

Product Information

**PKC ϵ , active, GST tagged, human
PRECISIO® Kinase
recombinant, expressed in *Sf9* cells**

Catalog Number **SRP5067**
Storage Temperature -70°C

Synonyms: PRKCE; MGC125656; MGC125657;
nPKC-epsilon

Product Description

PKC ϵ is a member of the protein kinase C (PKC) family of serine and threonine-specific protein kinases that can phosphorylate a wide variety of protein targets known to be involved in diverse cellular signaling pathways. PKC ϵ is involved in many different cellular functions, such as neuron channel activation, cardioprotection from ischemia,¹ heat shock response, as well as insulin exocytosis. Knockout studies in mice suggest this kinase is important for lipopolysaccharide (LPS)-mediated signaling in activated macrophages and may control anxiety-like behavior.²

Recombinant, full-length, human PKC ϵ was expressed by baculovirus in *Sf9* insect cells using an N-terminal GST tag. The gene accession number is NM_005400. Recombinant protein stored in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, 25% glycerol.

Molecular mass: ~110 kDa

Purity: 70–95% (SDS-PAGE, see Figure 1)

Specific Activity: 1,033–1,398 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 Typical Lot
70–95% (densitometry)

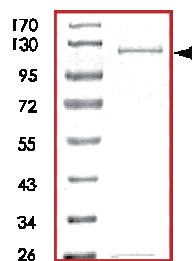
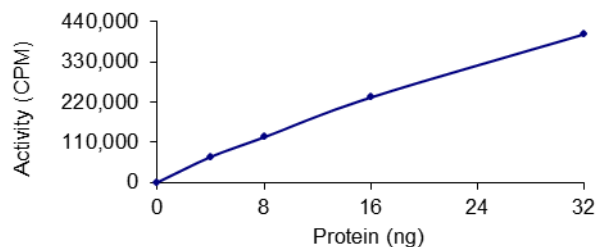


Figure 2.
Specific Activity of Typical Lot
1,033–1,398 nmole/min/mg



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.

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 .

Kinase Solution – Dilute the active PKC ϵ (0.1 μ g/ μ l) with Kinase Dilution Buffer to the desired concentration.

Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active PKC ϵ kinase for optimal results.

γ -³³P-ATP Assay Cocktail (250 μ M) – Combine 5.75 ml of Kinase Assay Buffer, 150 μ l of 10 mM ATP Stock Solution, 100 μ l of γ -³³P-ATP (1 mCi/100 μ l). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the synthetic peptide substrate (ERM ϵ PRKRQGSVRRRV) in distilled water at a final concentration of 1 mg/ml.

PKC Lipid Activator – 0.5 mg/ml phosphatidylserine and 0.05 mg/ml diacylglycerol in 20 mM MOPS, pH 7.2, containing 1 mM CaCl₂. (Sonicate lipid for 1 minute prior to use)

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 ³³P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active PKC ϵ , Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ -³³P-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 μ l:
 - 10 μ l of Kinase Solution
 - 5 μ l of Substrate Solution
 - 2.5 μ l of PKC Lipid Activator
 - 2.5 μ l of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5 μ l of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 μ l of the γ -³³P-ATP Assay Cocktail, bringing the final reaction volume to 25 μ l. 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 μ l of the reaction mixture onto an individually pre-cut strip of phosphocellulose P81 paper.

6. Air dry the pre-cut 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 -³³P-ATP counts introduced into the reaction. Spot 5 μ l of the γ -³³P-ATP Assay Cocktail on a pre-cut 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)

$$SR = \frac{\text{cpm of 5 } \mu\text{l of } \gamma\text{-}^{33}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

cpm – value from control (step 7)
nmole – 1.25 nmole (5 μ l of 250 μ M 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)

Δ cpm = 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. Chen, C.H. et al., Cardioprotection from ischemia by a brief exposure to physiological levels of ethanol: role of epsilon protein kinase C. Proc. Nat. Acad. Sci., **96**, 12784-12789 (1999).
2. Hodge, C.W. et al., Decreased anxiety-like behavior, reduced stress hormones, and neurosteroid supersensitivity in mice lacking protein kinase C-epsilon. J. Clin. Invest., **110**, 1003-1010 (2002).

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