

PKCgamma, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P66-10G

Lot # W198-2

Product Description

Recombinant full-length human PKC γ was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_002739](#).

Gene Aliases

PKCC; PKCG; SCA14; MGC57564; PKC-gamma; PRKCG

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.

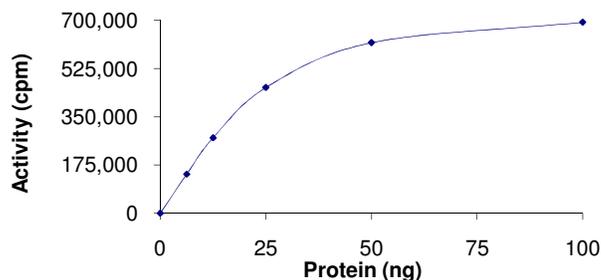
Scientific Background

PKC γ is a member of the protein kinase C (PKC) family of serine- and threonine-specific protein kinases that can phosphorylate a wide variety of protein targets known to be involved in diverse cellular signaling pathways. In the brain, PKC γ is translocated to cell membranes during ischemia and is rapidly removed or degraded during the second otherwise lethal ischemic insult in preconditioned brains. This suggests that ischemic preconditioning enhances downregulation of cell signaling mediated by PKC γ and may thereby provide neuroprotection (1).

References

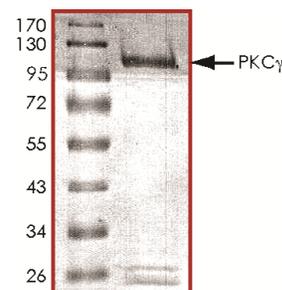
- Shamloo, M. et al: Rapid decline in protein kinase Cgamma levels in the synaptosomal fraction of rat hippocampus after ischemic preconditioning. Neuroreport. 1999 Apr 6;10(5):931-5.

Specific Activity



The specific activity of PKC γ was determined to be **586 nmol /min/mg** as per activity assay protocol.

Purity



The purity of PKC γ was determined to be **>85%** by densitometry, approx. MW **105kDa**.

PKCgamma, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	P66-10G
Specific Activity	586 nmol/min/mg
Specific Lot Number	W198-2
Purity	>85%
Concentration	0.1µg/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.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: P66-10G)

Active PKC γ (0.1 μ g/ μ l) 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 PKC γ 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 250 μ M [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 μ l of 10mM ATP Stock Solution (Catalog #: A50-09), 100 μ l [³³P]-ATP (1mCi/100 μ l), 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 200 μ l aliquots at -20°C.

Substrate (Catalog #: P15-58)

PKCtide peptide substrate (ERM₂PRKRQGSVRRRV) 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 γ , 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 20 μ l:
 - Component 1.** 10 μ l of diluted Active PKC γ (Catalog #P66-10G)
 - Component 2.** 5 μ l of 1mg/ml stock solution of substrate (Catalog #P15-58)
 - Component 3.** 2.5 μ l PKC lipid activator (0.5 mg/ml phosphatidylserine and 0.05mg/ml diacylglycerol in 20mM MOPS, pH 7.2, containing 1mM CaCl₂). Sonicate or vortex lipid for 1 minute prior to use. (Catalog #L51-39)
 - Component 4.** 2.5 μ l 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 μ l [³³P]-ATP Assay Cocktail bringing the final volume up to 25 μ l 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 μ l 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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

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

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

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

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

2

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.