

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 Ni²⁺/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 (KKLNRTLSSVA) 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.

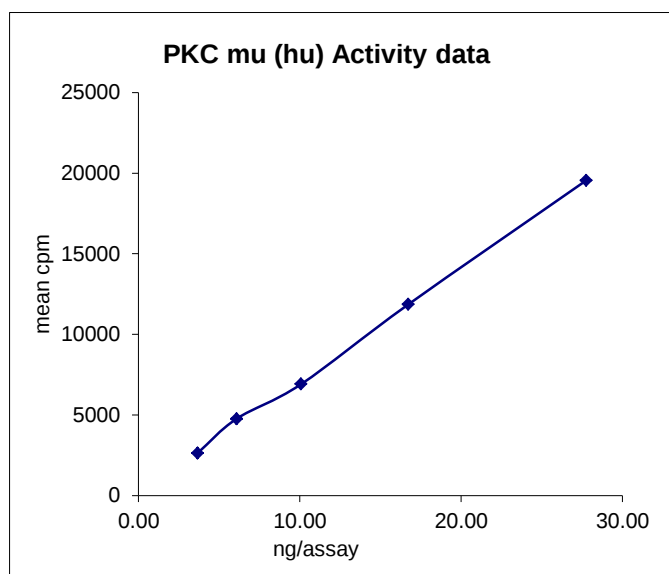
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

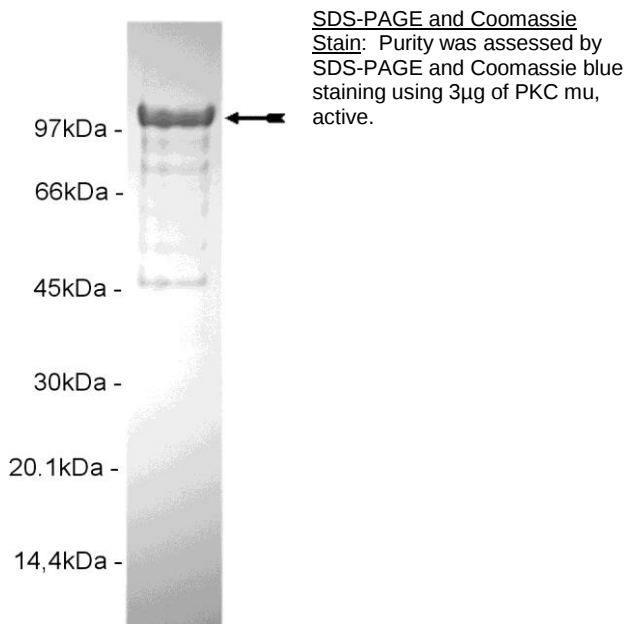
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 3.7–27.8ng of this lot of enzyme phosphorylated 30μM (KKLNRTLSSVA) 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.



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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 30 μ M. Make up a 30 μ M stock. Add 2.5 μ l of stock per assay point.
3. **PKC μ , active:** Dilute with 20mM HEPES/NaOH pH7.4. Use 3.7–27.8ng per assay point.
4. **[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 2.5 μ l of 10 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **(KKLNRTLSVA)**.
3. Add **2.5 μ l (3.7–27.8ng) PKC μ , active**.
4. Add 7.5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

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PKC mu Sequence Information

<u>Protein</u>	Human PKC mu
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M29 of the recombinant protein is equivalent to M1 of human PKC mu
<u>Accession number</u>	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 LMSKVVPHT
301 FVIHSYTRPT VCQYCKKLLK GLFRQGLQCK DCRFNCHKRC APKVPNNCLG EVTINGDLLS
361 PGAESDVVME EGSDDNDSEr NSGLMDDMEE AMVQDAEMAM AECQNDSGEM QDPDPDHEDA
421 NRTISPSTSN NIPLMRVVSQ VKHTKRKSST VMKEGWMVHY TSKDTLRKRH YWRLDSKCIT
481 LFQNDTGSRY YKEIPLSEIL SLEPVKTSAL IPNGANPHCF EITTANVVYY VGENVVNPSS
541 PSPNNSVLTS GVGADVARMW EIAIQHALMP VIPKGSSVGT GTNLHRDISV SISVSNCQIQ
601 ENVDISTVYQ IFPDEVLGSG QFGIVYGGKH RKTGRDVAIK IIDKLRFP TK QESQLRNEVA
661 ILQNLHHPGV VNLECMFETP ERVFVMEKL HGDMLEMILS SEKGRLEPHI TKFLITQILV
721 ALRHLHFKNi VHC DLKPENV LLASADFPFQ VKLCDFGFAR IIGESFRRS VVGTPAYLAP
781 EVLRNKGYNR SLDMWSVGVI IYVSLSGTFP FNEDEDIHDQ IQNAAFMYPP NPWKEISHEA
841 IDLINLLQV KMRKRYSDVK TLSHPWLQDY QTWLDLRELE CKIGERYITH ESDDLREWEKY
901 AGEQRLQYPT HLINPSASHS DTPETEEM KALGERVSIL

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Recombinant PKC mu nucleotide sequence:

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1  atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
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1441 ctctttcaga atgacacagg aagcaggtag tacaaggaaa ttcctttatc tgaaattttg
1501 tctctggaac cagtaaaaac ttcagcttta attcctaatt gggccaatcc tcattgtttc

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1561 gaaatcacta cggcaaagt agtgtattat gtgggagaaa atgtgggtcaa tccttcacgc
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1681 gagatagcca tccagcatgc ccttatgcc gtcattccca agggctcctc cgtgggtaca
1741 ggaaccaact tgcacagaga tatctctgtg agtatttcag tatcaaattg ccagattcaa
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2821 tga

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