PKC\(\delta\), Active
Full-length recombinant protein expressed in Sf9 cells

Catalog # P64-10G-10
Lot # M120-1

Product Description

Recombinant full-length human PKC\(\delta\) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_006254.

Gene Aliases

PRKCD

Concentration

0.1\(\mu\)g/\(\mu\)l

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\(^\circ\)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\(^\circ\)C from date of shipment. Product shipped on dry ice.

Scientific Background

Protein kinase C delta (PKC\(\delta\)) is a member of the protein kinase C (PKC) family of serine-threonine kinases. It is a 79 kDa protein kinase that shows strict dependence on the presence of phospholipids, but shows no activation by Ca\(^2+\) (1). Phosphatidylinoositol is the most potent activator of PKC\(\delta\). Northern blot analysis indicated that PKC\(\delta\) is widely distributed in almost all the tissues and is a major isoform of PKC expressed in hemopoietic cells (2). PKC\(\delta\) is involved in fundamental cellular functions regulated by diacylglycerols and mimicked by phorbol esters.

References


Purity

Figure 1. SDS-PAGE gel image
The purity of PKC\(\delta\) was determined to be \(>80\%\) by densitometry, approx. MW 104kDa.

Specific Activity

Figure 2. Radiometric Assay Data
The specific activity of PKC\(\delta\) was determined to be 337 nmol/min/mg as per activity assay protocol.

(For Radiometric Assay Protocol on this product please see pg. 2)

Figure 3. ADP-Glo\textsuperscript{TM} Assay Data
The specific activity of PKC\(\delta\) was determined to be 530 nmol/min/mg as per activity assay protocol.

(For ADP-Glo\textsuperscript{TM} Assay Protocol on this product please see pg. 3)
Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: P64-10G-10)
Active PKCδ (0.1µg/µl) diluted with Kinase Dilution Buffer I (Catalog #: K21-09) 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 PKCδ for optimal results).

Kinase Dilution Buffer I (Catalog #: K21-09)
Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with distilled H2O.

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 #: C50-58)
CREBtide synthetic peptide substrate (KRREILSRRPSYR) 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 PKCδ, 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 PKCδ (Catalog #P64-10G-10)
Component 2. 7.5µl of 1mg/ml stock solution of substrate (Catalog #C50-58)
Component 3. 2.5µl PKC lipid activator (Catalog # L51-39) (0.5 mg/ml phosphatidylserine and 0.05 mg/ml diacylglycerol in 20 mM MOPS, pH 7.2, containing 1 mM CaCl2). Sonicate lipid for 1 minute prior to use.

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 5µl [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 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 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 (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 [P33]-ATP Specific Activity (SA) (cpm/pmol)
Specific activity (SA) = cpm for 5µl [33P]-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 33P-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

PKCδ Kinase Enzyme System
(Promega, Catalog #: V3401)
- PKCδ, Active, 10μg (0.1μg/μl)
- CREBtide, substrate, 1ml (1mg/ml)
- Reaction Buffer A (5X), 1.5ml
- DTT solution (0.1M), 25μl
- PKC Lipid Activator (10X), 500μl

ADP-Glo™ Kinase Assay Kit
(Promega, Catalog #: V9101)
- Ultra Pure ATP, 10 mM (0.5ml)
- ADP, 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 MgCl2 and 0.5 mg/ml BSA.

Assay Protocol

The PKCδ assay is performed using the PKCδ Kinase Enzyme System (Promega; Catalog #: V3401) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The PKCδ 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 the ADP-Glo™ Kinase Assay, see the technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html.

Step 1. 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 2. Thaw the components of PKCδ Enzyme System, ADP and ATP on ice.

Step 3. Prepare 1ml of 2X Buffer by combining 400μl Reaction Buffer A, 1μl DTT and 599μl of dH2O.

Step 4. Prepare 1ml of 250µM ATP Assay Solution by adding 25μl ATP solution (10mM) to 500µl of 2X Buffer and 475µl of dH2O.

Step 5. Prepare diluted PKCδ 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 PKCδ for optimal results).

Step 6. 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 PKCδ
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 7. 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 H2O.

Step 8. At the same time as the PKCδ 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 9. Initiate the PKCδ 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 10. 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 11. Add 50μl of the Kinase Detection Reagent, shake the plate and then incubate the reaction mixture for another 30 minute at ambient temperature.

Step 12. Read the 96-well reaction plate using the Kinase-Glo™ Luminescence Protocol on a GloMax® Microplate Luminometer (Promega; Cat # E6501).

Step 13. 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 ADP-Glo™ Applications Database at http://www.promega.com/applications/cellularanalysis/cellsignaling.htm

Kinase Specific Activity (SA) (nmol/min/mg) = (ADP (step 6) – ADP (Step 7)) in nmol) / (Reaction time in min)*enzyme amount in mg

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