BioVision
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PKAcγ, Active

Full-length recombinant protein expressed in Sf9 cells
Catalog # 7728-5
Lot#: ______________________

Product Description

Recombinant full-length human PKAcγ was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_002732.

Gene Aliases

KAPG; PKAr; cAPKr

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.

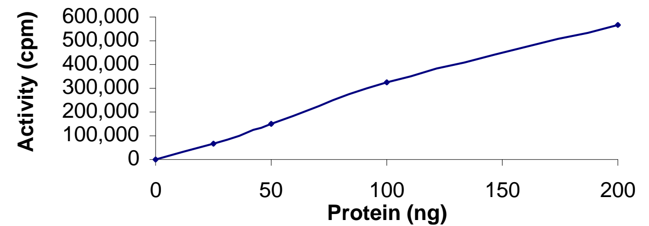
Scientific Background

PKA C-gamma (PKAcγ) is a third isoform of the catalytic subunit of cAMP-dependent protein kinase. It was isolated from a human testis cDNA library and was clearly derived from a gene distinct from C-alpha and C-beta and showed tissue-specific expression. Whereas at the amino acid level C-alpha and C-beta showed 93% homology, C-gamma showed only about 80% homology to both C-alpha and C-beta (1). The PRKACG gene is intronless, contains remnants of a poly (A) tail, is flanked by direct repeats, and is co-linear with the PRKACA gene(2).

References

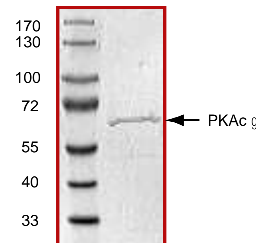
1. Beebe SJ et al: Molecular cloning of a tissue-specific catalytic subunit of cAMP-dependent protein kinase is a transcribed retroposon. *Genomics* 49: 290-297, 1998.

Specific Activity



The specific activity of PKAcγ was determined to be **145 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry
Approx. MW **65kDa**.

PKAcγ, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	7728-5
Specific Activity	145 nmol/min/mg
Specific Lot	
Purity	>90%
Concentration	0.1µg/µl
Stability	1yr At –70°C from date of shipment

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 #: 7728-5)

Active PKAcγ (0.1µg/µl) diluted with Kinase Dilution Buffer III (see below) 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 PKAcγ for optimal results).

Kinase Dilution Buffer III

Kinase Assay Buffer I: diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

Kinase Assay Buffer I

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.

[^{32}P]-ATP Assay Cocktail

Prepare 250µM [^{32}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution, 100µl [^{32P}]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I. Store 1ml aliquots at –20°C.

10mM ATP Stock Solution

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I. Store 200µl aliquots at –20°C.

Substrate

CREBtide synthetic peptide substrate (KRREILSRRPSYR) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{32}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active PKAcγ, 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 PKAcγ
- **Component 2.** 10µl of 1mg/ml stock solution of substrate

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 [^{32}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 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.

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

Corrected cpm from reaction / [(SA of ^{32}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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