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Certificate of Analysis

PKC mu, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-508, 14-508-K, 14-508M
Parent Lot # 25876U

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, full length, human PKC mu expressed in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 78.8% by SDS-PAGE and Coomassie blue staining. MW = 105kDa.

Specific Activity (Parent lot# 25876U): 414U/mg, where one unit of PKC mu, active activity is defined as 1nmol phosphate incorporated into 30µM (KKLNRTLSVA) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.222mg/ml of enzyme in 20mM Tris/HCl pH7.5, 0.02% Triton X-100, 5% glycerol, 1mM EGTA, 1mM EDTA, 10mM benzamidine, 1mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

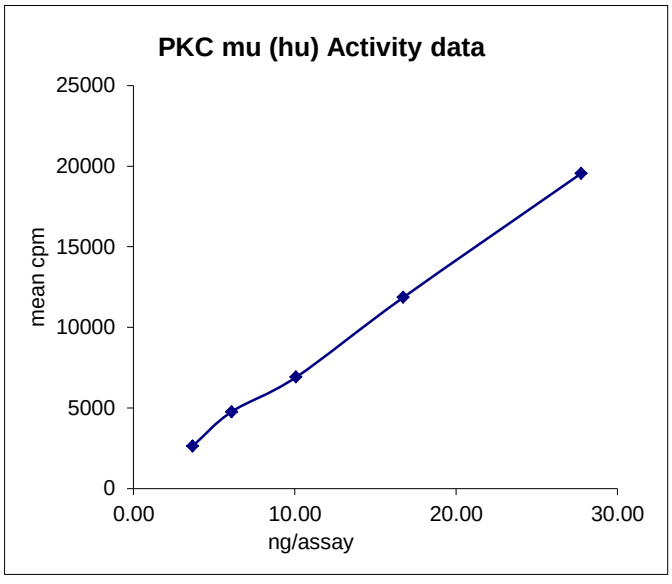
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

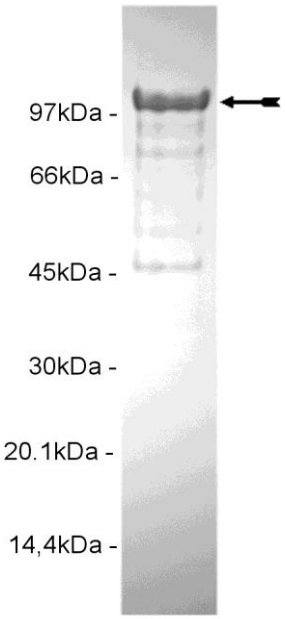
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 3.7–27.8ng of this lot of enzyme phosphorylated 30µM (KKLNRTLSVA) 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 mu 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 PKC mu, active.

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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. **(KKLNRTLSVA):** Use at a final assay concentration of 30µM. Make up a 30µM stock. Add 2.5µl of stock per assay point.
3. **PKC mu, active:** Dilute with 20mM HEPES/NaOH pH7.4. Use 3.7–27.8ng per assay point.
4. **[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 **(KKLNRTLSVA)**.
3. Add **2.5µl (3.7–27.8ng) PKC mu, active**.
4. Add 7.5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. 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 mu Sequence Information

Protein Human PKC mu

Tags *N*-terminal 6His

Native sequence M29 of the recombinant protein is equivalent to M1 of human PKC mu

Accession number GenBank NM_002742

Recombinant PKC mu amino acid sequence:

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   1 MSYYHHHHHH DYDIPTTENL YFQGAMGSMS APPVLRPPSP LLPVAAAAAA AAAALVPGSG
  61 PGPAPFLAPV AAPVGGISFH LQIGLSREPV LLLQDSSGDY SLAHVREMAC SIVDQKFPEC
 121 GFYGMYDKIL LFRHDPTSEN ILQLVKAASD IQEGDLIEVV LSASATFEDF QIRPHALFVH
 181 SYRAPAFCDH CGEMLWGLVR QGLKCEGCGL NYHKRCAFKI PNNCSGVRRR RLSNVSLTGV
 241 STIRTSSAEL STSAPDEPLL QKSPSESFIG REKRSNSQSY IGRPIHLDKI LMSKVKVPHT
 301 FVIHSYTRPT VCQYCKKLLK GLFRQGLQCK DCRFNCHKRC APKVPNNCLG EVTINGDLLS
 361 PGAESDVVME EGSDDNDSER NSGLMDDMEE AMVQDAEMAM AECQNDSGEM QDPDPDHEDA
 421 NRTISPSTSN NIPLMRVVQS VKHTKRKSST VMKEGWMVHY TSKDTLRKRH YWRLDSKCIT
 481 LFQNDTGSRY YKEIPLSEIL SLEPVKTSAL IPNGANPHCF EITTANVVYY VGENVVNPSS
 541 PSPNNSVLTS GVGADVARMW EIAIQHALMP VIPKGSSVGT GTNLHRDISV SISVSNCQIQ
 601 ENVDISTVYQ IFPDEVLGSG QFGIVYGGKH RKTGRDVAIK IIDKLRFPTK QESQLRNEVA
 661 ILQNLHHPGV VNLECMFETP ERVFVVMEKL HGDMLEMILS SEKGRLPEHI TKFLITQILV
 721 ALRHLHFKNI VHCDLKPENV LLASADPFPQ VKLCDFGFAR IIGEKSFRRS VVGTPAYLAP
 781 EVLRNKGYNR SLDMWSVGVI IYVSLSGTFP FNEDEDIHDQ IQNAAFMYPP NPWKEISHEA
 841 IDLINNLLQV KMRKRYSVDK TLSHPWLQDY QTWLDLRELE CKIGERYITH ESDDLRWEKY
 901 AGEQRLQYPT HLINPSASHS DTPETEETEM KALGERVSIL
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Recombinant PKC mu nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatggg atccatgagc gcccctccgg tcctgcggcc gcccagtccg
 121 ctgctgcccg tggcggcggc agctgccgca gcggccgccg cactggtccc agggtccggg
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 541 tcatacagag ctccagcttt ctgtgatcac tgtggagaaa tgctgtgggg gctggtacgt
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2821 tga
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Reviewed and approved by site quality representative.