

Certificate of Analysis

PKC gamma, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-483, 14-483-K, 14-483M

Parent Lot # WAC0324

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 full length PKC gamma expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 78% by SDS-PAGE and Coomassie Blue staining. MW = 78kDa.

Formulation: 0.22mg/ml of enzyme in 20mM Tris/HCl pH7.5, 5% glycerol, 10mM benzamidine, 1mM PMSF, 1mM EGTA, 1mM EDTA, 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.

Specific Activity (Parent lot# WAC0324): 2320U/mg, where one unit of PKC alpha, 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.

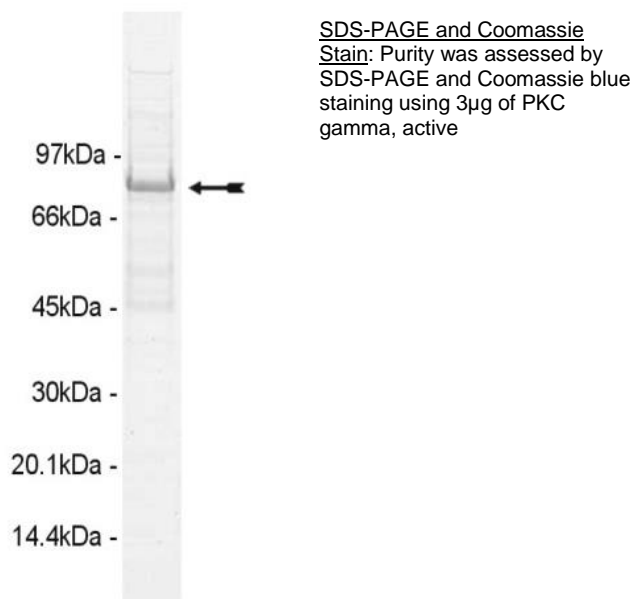
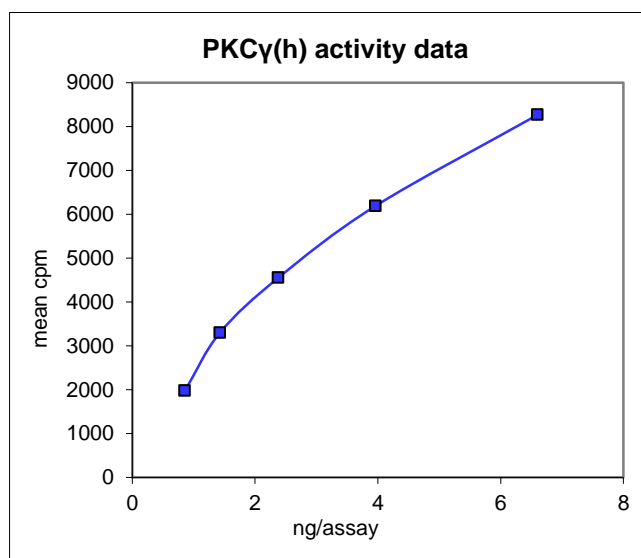
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

Kinase Assay: 0.86–6.60ng 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 product identity as PKC gamma with 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.
2. **CaCl₂:** Make up a 1mM stock in dH₂O. Add 2.5µl of stock per assay point.
3. **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.
4. **10 x Lipid Activator:** 0.3% Triton X-100, 1mg/ml phosphatidylserine, 0.1mg/ml diacylglycerol). Use 2.5µl of stock per assay point.
5. **PKC gamma, active:** Dilute with 20mM HEPES/NaOH pH7.4, 0.03% Triton X-100. Use 0.86–6.60ng per assay point.
6. **[γ-³³P]ATP:** 2.5x 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 **histone H1**.
3. Add 2.5µl of PKC lipid activator.
4. Add 2.5µl of 1mM CaCl₂.
5. Add **2.5µl (0.86–6.60ng) PKC gamma, active**.
6. Make up to 15µl with dH₂O.
7. Add 10µl of diluted [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.

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PKC gamma Sequence Information

Protein	human PKC gamma
Tags	N-terminal 6His
Native sequence	M8 of the recombinant is equivalent to M1 of the native sequence
Accession number	GenBank XM_017991

Recombinant PKC gamma amino acid sequence:

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1  MHHHHHMHAG  LGPGVGDSEG  GPRPLFCRKG  ALRQKVVHEV  KSHKFTARFF  KQPTFCSHCT
61  DFIWGIGKQG  LQCQVCSFV  HRRCHEFVTF  ECPGAGKGPQ  TDDPRNKHKF  RLHSYSSPTF
121 CDHCGSLLYG  LVHQGMKCS  CEMNVHRCV  RSVPSLCGVD  HTERRGRLQL  EIRAPTADEI
181 HVTVGEARNL  IPMDPNGLSD  PYVKLKLIPD  PRNLTKQKTR  TVKATLNPVW  NETFVFNLKP
241 GDVERRLSVE  VWDWDRSRN  DFMGAMSGV  SELLKAPVDG  WYKLLNQE  EYYNVPVADA
301 DNCSLLQKFE  ACNYPLELYE  RVRMGPS SSP  IPSPSPSP TD  PKRCFFGASP  GRLHISDFSF
361 LMLVGKGSFG  KVMLAERRGS  DELYAIKILK  KDVIQDDDV  DCTLVEKRVL  ALGGRGPGGR
421 PHFLTQLHST  FQTPDRLYFV  MEYVTGGDLM  YHIQQLGKFK  EPAAFYAAE  IAIGLFFLHN
481 QGIYRDLKL  DNVMLDAEGH  IKITDFGMCK  ENVFPGTTR  TFCGTPDYIA  PEIIAYQPYG
541 KSDVWWSFGV  LLYEMLAGQP  PFDGEDEEEL  FQAIMEQTVT  YPKSLSREAV  AICKGFLTKH
601 PGKRLGSGPD  GEPTIRAHGF  FRWIDWERLE  RLEIPPPFRP  RPCGRSGENF  DKFFTRAAPA
661 LTPPDRVLVA  SIDQADFQGF  TYVNPDFVHP  DARSPTSPVP  VPVM
    
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Recombinant PKC gamma nucleotide sequence:

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1  atgcatcacc  atcatcatca  tatggctggt  ctgggccccg  gcgtaggcga  ttcagagggg
61  ggaccccggc  ccctgtttt  cagaaagggg  gctctgaggc  agaaggtggt  ccacgaagtc
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2101 gtgcccgtca tgtaa
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