SignalChem
Specialists in Signaling Proteins
1-866-9 KINASE (54-6273)

PKC theta, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P74-10G-10
Lot # I124-2

Product Description

Recombinant full-length human PKCθ was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_006257.

Gene Aliases

PRKCQ, PRKCT, MGC126514, MGC141919, nPKC-theta

Concentration

0.1 µg/µl

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage, Shipping and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.
Stability is 1yr at –70°C from date of shipment. Product shipped on dry ice.

Scientific Background

Protein Kinase C, theta (PKCθ) is important component in the intracellular signaling cascade (1). Recent studies have suggested that 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).

References

1. Manicassamy, S. and Sun, Z. The critical role of protein kinase C-theta in Fas/Fas ligand-mediated apoptosis. J. Immunol. 2007;178(1):312-9.
2. Kim, J K. et al: PKC-theta knockout mice are protected from fat-induced insulin resistance. J. Clin. Invest. 2004;114(6):823-7.

Purity

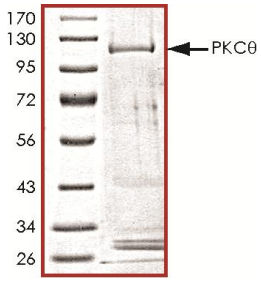
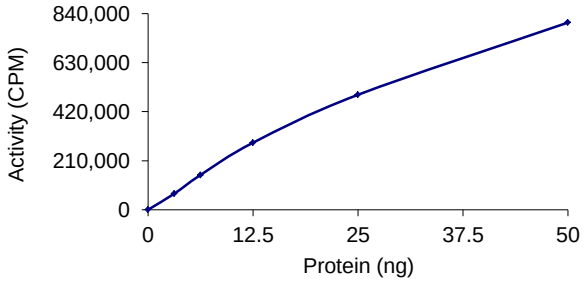


Figure 1. SDS-PAGE gel image

The purity of PKCθ was determined to be **>75%** by densitometry, approx. MW **110kDa**.

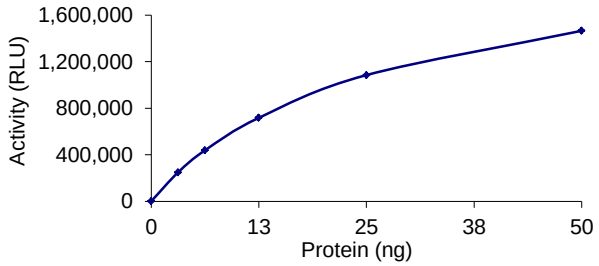
Specific Activity

Figure 2. Radiometric Assay Data



The specific activity of PKCθ was determined to be **910 nmol /min/mg** as per activity assay protocol.
(For Radiometric Assay Protocol on this product please see pg. 2)

Figure 3. ADP-Glo™ Assay Data



The specific activity of PKCθ was determined to be **1090 nmol /min/mg** as per activity assay protocol.
(For ADP-Glo™ Assay Protocol on this product please see pg. 3)

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: P74-10G)

Active PKCθ (0.1μg/μl) diluted with Kinase Dilution Buffer VII (Catalog #: K27-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active PKCθ for optimal results).

Kinase Dilution Buffer VII (Catalog #: K27-09)

Kinase Assay Buffer VII (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA and 5%glycerol solution

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{33P}]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200μl aliquots at –20°C.

Substrate (Catalog #: P15-58)

PKCtide peptide substrate (ERMRPRKRQGSVRRRV) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active PKCθ, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active PKCθ (Catalog #P74-10G)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #P15-58)
- **Component 1.** 2.5μl of PKC lipid activator (Catalog # L51-39). (sonicate or vortex lipid for 1 minute prior to use).
- **Component 3.** 2.5μl of distilled H_2O (4°C)

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5μl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25μl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity (cpm) on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5μl [^{33}P]-ATP / pmoles of ATP (in 5μl of a 250μM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

ADP-Glo™ Activity Assay Protocol

Reaction Components

PKC θ Kinase Enzyme System (Promega, Catalog #:V4040)

PKCθ, Active, 10µg (0.1µg/µl)
PKCtide, 1ml (1mg/ml)
Reaction Buffer A (5X), 1.5ml
DTT (0.1M), 25µl
PKC Lipid Activator (10X), 500µl

ADP-Glo™ Kinase Assay Kit (Promega, Catalog #: V9101)

Ultra Pure ATP solution, 10 mM (0.5ml)
ADP solution, 10 mM (0.5ml)
ADP-Glo™ Reagent (5ml)
Kinase Detection Buffer (10ml)
Kinase Detection Substrate (Lyophilized)

Reaction Buffer A (5X)

200mM Tris-HCl, pH 7. 5, 100mM $MgCl_2$ and 0.5 mg/ml BSA.

Assay Protocol

The PKCθ assay is performed using the PKCθ Kinase Enzyme System (Promega; Catalog #: V4040) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The PKCθ reaction utilizes ATP and generates ADP. Then the ADP- Glo™ Reagent is added to simultaneously terminate the kinase reaction and deplete the remaining ATP. Finally, the Kinase Detection Reagent is added to convert ADP to ATP and the newly synthesized ATP is converted to light using the luciferase/luciferin reaction. For more detailed protocol regarding t*he ADP-Glo™ Kinase Assay,* see the technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html.

Step 2. Thaw the ADP-Glo™ Reagents at ambient temperature. Then prepare Kinase Detection Reagent by mixing Kinase Detection Buffer with the Lyophilized Kinase Detection Substrate. Set aside.

Step 3. Thaw the components of PKCθ Enzyme System, ADP and ATP on ice.

Step 4. Prepare 1ml of 2X Buffer by combining 400µl Reaction Buffer A, 1µl DTT and 599µl of dH_20.

Step 5. Prepare 1ml of 250µM ATP Assay Solution by adding 25µl ATP solution (10mM) to 500µl of 2X Buffer and 475µl of dH_20.

Step 6. Prepare diluted PKCθ in 1X Buffer (diluted from 2X buffer) as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active PKCθ for optimal results).

Step 7. In a white 96-well plate (Corning Cat # 3912), add the following reaction components bringing the initial reaction volume up to 20µl:

- **Component 1.** 5µl of diluted Active PKCθ
- **Component 2.** 5µl of 1mg/ml stock solution of substrate
- **Component 3.** 2.5µl of PKC Lipid Activator (10X) (sonicate or vortex lipid for 1 minute prior to use)
- **Component 4.** 7.5µl of 2X Buffer

Step 8. Set up the blank control as outlined in step 6, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 9. At the same time as the PKCθ kinase reaction, set up an ATP to ADP conversion curve at 50µM ATP/ADP range as described in the *ADP-Glo™ Kinase Assay* technical Manual #TM313.

Step 10. Initiate the PKCθ reactions by the addition of 5µl of 250 µM ATP Assay Solution thereby bringing the final volume up to 25µl. Shake the plate and incubate the reaction mixture at 30°C for 15 minutes.

Step 11. Terminate the reaction and deplete the remaining ATP by adding 25µl of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minute at ambient temperature.

Step 12. Add 50µl of the Kinase Detection Reagent, shake the plate and then incubate the reaction mixture for another 30 minute at ambient temperature.

Step 13. Read the 96-well reaction plate using the Kinase-Glo™ Luminescence Protocol on a GloMax® plate reader (Promega; Cat# E7031).

Step 14. Using the conversion curve, determine the amount of ADP produced (nmol) in the presence (step 6) and absence of substrate (Step 7) and calculate the kinase specific activity as outlined below. For a detailed protocol of how to determine nmols from RLUs, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

Kinase Specific Activity (SA) (nmol/min/mg)

(ADP (step 6) – ADP (Step 7)) in nmol) / (Reaction time in min)*(Enzyme amount in mg)