC epsilon, amino acids 2–end, expressed in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 96.3% by SDS-PAGE and Coomassie blue staining. MW = 84.6kDa.

Specific Activity (Parent lot# D8PN019U): 2420U/mg, where one unit of PKC epsilon, active activity is defined as 1nmol phosphate incorporated into 50µM PKCtide (ERMRPRKRQGSVRRRV) in the presence of lipid activator (cat# 20-133) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.126mg/ml of enzyme in 20mM Tris/HCl pH 7.5, 1mM EGTA, 1mM EDTA, 0.02% Triton X-100, 5% glycerol, 1mM PMSF, 10mM benzamidine, 1mM DTT. Frozen solution.

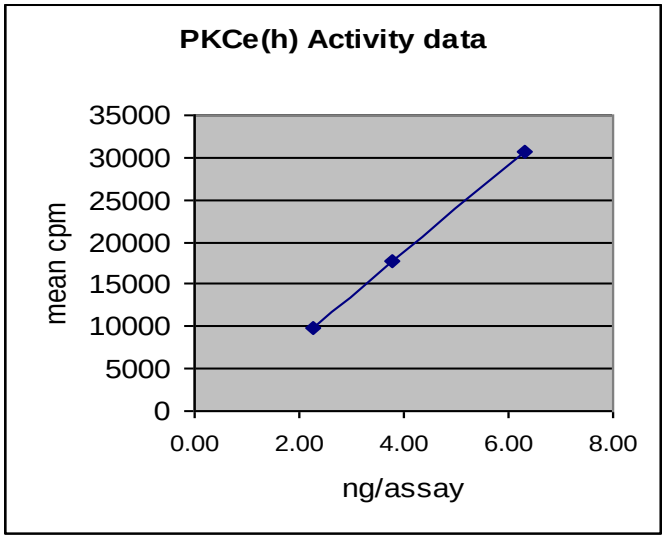
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

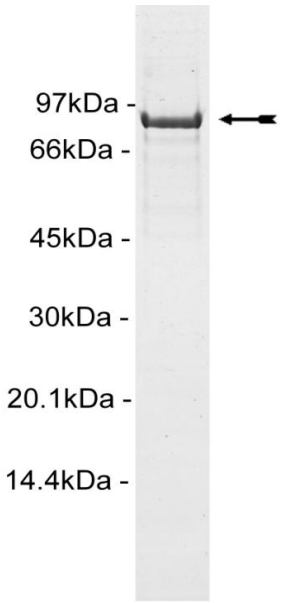
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 2.3–6.3ng of this lot of enzyme phosphorylated 50µM PKC tide in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as human PKC epsilon with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of PKC epsilon, active.

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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 200mM HEPES/NaOH pH7.4.
2. **PKCtide(ERMRPRKRQGSVRRRV):** Use at a final assay concentration of 50µM. Prepare a 500µM stock. Use 2.5µl of stock per assay point.
3. **10 x Lipid Activator:** 0.3% Triton X-100, 1mg/ml phosphatidylserine, 0.1mg/ml diacylglycerol). Use 2.5µl of stock per assay point.
4. **PKC epsilon, active:** Dilute with 20mM HEPES/NaOH pH7.4, 0.03% Triton X-100. Use 2.3–6.3ng per assay point.
5. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 2.5µl of 10 x reaction buffer per assay to wells.
2. Add 2.5µl of **PKCtide**.
3. Add 2.5µl of lipid activator.
4. Add **2.5µl (2.3–6.3ng) PKC epsilon, active**.
5. Make up to 15µl with dH_2O.
6. Add 10µl of diluted [γ-^{33}P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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PKC epsilon Sequence Information

Protein human PKC epsilon

Tags *N*-terminal 6His

Native sequence V8 of the recombinant protein is equivalent to V2 of human PKC epsilon

Accession number GenBank X65293

Recombinant PKC epsilon amino acid sequence:

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   1 MHHHHHHVVF NGLLKIKICE AVSLKPTAWS LRHAVGPRPQ TFLLDPYIAL NVDDSRIGQT
  61 ATKQKTNSPA WHDEFVTDVC NGRKIELAVF HDAPIGYDDF VANCTIQFEE LLQNGSRHFE
 121 DWIDLEPEGR VYVIIDLSGS SGEAPKDNEE RVFRERMRPR KRQGAVRRRV HQVNGHKFMA
 181 TYLRQPTYCS HCRDFIWGVI GKQGYQCQVC TCVVHKRCHE LIITKCAGLK KQETPDQVGS
 241 QRFSVNMPHK FGIHNYKVPT FCDHCGSLLW GLLRQGLQCK VCKMNVHRRC ETNVAPNCGV
 301 DARGIAKVLA DLGVTPDKIT NSGQRRKKLI AGAESPQPAS GSSPSEEDRS KSAPTSPCDQ
 361 EIKELENNIR KALSFDNRGE EHRAASSPDG QLMSPGENGE VRQGQAKRLG LDEFNFIKVL
 421 GKGSFGKVML AELKGKDEVY AVKVLKKDVI LQDDDVDCTM TEKRILALAR KHPYLTQLYC
 481 CFQTKDRLFF VMEYVNGGDL MFQIQRSRKF DEPRSRFYAA EVTSALMFLH QHGVIYRDLK
 541 LDNILLDAEG HCKLADFGMC KEGILNGVTT TTFCGTPDYI APEILQELEY GPSVDWWALG
 601 VLMYEMMAGQ PPFEADNEDD LFESILHDDV LYPVWLSKEA VSILKAFMTK NPHKRLGCVA
 661 SQNGEDAIKQ HPFFKEIDWV LLEQKKIKPP FKPRIKTKRD VNNFDQDFTR EEPVLTLVDE
 721 AIVKQINQEE FKGFSYFGED LMP
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Recombinant PKC epsilon nucleotide sequence:

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   1 atgcatcacc atcatcacca tgtagtgttc aatggccttc ttaagatcaa aatctgcgag
  61 gccgtgagct tgaagcccac agcctggtcg ctgcgccatg cggtgggacc ccggccgcag
 121 actttccttc tcgaccccta cattgccctc aatgtggacg actcgcgcat cggccaaacg
 181 gccaccaagc agaagaccaa cagcccggcc tggcacgacg agttcgtcac cgatgtgtgc
 241 aacggacgca agatcgagct ggctgtcttt cacgatgccc ccataggcta cgacgacttc
 301 gtggccaact gcaccatcca gtttgaggag ctgctgcaga acgggagccg ccacttcgag
 361 gactggattg atctggagcc agaaggaaga gtgtatgtga tcatcgatct ctcagggtcg
 421 tcgggtgaag cccctaaaga caatgaagag cgtgtgttca gggaacgcat gcggccgagg
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 541 acctatcttc ggcagcccac ctactgctcc cattgcagag acttcatctg gggtgtcata
 601 ggaaagcagg gataccagtg tcaagtctgc acctgcgtgg tccacaagcg gtgccacgag
 661 ctcataatca caaagtgtgc tgggttaaag aagcaggaga cccccgacca ggtgggctcc
 721 cagcggttca gcgtcaacat gccccacaag ttcggtatcc acaactacaa ggtccctacc
 781 ttctgcgatc actgtgggtc cctgctctgg ggactcttgc ggcagggttt gcagtgtaaa
 841 gtctgcaaaa tgaatgttca ccgtcgatgt gagaccaacg tggctcccaa ctgtggagtg
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 961 aacagcggcc agagaaggaa aaagctcatt gctggtgccg agtccccgca gcctgcttct
1021 ggaagctcac catctgagga agatcgatcc aagtcagcac ccacctcccc ttgtgaccag
1081 gaaataaaag aacttgagaa caacattcgg aaagccttgt catttgacaa ccgaggagag
1141 gagcaccggg cagcatcgtc tcctgatggc cagctgatga gccccggtga gaatggcgaa
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1501 atgtttcaga ttcagcgctc ccgaaaattc gacgagcctc gttcacggtt ctatgctgca
1561 gaggtcacat cggccctcat gttcctccac cagcatggag tcatctacag ggatttgaaa
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1621 ctggacaaca tccttctgga tgcagaaggt cactgcaagc tggctgactt cgggatgtgc
1681 aaggaaggga ttctgaatgg tgtgacgacc accacgttct gtgggactcc tgactacata
1741 gctcctgaga tcctgcagga gttggagtat ggcccctccg tggactggtg ggccctgggg
1801 gtgctgatgt acgagatgat ggctggacag cctccctttg aggccgacaa tgaggacgac
1861 ctatttgagt ccatcctcca tgacgacgtg ctgtacccag tctggctcag caaggaggct
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1981 tcgcagaatg gcgaggacgc catcaagcag cacccattct tcaaagagat tgactgggtg
2041 ctcctggagc agaagaagat caagccaccc ttcaaaccac gcattaaaac caaaagagac
2101 gtcaataatt ttgaccaaga ctttacccgg gaagagccgg tactcaccct tgtggacgaa
2161 gcaattgtaa agcagatcaa ccaggaggaa ttcaaaggtt tctcctactt tggtgaagac
2221 ctgatgccct ga
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Reviewed and approved by site quality representative.