PKCγ, Active
Full-length recombinant protein expressed in Sf9 cells

Catalog # P66-10G
Lot # B096-1

Product Description

Recombinant full-length human PKCγ 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, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 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

PKCγ 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, PKCγ 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 PKCγ and may thereby provide neuroprotection (1).

References


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Activity Assay Protocol

**Reaction Components**

**Active Kinase (Catalog #: P66-10G)**
Active PKCγ (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 PKCγ for optimal results).

**Kinase Dilution Buffer I (Catalog #: K21-09)**
Dilute Kinase Assay Buffer I (Catalog #: K01-09) 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.

**[32P]-ATP Assay Cocktail**
Prepare 250μM [32P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [32P]-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 H2O to a final concentration of 1mg/ml.

**Assay Protocol**

1. **Step 1.** Thaw [32P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
2. **Step 2.** Thaw the Active PKCγ, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
3. **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 PKCγ (Catalog #P66-10G)
   - **Component 2.** 7.5μl of 1mg/ml stock solution of substrate (Catalog #P15-58)
   - **Component 3.** 2.5μl PKC lipid activator (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. (Catalog #L51-39)
4. **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.
5. **Step 5.** Initiate the reaction by the addition of 5μl [32P]-ATP Assay Cocktail bringing the final volume up to 25μl and incubate the mixture in a water bath at 30°C for 15 minutes.
6. **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.
7. **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.
8. **Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. **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 [P32]-ATP Specific Activity (SA) (cpm/pmol)**
Specific activity (SA) = cpm for 5μl [32P]-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 32P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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