

## Certificate of Analysis

### PKC $\zeta$ , 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 PKC $\zeta$ , amino acids 2–end. Expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>/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 PKC $\zeta$ , active activity is defined as 1nmol phosphate incorporated into 50 $\mu$ M PKCtide (ERMRRPRKRQGSVRRRV) per minute at 30°C with a final ATP concentration of 100 $\mu$ M.

**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.

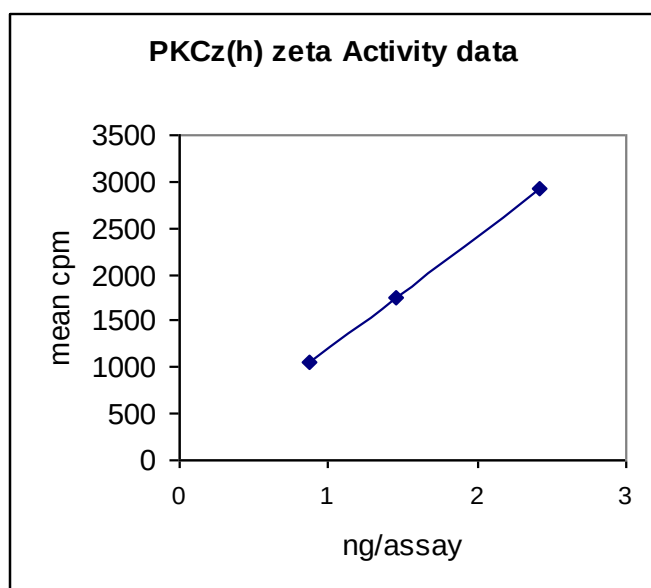
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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**

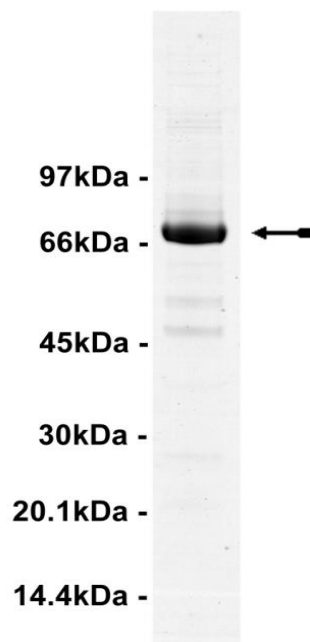
### Quality Control Testing

**Kinase Assay:** 0.9–2.4ng of this lot of enzyme phosphorylated 50 $\mu$ M 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 PKC $\zeta$  with the translated sequence listed on page three.

**SDS-PAGE and Coomassie Stain:** Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 $\mu$ g of PKC $\zeta$ , active.



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### Kinase Assay Protocol

#### Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **PKCtide (ERM<sub>1</sub>PRKRQGSVRRRV):** 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-<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[γ-<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-<sup>33</sup>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<sub>2</sub>O.
5. Add 10µl of diluted [γ-<sup>33</sup>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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## PKC $\zeta$ Sequence Information

|                                |                                                                         |
|--------------------------------|-------------------------------------------------------------------------|
| <b><u>Protein</u></b>          | Human PKC $\zeta$                                                       |
| <b><u>Tags</u></b>             | N-terminal 6His                                                         |
| <b><u>Native sequence</u></b>  | P10 of the recombinant protein is equivalent to P2 of human PKC $\zeta$ |
| <b><u>Accession number</u></b> | GenBank BC014270                                                        |

### Recombinant PKC $\zeta$ amino acid sequence:

```

1  MHHHHHHEFP SRTGPKMEGS GGRVRLKAHY GGDIFITSVD AATTFEELCE EVRDMCRLHQ
61  QHPLTLKWVD SEGDPCTVSS QMELEEAFLR ARQCRDEGLI IHVFPSTPEQ PGLPCPGEDK
121 SIYRRGARRW RKLVRANGHL FQAKRFNRRA YCQCQSERIW GLARQGYRCI NCKLLVHKRC
181 HGLVPLTCRK HMDSVMPSEQ 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 LRGEYGFVSF DWWALGVLMF EMMAGRSPFD IITDNPDMNT EDYLFQVILE
481 KPIRIPRFLS VKASHVLKGF LNKDPKERLG CRPQTGFSDI KSHAFFRSID WDLLEKKQAL
541 PPFQPQITDD YGLDNFDTQF TSEPVLTPD DEDAIKRIDQ SEFEGFEYIN PLLLSTEEVS

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### Recombinant PKC $\zeta$ nucleotide sequence:

```

1  atgcatcatc accatcacca tgaattcccc agcaggaccg gccccaagat ggaagggagc
61  ggcgcccgcg tccgcctcaa ggcgcattac ggggggggaca tcttcatcac cagcgtggac
121 gccgccacga ccttcgagga gctctgtgag gaagtgagag acatgtgtcg tctgcaccag
181 cagcacccgc tcaccctcaa gtgggtggac agcgaagggtg acccttgcac ggtgtcctcc
241 cagatggagc tggaaagggc tttccgcctg gcccgctcagt gcagggatga aggcctcatc
301 attcatgttt tcccagcac ccctgagcag cctggcctgc catgtccggg agaagacaaa
361 tctatctacc gccggggagc cagaagatgg aggaagctgt accgtgccaa cggccacctc
421 ttccaagcca agcgctttaa caggagagcg tactgcggtc agtgacgca gaggatatgg
481 ggcttcgca ggcaaggcta caggtgcatc aactgcaaac tgctggtcca taagcgctgc
541 caggcgctcg tcccgctgac ctgcaggaag catatggatt ctgtcatgcc ttccaagag
601 cctccagtag acgacaagaa cgaggacgcc gaccttcctt ccgaggagac agatggaatt
661 gcttacatct cctcatcccg gaagcatgac agcattaaag acgactcgga ggaccttaag
721 ccagttatcg atgggatgga tggaaatcaa atctctcagg ggcttgggct gcaggacttt
781 gacctaatca gagtcacgg gcgcgggagc tacgccaagg ttctcctggt gcggttgaa
841 aagaatgacc aaatttacgc catgaaagtg gtgaagaaag agctggtgca tgatgacgag
901 gatattgact gggtagacac agagaagcac gtgtttgagc aggcattccag caaccccttc
961 ctggctcgga tacactcctg cttccagacg acaagtcggt tgttcctggt cattgagtac
1021 gtcaacggcg gggacctgat gttccacatg cagaggcaga ggaagctccc tgaggagcac
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1141 atctacaggg acctgaagct ggacaacgtc ctctggatg cggacgggca catcaagctc
1201 acagactacg gcatgtgcaa ggaaggcctg ggccctgggt acacaacgag cactttctgc
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1321 gactggtggg cgctgggagt cctcatgttt gagatgatgg ccgggcgctc cccgttcgac
1381 atcatcaccg acaaccggga catgaacaca gaggactacc tttccaagt gatcctggag
1441 aagcccatcc ggatcccccg gttcctgtcc gtcaaagcct cccatgtttt aaaaggattt
1501 ttaataaagg accccaaaga gaggctcggc tgccggccac agactggatt ttctgacatc
1561 aagtcacacg cgttcttcg 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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