

Product Information

PKC η , Active human, recombinant GST-tagged, expressed in Sf9 cells

Catalog Number **K4143**
Lot Number 019K1627
Storage Temperature -70°C

Synonyms: PKCL; PKC-L; PRKCL; MGC5363;
MGC26269; nPKC-eta; PRKCH

Product Description

PKC η 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 η is predominantly expressed in squamous cell epithelia and induces terminal differentiation of keratinocytes. PKC η that is endogenously expressed or overexpressed is found to associate with the cyclin E/cdk2/p21 complex in keratinocytes of mice and humans.¹

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 006255. 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: ~103 kDa

Purity: $\geq 70\%$ (SDS-PAGE, see Figure 1)

Specific Activity: 73–99 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 019K1627:
>90% (densitometry)

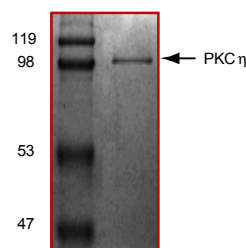
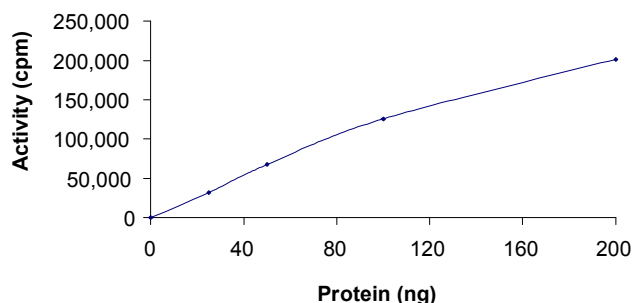


Figure 2.
Specific Activity of Lot Number 019K1627:
86 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl₂, 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 water.

Kinase Solution – Dilute the Active PKC η (0.1 $\mu\text{g}/\mu\text{l}$) 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 PKC η 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 $-20\text{ }^{\circ}\text{C}$.

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 $-20\text{ }^{\circ}\text{C}$.

Substrate Solution – Dissolve the synthetic peptide substrate (ERMRRPRKRQGSVRRRV) 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 PKC η , 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
 - 7.5 μl of Substrate Solution
 - 2.5 μl PKC lipid activator
(0.5 mg/ml phosphatidylserine and 0.05 mg/ml diacylglycerol in 20 mM MOPS, pH 7.2, containing 1 mM CaCl_2). Sonicate lipid for 1 minute prior to use.
3. Set up a blank control as outlined in step 2, substituting 7.5 μl of cold water ($4\text{ }^{\circ}\text{C}$) 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 $30\text{ }^{\circ}\text{C}$ for 15 minutes.

5. After the 15 minute incubation, stop the reaction by spotting 20 μl 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 - ^{32}P -ATP counts introduced into the reaction. Spot 5 μl of the γ - ^{32}P -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)

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

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)

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

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. Kashiwagi, M. et al., PKC η associates with cyclin E/cdk2/p21 complex, phosphorylates p21 and inhibits cdk2 kinase in keratinocytes. *Oncogene*, **19**, 6334-6341 (2000).

BKR,MAM 02/09-1

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.