SIGMA-ALDRICH®

Product Information

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

Catalog Number **P0061**
Lot Number 019K1628
Storage Temperature –70 °C

Synonyms: EPK2; PRKCN

Product Description

PKCν, also known as PKD3, is a member of the protein kinase C (PKC) family of serine/threonine kinases that play critical roles in the regulation of cellular differentiation and proliferation in many cell types. PKCν is composed of 890 amino acid residues and the protein has 77.3% similarity to human PKC mu (PKCμ) and 77.4% similarity to mouse PKD.[1] The PKCν mRNA is ubiquitously expressed in various tissues and the gene is located between markers WI-9798 and D2S177 on chromosome 2p21 region.

This recombinant product was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST-tag. The gene accession number is NM 005813. 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: ~142 kDa

Purity: ≥70% (SDS-PAGE, see Figure 1)

Specific Activity: 80–108 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.
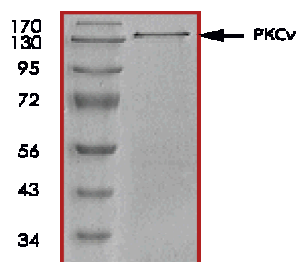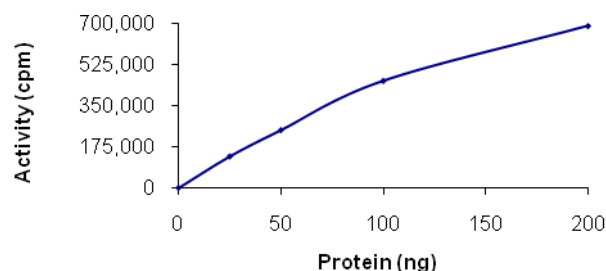SDS-PAGE Gel of Lot Number 019K1628:
>85% (densitometry)

Figure 2.
Specific Activity of Lot Number 019K1628:
108 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 a 50 ng/μl BSA solution.

Kinase Solution – Dilute the Active PKCν (0.1 µg/µl) with Kinase Dilution Buffer to the desired concentration. <u>Note</u>: 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 PKCν 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 the 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.

<u>Kinase Assay</u>
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 PKCν, 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
      5 µl of Substrate Solution
      5 µl of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5 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

<u>Calculations</u>:
1. Specific Radioactivity (SR) of ATP (cpm/nmole)

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

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

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. Hayashi, A. et al., PKC nu, a new member of the protein kinase C family, composes a fourth subfamily with PKC mu. Biochim. Biophys. Acta, **1450**, 99-106 (1999).

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