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Certificate of Analysis

PKC beta II

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-496, 14-496-K, 14-496M
Parent Lot # 28563U

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 beta II, amino acids 2–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 90.1% by SDS-PAGE and Coomassie blue staining. MW = 78.2kDa.

Specific Activity (Parent lot# 28563U): 1170U/mg, where one unit of PKC beta II activity is defined as 1nmol phosphate incorporated into 0.1mg/ml histone H1 per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.22mg/ml of enzyme in 20mM Tris/HCl pH7.5, 10mM benzamidine, 5% glycerol, 1mM EDTA, 1mM EGTA, 1mM PMSF, 0.1% 2-mercaptoethanol, 0.02% Triton X-100. Frozen solution.

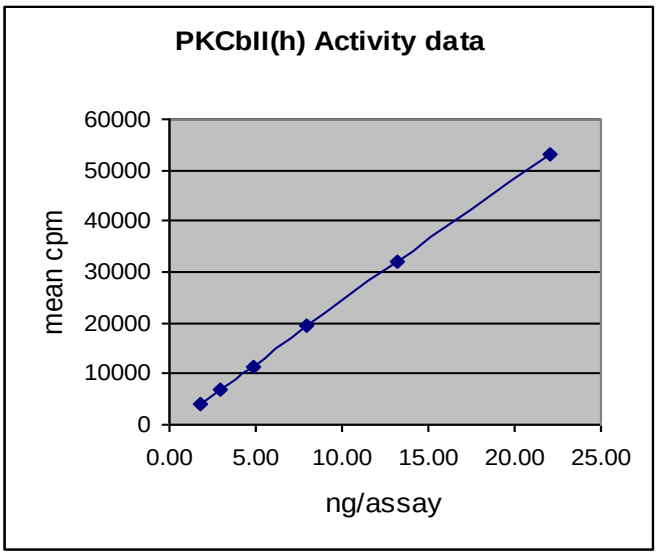
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

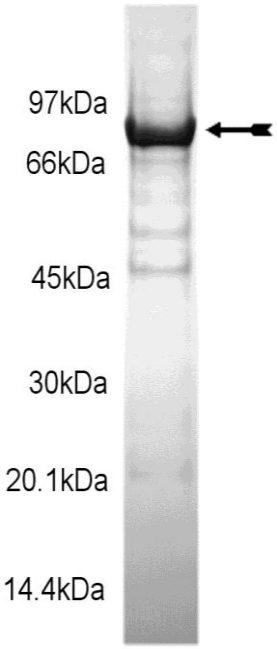
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 1.75–22ng 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 PKC beta II 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 beta II.

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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 200mM HEPES/NaOH pH7.4, 0.3% Triton X-100.
2. **Histone H1 Substrate:** Use at a final assay concentration of 0.1mg/ml. Prepare a 1mg/ml stock in 20mM MOPS pH7.0. Add 2.5µl of stock per assay point.
3. **PKC Lipid Activator (Catalogue# 20-133):** Use 2.5µl as supplied per assay point.
4. **$CaCl_2$:** Use at a final assay concentration of 0.1mM. Prepare a 1mM stock in dH_2O. Use 2.5µl per assay point.
5. **PKC beta II, active:** Dilute with 20mM HEPES/NaOH pH7.4, 0.03% Triton X-100. Use 1.75–22ng per assay point.
6. **[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 pate format)

1. Add 2.5µl of 10 x reaction buffer per assay to wells.
2. Add 2.5µl of **Histone H1**.
3. Add 2.5µl PKC Lipid Activator.
4. Add 2.5µl $CaCl_2$.
5. Add 2.5µl dH_2O.
6. Add **2.5µl (1.75–22ng) PKC beta II, active**.
7. Add 10µl [γ-^{33}P]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 II Sequence Information

Protein	human PKC beta II
Tags	*N*-Terminal 6His
Native sequence	A10 of the recombinant protein is equivalent to A2 of human PKC beta II
Accession number	GenBank X07109

Recombinant PKC beta II amino acid sequence:

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   1 MHHHHHHEFA DPAAGPPPSE GEESTVRFAR KGALRQKNVH EVKNHKFTAR FFKQPTFCSH
  61 CTDFIWGFGK QGFQCQVCCF VVHKRCHEFV TFSCPGADKG PASDDPRSKH KFKIHTYSSP
 121 TFCDHCGSLL YGLIHQGMKC DTCMMNVHKR CVMNVPSLCG TDHTERRGRI YIQAHIDRDV
 181 LIVLVRDAKN LVPMDPNGLS DPYVKLKLIP DPKSESKQKT KTIKCSLNPE WNETFRFQLK
 241 ESDKDRRLSV EIWDWDLTSR NDFMGSLSFG ISELQKASVD GWFKLLSQEE GEYFNVPVPP
 301 EGSEANEELR QKFERAKISQ GTKVPEEKTT NTVSKFDNNG NRDRMKLTDF NFLMVLGKGS
 361 FGKVMLSERK GTDELYAVKI LKKDVVIQDD DVECTMVEKR VLALPGKPPF LTQLHSCFQT
 421 MDRLYFVMEY VNGGDLMYHI QQVGRFKEPH AVFYAAEIAI GLFFLQSKGI IYRDLKLDNV
 481 MLDSEGHIKI ADFGMCKENI WDGVTTKTFC GTPDYIAPEI IAYQPYGKSV DWWAFGVLLY
 541 EMLAGQAPFE GEDEDELFQS IMEHNVAYPK SMSKEAVAIC KGLMTKHPGK RLGCGPEGER
 601 DIKEHAFFRY IDWEKLERKE IQPPYKPKAC GRNAENFDRF FTRHPPVLTP PDQEVIRNID
 661 QSEFEGFSFV NSEFLKPEVK S
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Recombinant PKC beta II nucleotide sequence:

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   1 atgcatcatc accatcacca tgaattcgct gacccggctg cggggccgcc gccgagcgag
  61 ggcgaggaga gcaccgtgcg cttcgcccgc aaaggcgccc tccggcagaa gaacgtgcat
 121 gaggtcaaga accacaaatt caccgcccgc ttcttcaagc agcccacctt ctgcagccac
 181 tgcaccgact tcatctgggg cttcgggaag cagggattcc agtgccaagt ttgctgcttt
 241 gtggtgcaca agcggtgcca tgaatttgtc acattctcct gccctggcgc tgacaagggt
 301 ccagcctccg atgacccccg cagcaaacac aagtttaaga tccacacgta ctccagcccc
 361 acgttttgtg accactgtgg gtcactgctg tatggactca tccaccaggg gatgaaatgt
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 541 ctcattgtcc tcgtaagaga tgctaaaaac cttgtaccta tggaccccaa tggcctgtca
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 721 gaatcggaca aagacagaag actgtcagta gagatttggg attgggattt gaccagcagg
 781 aatgacttca tgggatcttt gtcctttggg atttctgaac ttcagaaagc cagtgttgat
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 901 gaaggaagtg aggccaatga agaactgcgg cagaaatttg agagggccaa gatcagtcag
 961 ggaaccaagg tcccggaaga aaagacgacc aacactgtct ccaaatttga caacaatggc
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1081 tttggcaagg tcatgctttc agaacgaaaa ggcacagatg agctctatgc tgtgaagatc
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1381 ggtctgttct tcttacagag taagggcatc atttaccgtg acctaaaact tgacaacgtg
1441 atgctcgatt ctgagggaca catcaagatt gccgattttg gcatgtgtaa ggaaaacatc
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1681 atcatggaac acaacgtagc ctatcccaag tctatgtcca aggaagctgt ggccatctgc
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1741 aaagggctga tgaccaaaca cccaggcaaa cgtctgggtt gtggacctga aggcgaacgt
1801 gatatcaaag agcatgcatt tttccggtat attgattggg agaaacttga acgcaaagag
1861 atccagcccc cttataagcc aaaagcttgt gggcgaaatg ctgaaaactt cgaccgattt
1921 ttcacccgcc atccaccagt cctaacacct cccgaccagg aagtcatcag gaatattgac
1981 caatcagaat tcgaaggatt ttcctttgtt aactctgaat ttttaaaacc cgaagtcaag
2041 agctaa
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Reviewed and approved by site quality representative.