

Certificate of Analysis

PKC ϵ , 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 Ni²⁺/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 50 μ M PKCtide (ERM₁PRKRQGSVRRRV) in the presence of lipid activator (cat# 20-133) per minute at 30°C with a final ATP concentration of 100 μ M.

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.

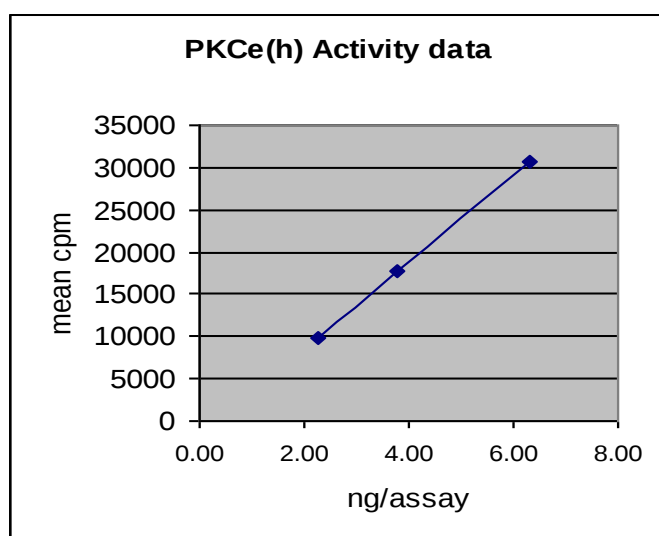
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

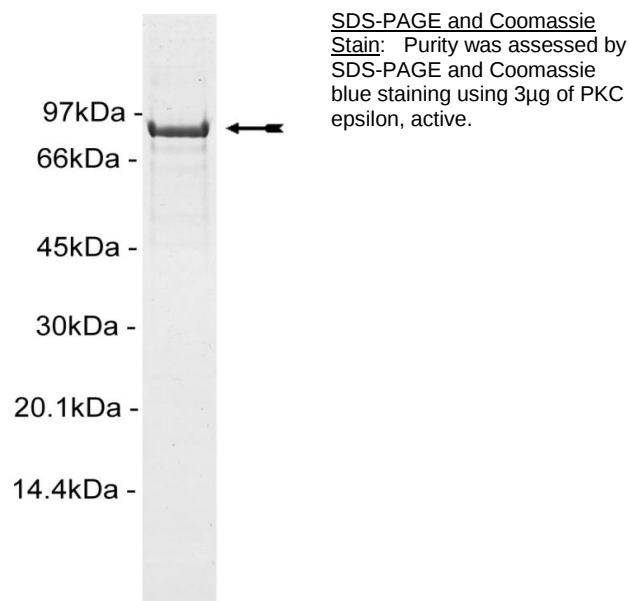
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2.3–6.3ng of this lot of enzyme phosphorylated 50 μ M 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.



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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 200mM HEPES/NaOH pH7.4.
2. **PKCtide(ERM⁺PRKRQGSVRRRV):** 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-³³P]ATP:** 2.5 x magnesium acetate/[γ-³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-³³P]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 dH₂O.
6. Add 10μl of diluted [γ-³³P]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

<u>Protein</u>	human PKC epsilon
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	V8 of the recombinant protein is equivalent to V2 of human PKC epsilon
<u>Accession number</u>	GenBank X65293

Recombinant PKC epsilon amino acid sequence:

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1  MHHHHHHVVF  NGLLIKICE  AVSLKPTAWS  LRHAVGPRPQ  TFLDPYIAL  NVDDSRIGQT
61  ATKQKTNSPA  WHDEFVTDVC  NGRKIELAVF  HDAPIGYDDF  VANCTIQFEE  LLQNGSRHFE
121 DWIDLEPEGR  VYVIDLSGS  SGEAPKDNEE  RVFRERMRPR  KRQGAVRRRV  HQVNGHKFMA
181 TYLRQPTYCS  HCRDFIWGVI  GKQGYQCQVC  TCVVHKRCHE  LIITKCAGLK  KQETPDQVGS
241 QRFSVNMPHK  FGIHNYKVPT  FCDHCGSLLW  GLLRQGLQCK  VCKMNVHRRR  ETNVAPNCGV
301 DARGIAKVLA  DLGVTDPKIT  NSGQRRKKLI  AGAESPQPAS  GSSPSEEDRS  KSAPTSPCDQ
361 EIKELENNIR  KALSFDNRGE  EHRAASSPDG  QLMSPGENGE  VRQGQAKRLG  LDEFNFIKVL
421 GKGSFGKVML  AELKGKDEVY  AVKVLKKDVI  LQDDVDVCTM  TEKRLALAR  KHPYLTQLYC
481 CFQTKDRLFF  VMEYVNGGDL  MFQIQSRKRF  DEPRSRFYAA  EVTSALMFLH  QHGVYIRDLE
541 LDNILLDAEG  HCKLADFGMC  KEGILNGVTT  TFCGTPDYI  APEILQELEY  GPSVDWWALG
601 VLMYEMMAGQ  PPFEADNEDD  LFESILHDDV  LYPVWLSKEA  VSILKAFMTK  NPHKRLGCVA
661 SQNGEDAIAKQ  HPFFKEIDWV  LLEQKKIKPP  FKPRIKTKRD  VNNFDQDFTR  EEPVLTLDVE
721 AIVKQINQEE  FKGSYFGEED  LMP

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Recombinant PKC epsilon nucleotide sequence:

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1  atgcatcacc  atcatcacca  tgtagtgttc  aatggccttc  ttaagatcaa  aatctgcgag
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481 aagcggcagg  gggccgtcag  gcgcagggtc  catcagggtc  acggccacaa  gttcatggcc
541 acctatcttc  ggcagccac  ctactgtccc  cattgcagag  acttcacatg  ggggtgtcata
601 ggaaagcagg  gataccagtg  tcaagtctgc  acctgcgtgg  tccacaagcg  gtgccacgag
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1441 tgcttccaga  ccaaggaccg  cctctttttc  gtcattggaat  atgtaaattg  tggagacctc
1501 atgtttcaga  ttcagcgctc  ccgaaaattc  gacgagcctc  gttcacggtt  ctatgtctga
1561 gaggtcacat  cggccctcat  gttcctccac  cagcatggag  tcattctacg  ggatttgaaa

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1741 gtccttgaga tcctgcagga gttggagtat ggcccctccg tggactggtg ggccctgggg
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1921 gtcagcatct tgaaagcttt catgacgaag aatccccaca agcgcctggg ctgtgtggca
1981 tcgcagaatg gcgaggacgc catcaagcag caccattct tcaaagagat tgactgggtg
2041 ctcctggagc agaagaagat caagccacc ttcaaaccac gcattaaaac caaaagagac
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2221 ctgatgccct ga

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