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Certificate of Analysis

PKCζ, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-525, 14-525-K, 14-525M
Parent Lot # 1718700

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 human PKCζ, amino acids 2–end. Expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 91.8% by SDS-PAGE and Coomassie blue staining. MW = 68.9kDa.

Specific Activity (Parent lot# 1718700): 1377U/mg, where one unit of PKCζ activity is defined as 1nmol phosphate incorporated into 50µM PKCtide (ERMRPRKRQGSVRRRV) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.538mg/ml of enzyme in 20mM Tris/HCl pH7.5, 0.02% Triton X-100, 5% glycerol, 1mM EDTA, 1mM EGTA, 0.1% 2-mercaptoethanol, 1mM PMSF, 10mM benzamidine. Frozen solution.

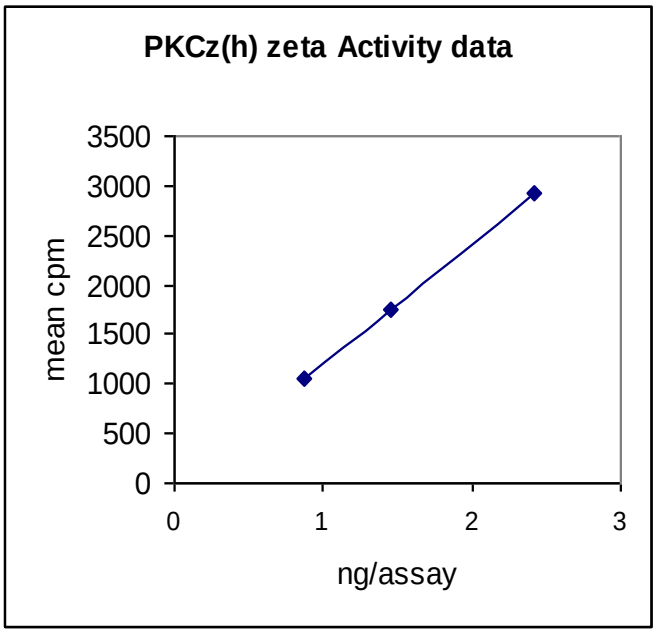
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge 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

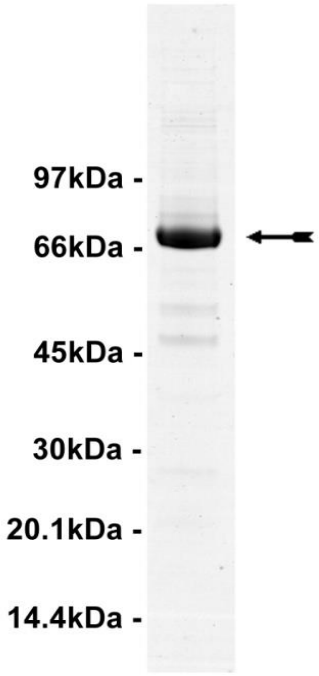
Quality Control Testing

Kinase Assay: 0.9–2.4ng of this lot of enzyme phosphorylated 50µM PKCtide 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 PKCζ 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 active PKCζ.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **PKCtide (ERMRPRKRQGSVRRRV):** Use at a final assay concentration of 50µM. Prepare a 500µM stock and add 2.5µl of stock per assay point.
3. **PKCζ, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.9–2.4ng per assay point.
4. **[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 assay):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **PKCtide**.
3. Add **2.5µl (0.9–2.4ng) PKCζ, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
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 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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PKCζ Sequence Information

Protein Human PKCζ

Tags *N*-terminal 6His

Native sequence P10 of the recombinant protein is equivalent to P2 of human PKCζ

Accession number GenBank BC014270

Recombinant PKCζ amino acid sequence:

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   1 MHHHHHHEFP SRTGPKMEGS GGRVRLKAHY GGDIFITSVD AATTFEELCE EVRDMCRLHQ
  61 QHPLTLKWVD SEGDPCTVSS QMELEEAFRL ARQCRDEGLI IHVFPSTPEQ PGLPCPGEDK
 121 SIYRRGARRW RKLYRANGHL FQAKRFNRRA YCGQCSERIW GLARQGYRCI NCKLLVHKRC
 181 HGLVPLTCRK HMDSVMPSQE PPVDDKNEDA DLPSEETDGI AYISSSRKHD SIKDDSEDLK
 241 PVIDGMDGIK ISQGLGLQDF DLIRVIGRGS YAKVLLVRLK KNDQIYAMKV VKKELVHDDE
 301 DIDWVQTEKH VFEQASSNPF LVGLHSCFQT TSRLFLVIEY VNGGDLMFHM QRQRKLPEEH
 361 ARFYAAEICI ALNFLHERGI IYRDLKLDNV LLDADGHIKL TDYGMCKEGL GPGDTTSTFC
 421 GTPNYIAPEI LRGEEYGFSV DWWALGVLMF EMMAGRSPFD IITDNPDMNT EDYLFQVILE
 481 KPIRIPRFLS VKASHVLKGF LNKDPKERLG CRPQTGFSDI KSHAFFRSID WDLLEKKQAL
 541 PPFQPQITDD YGLDNFDTQF TSEPVQLTPD DEDAIKRIDQ SEFEGFEYIN  PLLLSTEESV
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Recombinant PKCζ nucleotide sequence:

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   1 atgcatcatc accatcacca tgaattcccc agcaggaccg gccccaagat ggaagggagc
  61 ggcggccgcg tccgcctcaa ggcgcattac gggggggaca tcttcatcac cagcgtggac
 121 gccgccacga ccttcgagga gctctgtgag gaagtgagag acatgtgtcg tctgcaccag
 181 cagcacccgc tcaccctcaa gtgggtggac agcgaaggtg acccttgcac ggtgtcctcc
 241 cagatggagc tggaagaggc tttccgcctg gcccgtcagt gcagggatga aggcctcatc
 301 attcatgttt tcccgagcac ccctgagcag cctggcctgc catgtccggg agaagacaaa
 361 tctatctacc gccggggagc cagaagatgg aggaagctgt accgtgccaa cggccacctc
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 601 cctccagtag acgacaagaa cgaggacgcc gaccttcctt ccgaggagac agatggaatt
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1141 atctacaggg acctgaagct ggacaacgtc ctcctggatg cggacgggca catcaagctc
1201 acagactacg gcatgtgcaa ggaaggcctg ggccctggtg acacaacgag cactttctgc
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1321 gactggtggg cgctgggagt cctcatgttt gagatgatgg ccgggcgctc cccgttcgac
1381 atcatcaccg acaacccgga catgaacaca gaggactacc ttttccaagt gatcctggag
1441 aagcccatcc ggatcccccg gttcctgtcc gtcaaagcct cccatgtttt aaaaggattt
1501 ttaaataagg accccaaaga gaggctcggc tgccggccac agactggatt ttctgacatc
1561 aagtcccacg cgttcttccg cagcatagac tgggacttgc tggagaagaa gcaggcgctc
1621 cctccattcc agccacagat cacagacgac tacggtctgg acaactttga cacacagttc
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1681 accagcgagc ccgtgcagct gaccccagac gatgaggatg ccataaagag gatcgaccag
1741 tcagagttcg aaggctttga gtatatcaac ccattattgc tgtccaccga ggagtcggtg
1801 tga
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Reviewed and approved by site quality representative,