

Certificate of Analysis

PKC beta I, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-503, 14-503-K, 14-503M

Parent Lot # D8HN062U

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 amino acids 2–end of human PKC beta I expressed in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 91% by SDS-PAGE and Coomassie blue staining. MW = 78kDa.

Specific Activity (Parent lot# D8HN062U): 2552U/mg, where one unit of PKC beta I activity is defined as 1nmol phosphate incorporated into 0.1mg/ml histone H1 (in the presence of lipid activator and 0.1mM CaCl₂) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.194mg/ml of enzyme in 20mM Tris/HCl pH7.5, 0.02% Triton X-100, 1mM DTT, 1mM PMSF, 10mM benzamidine, 5% glycerol, 1mM EDTA, 1mM EGTA. Frozen solution.

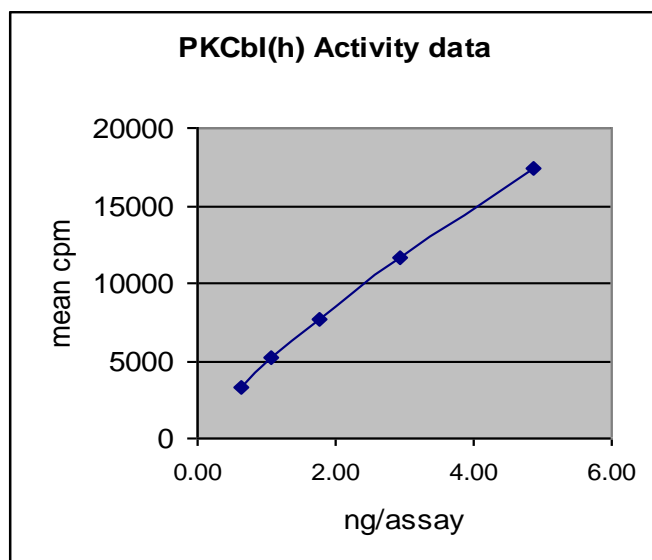
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

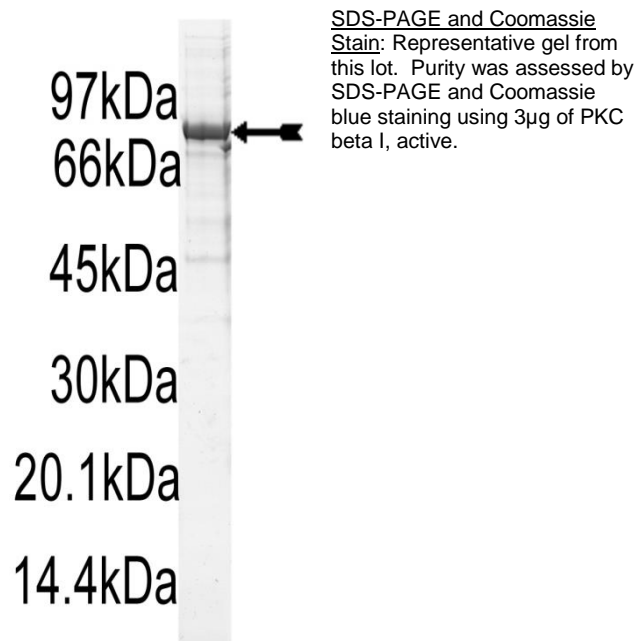
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.64–4.85ng of this lot of enzyme phosphorylated 0.1mg/ml histone H1 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 beta I 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. CaCl₂:** Make up a 1mM stock in dH₂O. Add 2.5µl of stock per assay point.
- 3. Histone H1:** Use at a final assay concentration of 0.1mg/ml. Make up a 1mg/ml stock in 20mM MOPS pH7.0. Add 2.5µl of stock per assay point.
- 4. 10 x Lipid Activator:** 0.3% Triton X-100, 1mg/ml phosphatidylserine, 0.1mg/ml diacylglycerol). Use 2.5µl of stock per assay point.
- 5. PKC beta I, active:** Dilute with 20mM HEPES/NaOH pH7.4, 0.03% Triton X-100. Use 0.64–4.85ng per assay point.
- 6. [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.
2. Add 2.5µl of **histone H1**.
3. Add 2.5µl of PKC lipid activator.
4. Add 2.5µl of 1mM CaCl₂.
5. Add **2.5 µl (0.64–4.85ng) PKC beta I, active**.
6. Add 2.5µl with dH₂O.
7. Add 10µl of diluted [γ-³³P] ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5µl of 3% phosphoric acid.
10. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
11. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
12. Wash the filtermat once for 2 minutes with methanol.
13. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
14. 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 beta I Sequence Information

<u>Protein</u>	Human PKC beta I
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	A2 of the recombinant protein is equivalent to A10 of human PKC beta I
<u>Accession number</u>	GenBank X06318

Recombinant PKC beta I amino acid sequence:

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1 MHHHHHHEFA DPAAGPPPSE GEESTVRFAR KGALRQKNVH EVKNHKFTAR FFKQPTFCSH
61 CTDFIWGFGK QGFQCQVCCF VVHKRCHEFV TFSCPGADKG PASDDPRSKH KFKIHTYSSP
121 TFCDHCGSL YGLIHQGMKC DTCMMNVHKR CVMNVPSLCG TDHTERRGRI YIQAHIDRDV
181 LIVLVRDAKN LVPMDPNGLS DPYVKLKLIP DPKSESKQKT KTIKCSLNPE WNETFRFQLK
241 ESKDRRLSV EIWDWDLTSR NDFMGSLFSG ISELQKASVD GWFKLLSQEE GEYFNVPVPP
301 EGSEANEELR QKFERAKISQ GTKVPEEKTT NTVSKFDNNG NRDRMKLTFD NFLMVLGKGS
361 FGKVMLSERK GTDELYAVKI LKKDVVIQDD DVECTMVEKR VLALPGKPPF LTQLHSCFQT
421 MDRLYFVMEY VNGGDLMYHI QQVGRFKEPH AVFYAAEIAI GLFFLQSKGI IYRDLKLDNV
481 MLDSEGHIKI ADFGMCKENI WDGVTTKTFC GTPDYIAPEI IAYQPYGKSV DWWAFGVLLY
541 EMLAGQAPFE GEDEDELQFS IMEHNVAYPK SMSKEAVAIC KGLMTKHPGK RLGCPEGER
601 DIKEHAFFRY IDWEKLERKE IQPPYKPKAR DKRDTSNFDK EFTRQPVVELT PTDKLFIMNL
661 DQNEFAGFSY TNPEFVINV
  
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Recombinant PKC beta I nucleotide sequence:

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1 atgcatcatc accatcacca tgaattcgct gaccgcgctg cggggcgcgc gccgagcgag
61 ggcgaggaga gcaccgtgcg ctctgcccgc aaaggcgccc tccggcagaa gaacgtgcat
121 gaggtcaaga accacaaatt caccgcccgc ttcttcaagc agcccacctt ctgcagccac
181 tgcaccgact tcactgggg ctctgggaag cagggattcc agtgccaagt ttgctgcttt
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901 gaaggaagtg agccaatga agaactgcgg cagaaatttg agagggccaa gatcagtcag
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1681 atcatggaac acaacgtagc ctatcccagg tctatgtcca aggaagctgt ggccatctgc
1741 aaagggtgca tgaccaaaaca cccaggcaaa cgtctgggtt gtggacctga aggcgaacct
  
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1801 gatatcaaag agcatgcatt tttccggtat attgattggg agaaacttga acgcaaagag
1861 atccagcccc cttataagcc aaaagctaga gacaagagag acacctccaa cttcgacaaa
1921 gagttcacca gacagcctgt ggaactgacc cccactgata aactcttcat catgaacttg
1981 gacaaaaatg aatttgctgg cttctcttat actaaccag agtttgtcat taatgtgtag
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