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Certificate of Analysis

PKCα, 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 PKCα expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/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 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: 0.203mg/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.

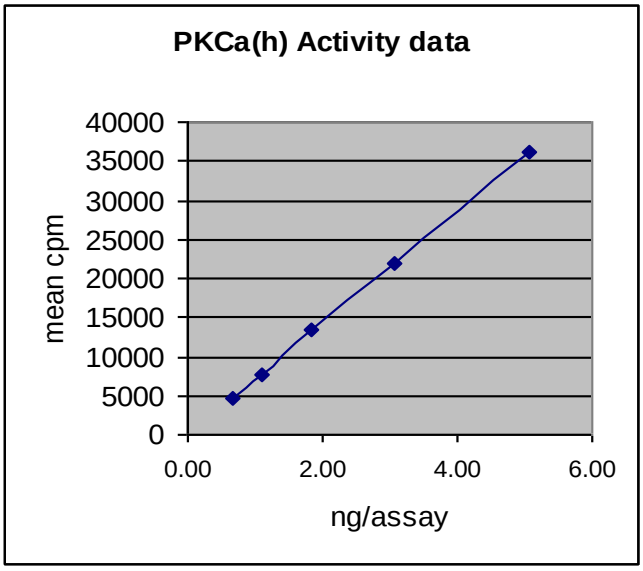
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

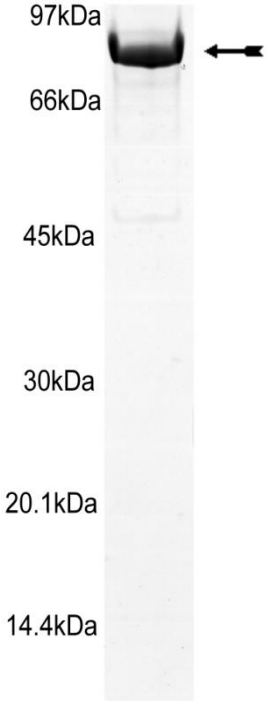
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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 PKCα 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 PKCα, active.

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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 200mM HEPES/NaOH pH7.4.
2. **$CaCl_2$:** Make up a 1mM stock in dH_2O. 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-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]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 $CaCl_2$
5. Add **2.5µl (0.67–5.08ng) PKCα, active**.
6. Add 2.5µl of dH_2O.
7. Add 10µl of diluted [γ-^{33}P]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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PKCα Sequence Information

Protein human PKCα

Tags *N*-terminal 6His

Native sequence M8 of the recombinant protein is equivalent to M1 of human PKCα

Accession number GenBank X52479

Recombinant PKCα amino acid sequence:

```
   1 MHHHHHHMAD VFPGNDSTAS QDVANRFARK GALRQKNVHE VKDHKFIARF FKQPTFCSHC
  61 TDFIWGFGKQ GFQCQVCCFV VHKRCHEFVT FSCPGADKGP DTDDPRSKHK FKIHTYGSPT
 121 FCDHCGSLLY GLIHQGMKCD TCDMNVHKQC VINVPSLCGM DHTEKRGRIY LKAEVADEKL
 181 HVTVRDAKNL IPMDPNGLSD PYVKLKLIPD PKNESKQKTK TIRSTLNPQW NESFTFKLKP
 241 SDKDRRLSVE IWDWDRTTRN DFMGSLSFGV SELMKMPASG WYKLLNQEEG EYYNVPIPEG
 301 DEEGNMELRQ KFEKAKLGPA GNKVISPSED RKQPSNNLDR VKLTDFNFLM VLGKGSFGKV
 361 MLADRKGTEE LYAIKILKKD VVIQDDDVEC TMVEKRVLAL LDKPPFLTQL HSCFQTVDRL
 421 YFVMEYVNGG DLMYHIQQVG KFKEPQAVFY AAEISIGLFF LHKRGIIYRD LKLDNVMLDS
 481 EGHIKIADFG MCKEHMMDGV TTRTFCGTPD YIAPEIIAYQ PYGKSVDWWA YGVLLYEMLA
 541 GQPPFDGEDE DELFQSIMEH NVSYPKSLSK EAVSICKGLM TKHPAKRLGC GPEGERDVRE
 601 HAFFRRIDWE KLENREIQPP FKPKVCGKGA ENFDKFFTRG QPVLTPPDQL VIANIDQSDF
 661 EGFSYVNPQF VHPILQSAV
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Recombinant PKCα nucleotide sequence:

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   1 atgcatcatc accatcacca tatggctgac gttttcccgg gcaacgactc cacggcgtct
  61 caggacgtgg ccaaccgctt cgcccgcaaa ggggcgctga ggcagaagaa cgtgcacgag
 121 gtgaaggacc acaaattcat cgcgcgcttc ttcaagcagc ccaccttctg cagccactgc
 181 accgacttca tctgggggtt tgggaaacaa ggcttccagt gccaagtttg ctgttttgtg
 241 gtccacaaga ggtgccatga atttgttact ttttcttgtc cgggtgcgga taagggaccc
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 481 gatcacactg agaagagggg gcggatttac ctaaaggctg aggttgctga tgaaaagctc
 541 catgtcacag tacgagatgc aaaaaatcta atccctatgg atccaaacgg gctttcagat
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 661 accatccgct ccacactaaa tccgcagtgg aatgagtcct ttacattcaa attgaaacct
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1021 gtgaaactca cggacttcaa tttcctcatg gtgttgggaa aggggagttt tggaaaggtg
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1561 ccgtatggaa aatctgtgga ctggtgggcc tatggcgtcc tgttgtatga aatgcttgcc
1621 gggcagcctc catttgatgg tgaagatgaa gacgagctat ttcagtctat catggagcac
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1681 aacgtttcct atccaaaatc cttgtccaag gaggctgttt ctatctgcaa aggactgatg
1741 accaaacacc cagccaagcg gctgggctgt gggcctgagg gggagaggga cgtgagagag
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 tcttacagag tgcagtatga
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Reviewed and approved by site quality representative.