

## Certificate of Analysis

### PKC Theta, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-444, 14-444-K, 14-444M

Parent Lot # WAE0350

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:** C-terminal 6His-tagged, recombinant, full-length, human PKC theta, expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>/NTA-agarose. Purity 62% by SDS-PAGE and Coomassie blue staining. MW = 82.8kDa.

**Specific Activity (Parent lot# WAE0350):** 57U/mg, where one unit of PKC theta, active 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:** 0.18mg/ml of enzyme in 50mM Hepes/NaOH pH7.5, 150mM NaCl, 0.03% Brij-35, 0.1mM EGTA, 5% glycerol, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

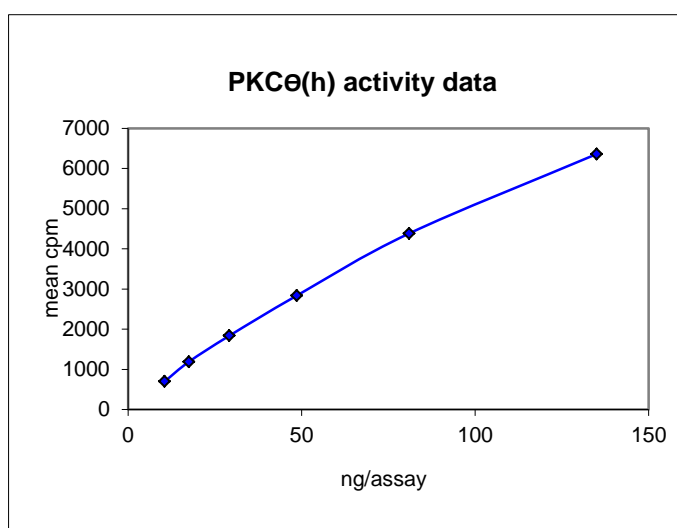
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

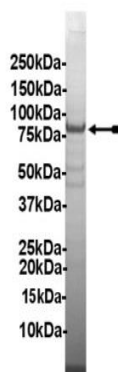
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 10.50–135.00ng of this lot of enzyme phosphorylated 0.1mg/ml Histone Hi 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 theta 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 theta, active.

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## Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. 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.
- 3. PKC theta, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 10.50–135.00ng per assay point.
- 4. [ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP specific activity approximately 500 – 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl **histone H1**.
3. Add **2.5µl (10.50–135.00ng) PKC theta, active**.
4. Add 5µl of dH<sub>2</sub>O.
5. Add 10µl of diluted [ $\gamma$ -<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 the 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 Theta Sequence Information

<b><u>Protein</u></b>	Human PKC theta
<b><u>Tags</u></b>	C-terminal 6His
<b><u>Native sequence</u></b>	M1 of the fusion protein is equivalent to M1 of human PKC theta
<b><u>Accession number</u></b>	GenBank L07032

### Recombinant PKC theta amino acid sequence:

```

1 MSPFLRIGLS NFDGSCQSC QGEAVNPYCA VLVKEYVESE NGQMYIQKKP TMYPPWDSTF
61 DAHINKGRVM QIIIVGKNVD LISETTVELY SLAERCCKNN GKTEIWLELK PQGRMLMNAR
121 YFLEMSDTKD MNEFETEGFF ALHQRRGAIK QAKVHHVKCH EFTATFFPQP TFCSVCHEFV
181 WGLNKQGYQC RQCNAAIHKK CIDKVIKCT GSAINSRETM FHKERFKIDM PHRFKVYNYK
241 SPTFCEHCGT LLWGLARQGL KCDACGMNVH HRCQTKVANL CGINQKLMAE ALAMIESTQQ
301 ARCLRDTEQI FREGPVEIGL PCSIKNEARP PCLPTPGKRE PQGISWESPL DEVDKMCHLP
361 EPELNKERPS LQIKLKIEDF ILHKMLGKGS FGKVFLAEFK KTNQFFAIKA LKKDVLMDDD
421 DVECTMVEKR VLSLAWEHF LTHMFCTFQT KENLFFVMEY LNGGDLMYHI QSCHKFDLSR
481 ATFYAAEIIIL GLQLHSGKI VYRDLKLDNI LLDKDGHIKI ADFGMCKENM LGDAKTNTFC
541 GTPDYIAPEI LLGQKYNHSV DWWSFGVLLY EMLIGQSPFH GQDEEELFHS IRMDNPFYPR
601 WLEKEAKDLL VKLFVREPEK RLGVRGDIRQ HPLFREINWE ELERKEIDPP FRPKVKSPFD
661 CSNFDKEFLN EKPRLSFADR ALINSMDQNM FRNFSFMNPG MERLISHHHH HH
    
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### Recombinant PKC theta nucleotide sequence:

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1 atgtcgccat ttcttcggat tggcttgtcc aactttgact gcgggtcctg ccagtcttgt
61 cagggcgagg ctgtaacc ttactgtgct gtgctcgtca aagagtatgt cgaatcagag
121 aacgggcaga tgtatatcca gaaaaagcct accatgtacc caccctggga cagcactttt
181 gatgcccata tcaacaaggg aagagtcattg cagatcattg tgaaaggcaa aaacgtggac
241 ctatctctg aaaccaccgt ggagctctac tcgctggctg agaggtgcag gaagaacaac
301 gggaagacag aaatatgggt agagctgaaa cctcaaggcc gaatgctaataaat gaatgcaaga
361 tactttctgg aaatgagtga cacaaaggac atgaatgaat ttgagacgga aggcttcttt
421 gctttgcatc agcgcggggg tgccatcaag caggcaaagg tccaccacgt caagtgccac
481 gagttcactg ccaccttctt cccacagccc acattttgct ctgtctgcca cgagtttgtc
541 tggggcctga acaaacaggg ctaccagtgc cgacaatgca atgcagcaat tcacaagaag
601 tgtattgata aagttatagc aaagtgcaca ggatcagcta tcaatagccg agaaaccatg
661 ttccacaagg agagattcaa aattgacatg ccacacagat ttaaagtcta caattacaag
721 agcccacact tctgtgaaca ctgtgggacc ctgctgtggg gactggcacg gcaaggactc
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961 ccatgctcca tcaaaaatga agcaaggccg ccatggttac cgacaccggg aaaaagagag
1021 cctcagggca tttctggga gtctccgttg gatgagggtg ataaaatgtg ccatcttcca
1081 gaacctgaac tgaacaaaga aagaccatct ctgcagatta aactaaaaat tgaggatattt
1141 atcttgacaa aaatgttggg gaaaggaagt tttggcaagg tcttctggc agaattcaag
1201 aaaaccaatc aattttcgc aataaaggcc ttaaagaaag atgtggtctt gatggacgat
1261 gatgttgagt gcacgatggt agagaagaga gttctttcct tggcctggga gcatccgttt
1321 ctgacgcaca tgttttgtac attccagacc aaggaaaacc tctttttgt gatggagtac
1381 ctcaacggag gggacttaat gtaccacatc caaagctgcc acaagttcga cctttccaga
1441 gcgacgtttt atgctgctga aatcattctt ggtctgcagt tccttcattc caaaggaata
1501 gtctacaggg acctgaagct agataacatc ctgtagaca aagatggaca tatcaagatt
1561 gcggattttg gaatgtgcaa ggagaacatg ttaggatg ccaagacgaa taccttctgt
    
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```
1621 gggacacctg actacatcgc cccagagatc ttgctgggtc agaaatacaa ccaactctgtg
1681 gactggtggt ccttcggggt tctcctttat gaaatgctga ttggtcagtc gcctttccac
1741 gggcaggatg aggaggagct cttccactcc atccgcatgg acaatccctt ttaccacacgg
1801 tggctggaga aggaagcaaa ggaccttctg gtgaagctct tcgtgagaga acctgagaag
1861 aggctgggcg tgaggggaga catccgccag cacccttgtg ttcgggagat caactgggag
1921 gaacttgaac ggaaggagat tgaccaccg ttccggccga aagtgaaatc accatttgac
1981 tgcagcaatt tcgacaaaga attcttaaac gagaagcccc ggctgtcatt tgccgacaga
2041 gcactgatca acagcatgga ccagaatatg ttcaggaact tttccttcat gaacccggg
2101 atggagcggc tgatatccca tcacatcat caccattga
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