

Certificate of Analysis

PKC delta, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-504, 14-504-K, 14-504M

Parent Lot # D8PN018U

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 delta expressed in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 98% by SDS-PAGE and Coomassie blue staining. MW = 78.7kDa.

Specific Activity (Parent lot# D8PN018U): 1280U/mg, where one unit of PKC delta, active activity is defined as 1nmol phosphate incorporated into 50µM PKCtide (ERM₂PRKRQGSVRRRV) in the presence of lipid activator (cat# 20-133) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.216mg/ml of enzyme in 50mM Tris/HCl pH7.5, 270mM sucrose, 150mM NaCl, 1mM benzamidine, 0.2mM PMSF, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.03% Brij-35. Frozen solution.

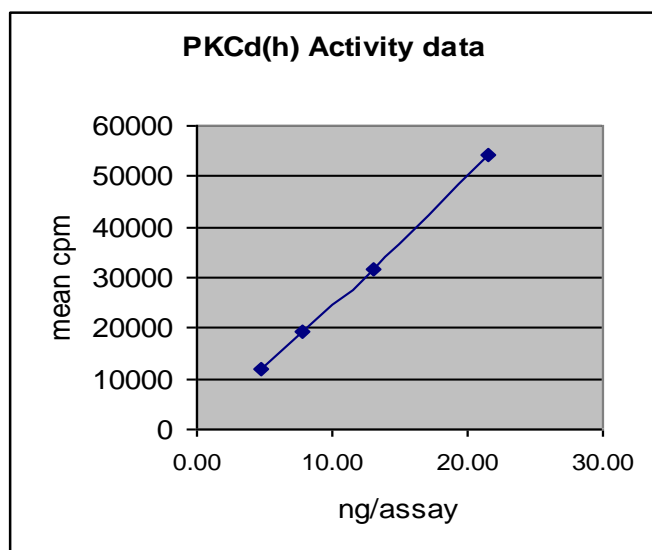
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

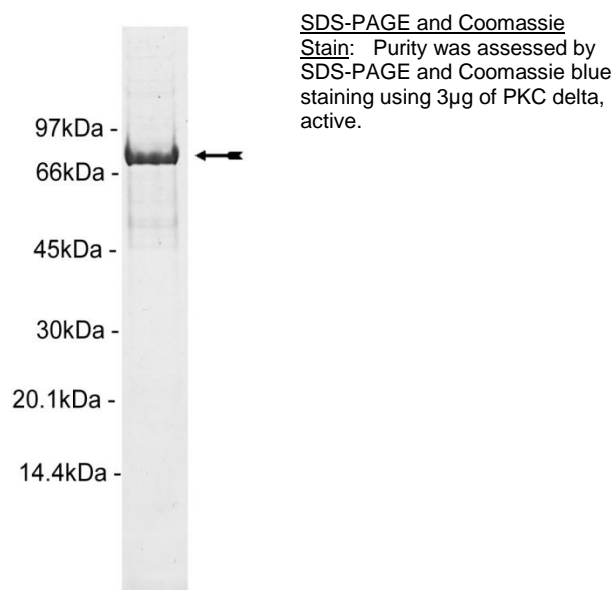
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 4.7–21.6ng of this lot of enzyme phosphorylated 50µM PKCtide 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 human PKC delta with of 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, 0.3% Triton X-100.
- 2. 10 x Lipid Activator:** 0.3% Triton X-100, 1mg/ml phosphatidylserine, 0.1mg/ml diacylglycerol). Use 2.5µl of stock per assay point.
- 3. PKCtide:** Use at a final assay concentration of 50µM. Make up a 500µM stock. Add 2.5µl of stock per assay point.
- 4. PKC delta, active:** Dilute with 20mM HEPES/NaOH pH7.4, 0.03% Triton X-100. Use 4.7–21.6ng 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 per assay.
2. Add 2.5µl of **PKCtide (ERM RPRKRQGSVRRRV)**.
3. Add 2.5µl of PKC lipid activator.
4. Add **2.5µl (4.7–21.6ng) PKC delta, active**.
5. Add 5µl of dH₂O.
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 and 1µl of 30% phosphoric acid.

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PKC delta Sequence Information

<u>Protein</u>	Human PKC delta
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	A10 of the recombinant protein is equivalent to A2 of human PKC delta
<u>Accession number</u>	GenBank L07861

Recombinant PKC delta amino acid sequence:

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1  MHHHHHHEFA PFLRIAFNSY ELGSLQAEDE ANQPFCAVKM KEALSTERGK TLVQKKPTMY
61  PEWKSTFDAH IYEGRVIQIV LMRAAEEPVS EVTVGVSVLA ERCKKNNNGKA EFWLDLQPQA
121 KVLMSVQYFL EDVDCKQSMR SEDEAKFPTM NRRGAIKQAK IHYIKNHEFI ATFFGQPTFC
181 SVCKDFVWGL NKQGYKCRQC NAAIHKKCID KIIGRCTGTA ANSRDTIFQK ERFNIDMPHR
241 FKVHNYMSPT FCDHCGSLW GLVKQGLKCE DCGMNVHHC REKVANLCGI NQKLLAEALN
301 QVTQRASRRS DSASSEPVGI YQGFEKKTGV AGEDMQDNSG TYGKIWEGSS KCNINNFIFH
361 KVLGKGSFGK VLLGELKGRG EYFAIKALKK DVVLIDDDVE CTMVEKRVLT LAENPFLTH
421 LICTFQTKDH LFFVMEFLNG GDLMYHIQDK GRFELYRATF YAAEIMCGLQ FLHSGI IYR
481 DLKLDNVLLD RDGHIKIADF GMCKENIFGE SRASTFCGTP DYIAPEILQG LKYTFVSVDWW
541 SFGVLLYEML IGQSPFHGDD EDELFE SIRV DTPHYPRWIT KESKDILEKL FEREPKRLG
601 VTGNIKIHPF FKTINWTLLE KRRLEPPFRP KVKS PRDYSN FDQEFLNEKA RLSYSDKNLI
661 DSMDQSAFAG FSFVNPKFEH LLED

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Recombinant PKC delta nucleotide sequence:

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1  atgcatcatc accatcacca tgaattcgcg cgttcctgc gcatgcctt caactcctat
61  gagctgggct ccctgcaggc cgaggacgag gccaaccagc cttctgtgc cgtgaagatg
121 aaggaggcgc tcagcacaga gcgtgggaaa aactggtgc agaagaagcc gaccatgtat
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1501 gggatgtgca aagagaacat attcggggag agccgggcca gcaccttctg cggcaccctt
1561 gactatatcg cccctgagat cctacagggc ctgaagtaca cattctctgt ggactggtgg
1621 tctttcgggg tccttctgta cgagatgctc attggccagt ccccttcca tggatgatg
1681 gaggatgaac tcttcgagtc catcctgtgt gacacgccac attatccccg ctggatcacc
1741 aaggagtcta aggacatcct ggagaagctc tttgaaaggg aaccaacca gaggctggga

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1801 gtgacgggaa acatcaaaat ccacccttc ttcaagacca taaactggac tctgctggaa
1861 aagcggaggt tggagccacc cttcaggccc aaagtgaagt cacccagaga ctacagtaac
1921 tttgaccagg agttcctgaa cgagaaggcg cgcctctcct acagcgacaa gaacctcatc
1981 gactccatgg accagtctgc attcgtggc ttctcctttg tgaaccccaa attcgagcac
2041 ctcttgaag attga
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