

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 Ni²⁺/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.

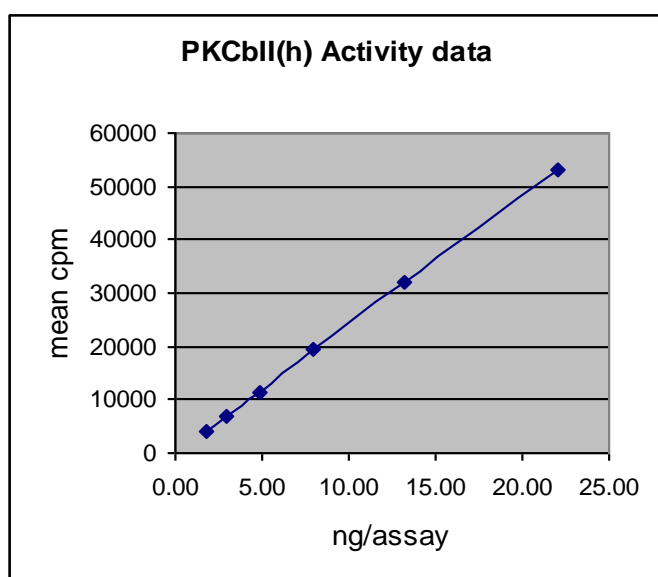
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

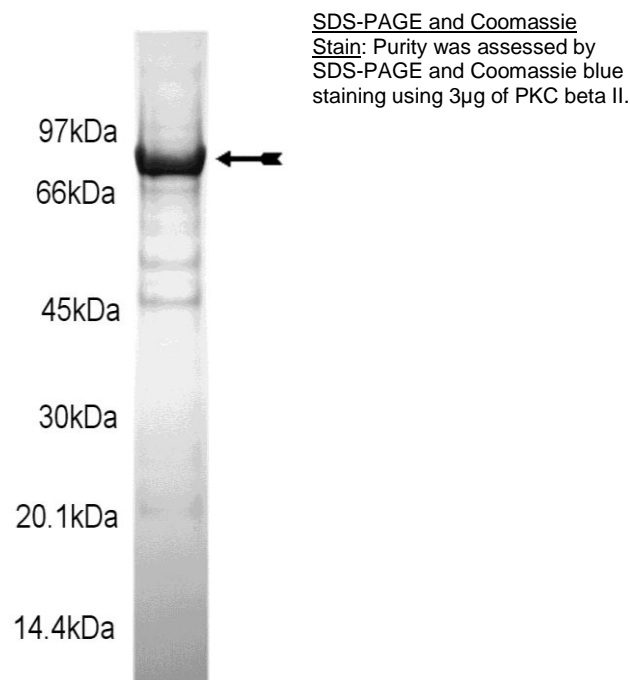
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 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.



Certificate of Analysis

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. CaCl₂:** Use at a final assay concentration of 0.1mM. Prepare a 1mM stock in dH₂O. 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-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 **Histone H1**.
3. Add 2.5µl PKC Lipid Activator.
4. Add 2.5µl CaCl₂.
5. Add 2.5µl dH₂O.
6. Add **2.5µl (1.75–22ng) PKC beta II, active**.
7. Add 10µl [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.

Certificate of Analysis

PKC beta II Sequence Information

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

Recombinant PKC beta II amino acid sequence:

```

1 MHHHHHHEFA DPAAGPPPSE GEESTVRFAR KGALRQKNVH EVKNHKFTAR FFKQPTFCSH
61 CTDFIWGFGK QGFQCQVCCF VVHKRCHEFV TFSCPGADKG PASDDPRSKH KFKIHTYSSP
121 TFDHCGSLL YGLIHQGMKC DTCMMNVHKR CVMNVPSLCG TDHTERRGRI YIQAHIDRDV
181 LIVLVRDAKN LVPMDPNGLS DPYVKLKLIP DPKSESKQKT KTIKCSLNPE WNETFRFQLK
241 ESDKDRRLSV EIWDWDLTSR NDFMGSLSFG ISELQKASVD GWFKLLSQEE GEYFNVPVPP
301 EGSEANEELR QKFERAKISQ GTKVPEEKT 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 RLGCGPEGER
601 DIKEHAFFRY IDWEKLERKE IQPPYKPKAC GRNAENFDRF FTRHPPVLT P DQEVIRNID
661 QSEFEGFSFV NSEFLKPEVK S

```

Recombinant PKC beta II nucleotide sequence:

```

1 atgcatcatc accatcacca tgaattcgct gaccggtcg cggggccgcc gccgagcgag
61 ggcgaggaga gcaccgtgcg cttegcccgc aaaggcgccc tccggcagaa gaacgtgcat
121 gaggtcaaga accacaaatt caccgcccgc ttcttcaagc agcccacctt ctgcagccac
181 tgcaccgact tcatctgggg cttegggaag cagggattcc agtgccaagt ttgctgcttt
241 gtggtgcaca agcggtgcca tgaatttgct acattctcct gccctggcgc tgacaagggt
301 ccagcctccg atgacccccg cagcaaacac aagtttaaga tccacacgta ctccagcccc
361 acgttttggt accactgtgg gtcactgctg tatggactca tccaccaggg gatgaaatgt
421 gacacctgca tgatgaatgt gcacaagcgc tgcgtgatga atgttcccag cctgtgtggc
481 acggaccaca cggagcgccg cggcgcgcat tacatccagg cccacatcga cagggacgtc
541 ctcattgtcc tcgtaagaga tgctaaaaac cttgtacctg tggaccccaa tggcctgtca
601 gatcccctacg taaaactgaa actgattccc gatccccaaa gtgagagcaa acagaagacc
661 aaaaccatca aatgctccct caaccctgag tggaatgaga catttagatt tcagctgaaa
721 gaatcggaca aagacagaag actgtcagta gagatttggg attgggattt gaccagcagg
781 aatgacttca tgggatcttt gtcctttggg atttctgaac ttcagaaagc cagtgttgat
841 ggctggttta agttactgag ccagaggaaa ggcgagtact tcaatgtgcc tctgccacca
901 gaaggaagtg agccaatga agaactgagg cagaaatttg agagggccaa gatcagtcag
961 ggaaccaagg tcccgaaga aaagacgacc aacctgtct ccaaatttga caacaatggc
1021 aacagagacc ggatgaaact gaccgatttt aacttcctaa tgggtgctggg gaaaggcagc
1081 tttggcaagg tcatgctttc agaacgaaaa ggcacagatg agctctatgc tgtgaagatc
1141 ctgaagaagg acgttgatgat ccaagatgat gacgtggagt gcaactatggg ggagaagcgg
1201 gtgttgcccc tgccctgggaa gccgcccttc ctgaccagc tccactcctg cttccagacc
1261 atggaccgcc tgtactttgt gatggagtac gtgaatgggg gcgacctcat gtatcacatc
1321 cagcaagtgc gccggttcaa ggagccccat gctgtatttt acgctgcaga aattgccatc
1381 ggtctgttct tcttacagag taagggcac tttaccgtg acctaaaact tgacaacgtg
1441 atgctcgatt ctgagggaca catcaagatt gccgattttg gcatgtgtaa ggaaaacatc
1501 tgggatgggg tgacaaccaa gacattctgt ggcactccag actacatcgc ccccagata
1561 attgcttacc agccctatgg gaagtccgtg gattggtggg catttgagat cctgctgtat
1621 gaaatggttg ctgggcaggc accctttgaa ggggaggatg aagatgaact cttccaatcc
1681 atcatggaac acaacgtagc ctatccaag tctatgtcca aggaagctgt ggccatctgc

```

Certificate of Analysis

```
1741 aaagggctga tgaccaaaca cccaggcaaa cgtctggggtt gtggacctga aggcgaacgt
1801 gatatcaaag agcatgcatt tttccggtat attgattggg agaaacttga acgcaaagag
1861 atccagcccc cttataagcc aaaagcttgt gggcgaaatg ctgaaaactt cgaccgattt
1921 ttcaccgccc atccaccagt cctaacacct cccgaccagg aagtcacagc gaatattgac
1981 caatcagaat tcgaaggatt ttcctttggt aactctgaat ttttaaacc cgaagtcaag
2041 agctaa
```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services.