PKCθ, Active
Full-length human recombinant protein expressed in Sf9 cells

Catalog # P74-10G
Lot # A1472-2

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

Gene Aliases
PRKCA, PRKCT, MGC126514, MGC141919, nPKC-theta

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 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.

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

Specific Activity
The specific activity of PKC theta was determined to be 900 nmol/min/mg as per activity assay protocol.

Purity
The purity of PKC theta was determined to be >90% by densitometry, approx. MW 110kDa.

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Full-length recombinant protein expressed in Sf9 cells

Catalog Number P74-10G
Specific Activity 900 nmol/min/mg
Specific Lot Number A1472-2
Purity >90%
Concentration 0.1 µg/µl
Stability 1yr at –70°C from date of shipment
Storage & Shipping 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. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: P74-10G)
Active PKC theta [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 PKC theta for optimal results).

Kinase Assay Buffer VII (Catalog #: K27-09)
Kinase Assay Buffer I (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 MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail
Prepare 250µM [³³P]-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 synthetic peptide substrate (ERMRPRKRGSVRRRV) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
Step 2. Thaw the Active PKC theta, 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 PKC theta (Catalog #P74-10G)
Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #P15-58)
Component 3. 2.5µl PKC lipid activator (Catalog #L51-39). Sonicate it for 1 minute prior to use.
Component 4. 2.5µl distilled H₂O (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 H₂O.

Step 5. Initiate the reaction by the addition of 5 µl [³³P]-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 20 µl 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 H₂O) 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 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 [P³³]-ATP Specific Activity (SA) (cpm/pmol)

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

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

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

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