

## Product Information

**PKC  $\alpha$ , active, GST-tagged, *Xenopus*  
PRECISIO® Kinase  
recombinant, expressed in *Sf9* cells**

Catalog Number **SRP5251**  
Storage Temperature  $-70^{\circ}\text{C}$

Synonyms: PRKCA

### Product Description

PKC  $\alpha$  is a member of the protein kinase C (PKC) family of serine and threonine-specific protein kinases that can be activated by calcium and the second messenger diacylglycerol. PKC  $\alpha$  has been reported to play roles in many different cellular processes, such as cell adhesion, cell transformation, cell cycle checkpoint, and cell volume control.<sup>1</sup> PKC  $\alpha$  has been assigned to the chromosome region 17q22-q23.2 and has been identified as a fundamental regulator of cardiac contractility and  $\text{Ca}^{2+}$  handling in myocytes.<sup>2</sup>

Recombinant full-length *Xenopus* PKC  $\alpha$  was expressed by baculovirus in *Sf9* insect cells using an N-terminal GST-tag. The gene accession number is BC078065. It is supplied 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, and 25% glycerol.

Molecular mass: ~103 kDa

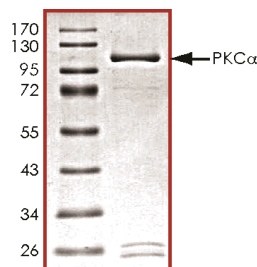
### Precautions and Disclaimer

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.

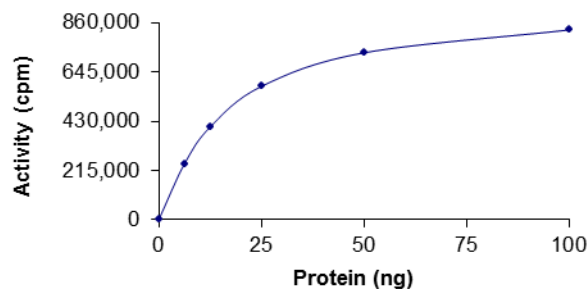
### Storage/Stability

The product ships on dry ice and storage at  $-70^{\circ}\text{C}$  is recommended. After opening, aliquot into smaller quantities and store at  $-70^{\circ}\text{C}$ . Avoid repeated handling and multiple freeze/thaw cycles.

**Figure 1.**  
SDS-PAGE Gel of Typical Lot:  
 $\geq 70\%$  (SDS-PAGE, densitometry)



**Figure 2.**  
Specific Activity of Typical Lot:  
186–290 nmole/min/mg



### Procedure

#### Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM  $\text{MgCl}_2$ , 5 mM EGTA, 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 distilled water.

Kinase Solution – Dilute the active PKC  $\alpha$  (0.1  $\mu\text{g}/\mu\text{L}$ ) 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  $\alpha$  kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 mL of Kinase Assay Buffer. Store in 200  $\mu\text{L}$  aliquots at  $-20\text{ }^{\circ}\text{C}$ .

$\gamma$ - $^{33}\text{P}$ -ATP Assay Cocktail (250  $\mu\text{M}$ ) – Combine 5.75 mL of Kinase Assay Buffer, 150  $\mu\text{L}$  of 10 mM ATP Stock Solution, 100  $\mu\text{L}$  of  $\gamma$ - $^{33}\text{P}$ -ATP (1 mCi/100  $\mu\text{L}$ ). Store in 1 mL aliquots at  $-20\text{ }^{\circ}\text{C}$ .

Substrate Solution – Dissolve the protein substrate (KRREILSRPSYR) in distilled 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  $^{33}\text{P}$  radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active PKC  $\alpha$ , Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The  $\gamma$ - $^{33}\text{P}$ -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  $\mu\text{L}$ :
  - 10  $\mu\text{L}$  of Kinase Solution
  - 5  $\mu\text{L}$  of Substrate Solution
  - 5  $\mu\text{L}$  of cold water ( $4\text{ }^{\circ}\text{C}$ )
3. Set up a blank control as outlined in step 2, substituting 5  $\mu\text{L}$  of cold water ( $4\text{ }^{\circ}\text{C}$ ) for the Substrate Solution.
4. Initiate each reaction with the addition of 5  $\mu\text{L}$  of the  $\gamma$ - $^{33}\text{P}$ -ATP Assay Cocktail, bringing the final reaction volume to 25  $\mu\text{L}$ . 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  $\mu\text{L}$  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  $\sim 10$  minutes each.
7. Set up a radioactive control to measure the total  $\gamma$ - $^{33}\text{P}$ -ATP counts introduced into the reaction. Spot 5  $\mu\text{L}$  of the  $\gamma$ - $^{33}\text{P}$ -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)

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

cpm – value from control (step 7)  
nmole – 1.25 nmole (5  $\mu\text{L}$  of 250  $\mu\text{M}$  ATP Assay Cocktail)

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

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

SR = specific radioactivity of the ATP (cpm/nmole ATP)

$\Delta\text{cpm}$  = 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. Coussens, L. et al., Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. *Science*, **233**, 859-866 (1986).
2. Braz, J.C. et al., PKC- $\alpha$  regulates cardiac contractility and propensity toward heart failure. *Nature Med.*, **10**, 248-254 (2004).

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