

Certificate of Analysis

PKC α , active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-484, 14-484-K, 14-484M

Parent Lot # 33245U

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 α expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 99% by SDS-PAGE and Coomassie Blue staining. MW = 78kDa.

Specific Activity (Parent lot# 33245U): 3597U/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 100 μ M.

Formulation: 0.203mg/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.

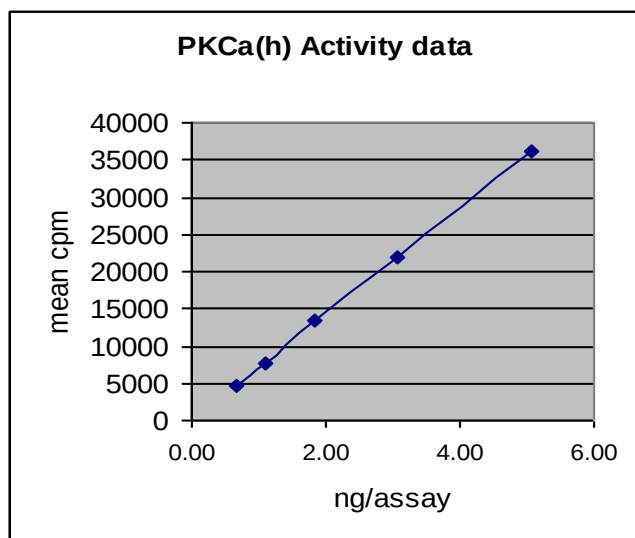
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

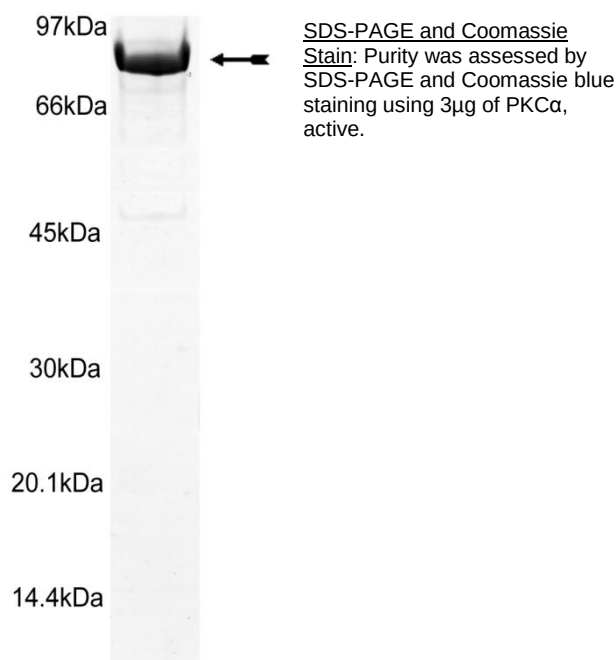
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.67–5.08ng 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 as PKC α 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 pH 7.0. 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. **PKCα, active:** Dilute with 20mM HEPES/NaOH pH7.4, 0.03% Triton X-100. Use 0.67–5.08ng 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 of PKC lipid activator.
4. Add 2.5µl of 1mM CaCl₂
5. Add **2.5µl (0.67–5.08ng) PKCα, active**.
6. Add 2.5µl of 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 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 α Sequence Information

<u>Protein</u>	human PKC α
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M8 of the recombinant protein is equivalent to M1 of human PKC α
<u>Accession number</u>	GenBank X52479

Recombinant PKC α amino acid sequence:

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1  MHHHHHMHAD  VFPGNDSTAS  QDVANRFARK  GALRQKNVHE  VKDHKFIARF  FKQPTFCSHC
61  TDFIWGFGKQ  GFQCQVCCFV  VHKRCHFEVT  FSCPGADKGP  DTDDPRSKHK  FKIH TYGSPT
121 FCDHCGSLLY  GLIHQGMKCD  TCDMNVHKQC  VINVPSLCGM  DHTEKRGRIY  LKAEVADEKL
181 HVTVRDAKNL  IPMDPNGLSD  PYVKLKLIPD  PKNESKQKTK  TIRSTLN PQW  NESFTFKLKP
241 SDKDRRLSVE  IWDWDRTRN  DFMGSLSFV  SELMKMPASG  WYKLLNQE EG  EYYNVP IPEG
301 DEEGNME LRQ  KFEKAKLGPA  GNKVISPSED  RKQPSNNLDR  VKLTDFNFLM  VLGKGSF GKV
361 MLADRKGT EE  LYAIKILK KD  VVIQDD DVEC  TMVEKRV LAL  LDKPPFL TQL  HSCFQTVD RL
421 YFVMEYVNGG  DLMYHIQQVG  KFKEPQAVFY  AAEISIGLFF  LHKRGIIYRD  LKLDNVM LDS
481 EGHKIADFG  MCKEHMMDGV  TTRTFCGTPD  YIAPEIIAYQ  PYGKSVDW WA  YGVLLY EMLA
541 GQPPFDGEDE  DELFQSIMEH  NVSPKSLSK  EAVSICKGLM  TKHPAKRLGC  GPEGERD VRE
601 HAFRRRIDWE  KLENREIQPP  FKPKVC GKGA  ENFDKFFTRG  QPVLTPDQL  VIANIDQ SDF
661 EGSYVNPQF  VHPILQSAV

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Recombinant PKC α nucleotide sequence:

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1  atgcatcatc  accatcacca  tatggctgac  gttttcccgg  gcaacgactc  cacggcgtct
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1501  acgaccagga  ctttctgtgg  gactccagat  tatatcgccc  cagagataat  cgcttatcag
1561  ccgtatggaa  aatctgtgga  ctggtgggcc  tatggcgtcc  tgttgtagta  aatgcttgcc
1621  gggcagcctc  cattgatgg  tgaagatgaa  gacgagctat  ttcagtctat  catggagcac

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1801 catgccttct tccggaggat cgactgggaa aaactggaga acagggagat ccagccacca
1861 ttcaagccca aagtgtgtgg caaaggagca gagaactttg acaagttctt cacacgagga
1921 cagcccgtct taacaccacc tgatcagctg gttattgcta acatagacca gtctgatttt
1981 gaagggttct cgtatgtcaa cccccagttt gtgcacccca ttttacagag tgcagtatga
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