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Product Information

PKCμ Active
human, recombinant
GST-tagged, expressed in *Sf*9 cells

Catalog Number **K4268**
Lot Number 019K0631
Storage Temperature –70 °C

Synonyms: PKD; PKCM; PRKCM

Product Description

Protein kinase C mu (PKCμ) is a member of the protein kinase C (PKC) family that differs from the other PKC isoenzymes in structural and enzymatic properties. PKCμ is ubiquitous in nature with the highest expression in the thymus, lung, and peripheral blood mononuclear cells.[1] PKCμ forms a complex *in vivo* with a phosphatidylinositol 4-kinase and a phosphatidyl-inositol-4-phosphate 5-kinase. A region of PKCμ between the amino-terminal transmembrane domain and the pleckstrin homology domain is shown to be involved in the association with the lipid kinases.[2]

This recombinant product was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST-tag. The gene accession number is X75756. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~131kDa

Purity: ≥70% (SDS-PAGE, see Figure 1)

Specific Activity: 578–782 nmole/min/mg (see Figure 2)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Lot Number 019K0631:
>90% (densitometry)

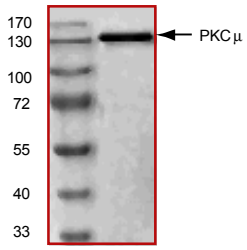
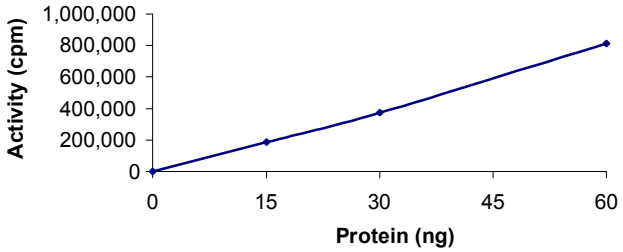


Figure 2.
Specific Activity of Lot Number 019K0631:
680 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM $MgCl_2$, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with distilled water.

Kinase Solution – Dilute the Active PKCμ (0.1 μg/μl) with Kinase Dilution Buffer to the desired concentration. Note: The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended that the researcher perform a serial dilution of Active PKCμ kinase for optimal results

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 μl aliquots at –20 °C.

γ-^{32}P-ATP Assay Cocktail (250 μM) – Combine 5.75 ml of Kinase Assay Buffer, 150 μl of 10 mM ATP Stock Solution, 100 μl of γ-^{32}P-ATP (1 mCi/100 μl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve CREBtide synthetic peptide substrate (KRREILSRRPSYR) in water at a final concentration of 1 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay

This assay involves the use of the ^{32}P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the Active PKCμ, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-^{32}P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 μl:
   10 μl of Kinase Solution
   10 μl of Substrate Solution
3. Set up a blank control as outlined in step 2, substituting 10 μl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 μl of the γ-^{32}P-ATP Assay Cocktail, bringing the final reaction volume to 25 μl. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 μl of the reaction mixture onto an individually precut strip of phosphocellulose P81 paper.
6. Air dry the precut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ-^{32}P-ATP counts introduced into the reaction. Spot 5 μl of the γ-^{32}P-ATP Assay Cocktail on a precut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of 5 μl of γ-}^{32}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

   cpm – value from control (step 7)
   nmole – 1.25 nmole (5 μl of 250 μM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)
Δcpm = cpm of the sample – cpm of the blank (step 3)
25 = total reaction volume
20 = spot volume
T = reaction time (minutes)
E = amount of enzyme (mg)

References

1. Rennecke, J. et al., Immunological demonstration of protein kinase C mu in murine tissues and various cell lines. Eur. J. Biochem., **242**(2), 428-32 (1996).
2. Nishikawa, K. et al., Association of protein kinase C mu with type II phosphatidylinositol 4-kinase and type phosphatidylinositol-4-phosphate 5-kinase. J. Biol. Chem., **273**(36), 23126-33 (1998).

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