

Certificate of Analysis

PKC eta, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-497, 14-497-K, 14-497M

Parent Lot # 25609U

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 eta, amino acids 2–end expressed by baculovirus in SF21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 80.5% by SDS-PAGE and Coomassie blue staining. MW = 78.6kDa.

Specific Activity (Parent lot# 25609U): 530U/mg, where one unit of PKC eta, active activity is defined as 1nmol phosphate incorporated into 50µM PKC tide per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.154mg/ml of enzyme in 20mM Tris/HCl pH7.5, 10mM benzamidine, 5% glycerol, 1mM EDTA, 1mM EGTA, 1mM PMSF, 0.02% Triton X-100, 0.1% 2-mercaptoethanol. Frozen solution.

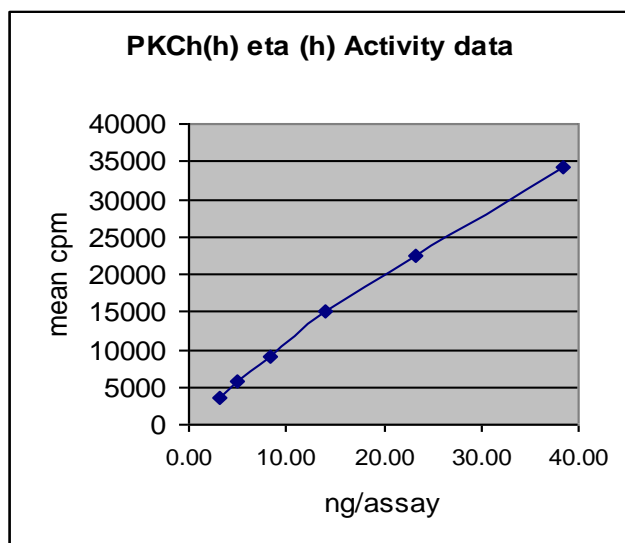
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

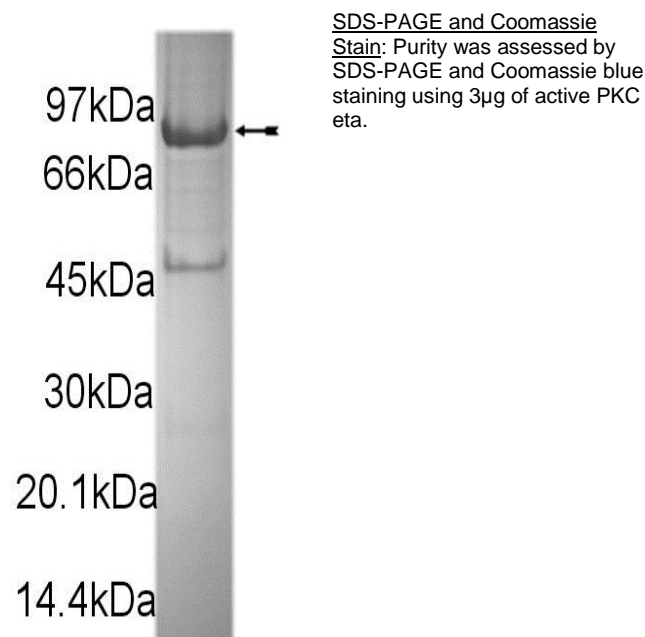
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 3.1–38.5ng of this lot of enzyme phosphorylated 50µM PKC tide 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 eta with the translated sequence identified on page three.



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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. PKCtide (ERM_RPRKRQGSVRRRV):** Make a 500µM stock in H₂O and use 2.5µl per assay point.
- 3. PKC Lipid Activator (Catalogue# 20-133):** Use 2.5µl as supplied.
- 4. PKC eta, active:** Dilute with 20mM Hepes/NaOH pH7.4, 0.03% Triton X-100. Use 3.1–38.5ng per assay point.
- 5. [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.
2. Add 2.5µl of **PKCtide (ERM_RPRKRQGSVRRRV)**.
3. Add 2.5µl PKC Lipid Activator.
4. Add 5µl H₂O.
5. Add **2.5µl (3.1–38.5ng) PKC eta, active**.
6. Add 10µl of diluted [γ -³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. 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 eta Information

<u>Protein</u>	Human PKC eta
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	S8 of the recombinant protein is equivalent to S2 of human PKC eta
<u>Accession number</u>	GenBank NM_006255. Translation conflicts with NM_006255, V108L and G109R reported in dbEST BG719560 and BM549890, and L392Q reported in dbEST BI913495, BQ051772 and AU136862.

Recombinant PKC eta amino acid sequence:

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1 MHHHHHSSG TMKFNGYLRV RIGEAVGLQP TRWSLRHSLF KKGHQLLDPY LTVSVDQVRV
61 GQTSTKQKTN KPTYNEEFCA NVTDGGHLEL AVFHETPLGY DFVANCTLQF QELLRRTTGAS
121 DTFEGWVDLE PEGKVFVVIT LTGSFTEATL QDRIRFKHFT RKRQRAMRRR VHQINGHKFM
181 ATYLRQPTYC SHCREFIWGV FGKQGYQCQV CTCVVKRCH HLIVTACTCQ NNINKVDSKI
241 AEQRFGINIP HKFSIHNYKV PTFCDHCGSL LWGIMRQGLQ CKICKMNVHI RCQANVAPNC
301 GVNAVELAKT LAGMGLQPGN ISPTSKLVSF STLRRQKES SKEGNGIGVN SSNRLGIDNF
361 EFIRVLGKGS FGKVMLARVK ETGDLYAVKV LKKDVLQDD DVECTMTEKR ILSLARNHPF
421 LTQLFCCFQT PDRLFFVMEF VNGGDLMFHI QKSRRFDEAR ARFYAAEIIIS ALMFLHDKGI
481 IYRDLKLDNV LLDHEGHCKL ADFGMCKEGI CNGVTTATFC GTPDYIAPEI LQEMLYGPAV
541 DWWAMGVLLY EMLCGHAPFE AENEDDLFEA ILNDEVVYPT WLHEDATGIL KSFMTKNPTM
601 RLGSLTQGGG HAILRHPFFK EIDWAQLNHR QIEPPFRPRI KSREDVSNFD PDFIKEEPVL
661 TPIDEGHLPM INQDEFNFS YVSPQLQP
    
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Recombinant PKC eta nucleotide sequence:

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1 atgcatcacc atcatcacca ttcgtctggc accatgaagt tcaatggcta tttgagggtc
61 cgcacggtg aggcagtggt gctgcagccc acccgcctgt ccctgcgcca ctgcctctc
121 aagaagggcc accagctgct ggaccctat ctgacggtga gcgtggacca ggtgcgcgtg
181 ggcagacca gcaccaagca gaagaccaac aaaccacgt acaacgagga gttttgcgct
241 aacgtcaccg acggcggcca cctcagttg gccgtcttc acgagacgcc cctgggctac
301 gacttcgtgg ccaactgcac cctgcagttc caggagctgc tgcgcacgac cggcgcctcg
361 gacaccttcg agggttgggt ggatctcgag ccagaggga aagtatttgt ggtaataacc
421 cttaccggga gtttactga agtactctc cagagagacc ggatcttcaa acattttacc
481 aggaagcgcc aaaggctat gcgaaggcga gtccaccaga tcaatggaca caagttcatg
541 gccacgtatc ttaggcagcc cacctactgc tctcactgca gggagtttat ctggggagtg
601 tttgggaaac agggttatca gtgccagtg tgcacctgtg tcgtccataa acgctgccat
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781 ccgacattct gcgactactg tggctactg ctctggggaa taatcgaca aggacttcag
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1261 ctactcagt tgttctgctg ctttcagacc cccgatcgtc tgttttttgt gatggagttt
1321 gtgaatgggg gtgacttgat gttccacatt cagaagtctc gtcgttttga tgaagcacga
1381 gctcgcttct atgctgcaga aatcatttcg gctctcatgt tcctccatga taaaggaatc
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1501 gcagacttcg gaatgtgcaa ggaggggatt tgcaatggtg tcaccacggc cacattctgt
1561 ggcacgccag actatatcgc tccagagatc ctccaggaaa tgctgtacgg gcctgcagta
    
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1621 gactggtggg caatgggctg gttgctctat gagatgctct gtggtcacgc gccttttgag
1681 gcagagaacg aagatgacct ctttgaggcc atactgaatg atgaggtggg ctaccctacc
1741 tggctccatg aagatgccac agggatccta aaatctttca tgaccaagaa ccccaccatg
1801 cgcttgggca gcctgactca gggaggcgag cacgccatct tgagacatcc tttttttaag
1861 gaaatcgact gggcccagct gaaccatcgc caaatagaac cgcctttcag acccagaatc
1921 aaatcccagag aagatgtcag taattttgac cctgacttca taaaggaaga gccagtttta
1981 actccaattg atgagggaca tcttccaatg attaaccagg atgagtttag aaacttttcc
2041 tatgtgtctc cagaattgca accatag
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