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Product Information

PKCθ, active, GST-tagged, human
PRECISIO® Kinase
recombinant, expressed in *Sf*9 cells

Catalog Number **K4643**
Lot Number 021M0604
Storage Temperature –70 °C

Synonyms: PRKCQ, PRKCT, MGC126514, MGC141919, nPKC-theta

Product Description

Protein Kinase C, theta (PKCθ) is an important component in the intracellular signaling cascade.[1] Recent studies have suggested local accumulation of fat metabolites inside skeletal muscle may activate a serine kinase cascade involving PKCθ leading to defects in insulin signaling and glucose transport in skeletal muscle.[2] Insulin resistance plays a primary role in the development of type 2 diabetes and may be related to alterations in fat metabolism. PKCθ is a crucial component mediating fat-induced insulin resistance in skeletal muscle and is a potential therapeutic target for the treatment of type 2 diabetes.[2]

This recombinant product was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST-tag. The gene accession number is NM 006257. 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: ~110 kDa

Purity: ≥70% (SDS-PAGE, see Figure 1)

Specific Activity: 673–911 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 021M0604:
>75% (densitometry)

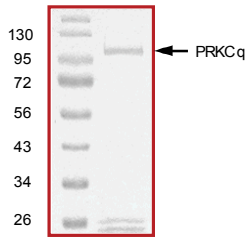
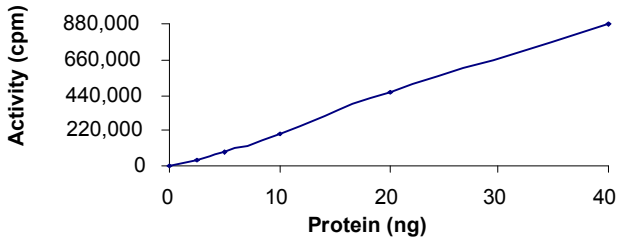


Figure 2.
Specific Activity of Lot Number 021M0604:
792 nmole/min/mg



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 and 5% glycerol solution.

Kinase Solution – Dilute the active PKCθ (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 the researcher perform a serial dilution of active PKCθ 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 (ERMRPRKRQGSVRRRV) 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 PKCθ, 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 °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. Manicassamy, S. and Sun, Z., The critical role of protein kinase C-theta in Fas/Fas ligand-mediated apoptosis. J. Immunol., **178**, 312-319 (2007).
2. Kim, J K. et al., PKC-theta knockout mice are protected from fat-induced insulin resistance. J. Clin. Invest., **114**, 823-827 (2004).

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