

PKAc γ , Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # 7728-5

Lot#: _____

Product Description

Recombinant full-length human PKAc γ 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; cAPK γ

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.

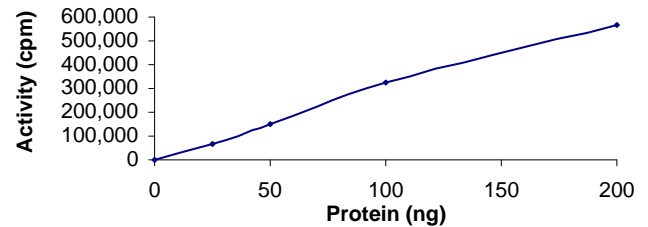
Scientific Background

PKA C-gamma (PKAc γ) 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

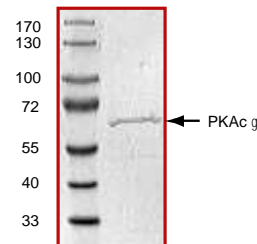
1. Beebe, S. J. et al: Molecular cloning of a tissue-specific protein kinase (C gamma) from human testis—representing a third isoform for the catalytic subunit of cAMP-dependent protein kinase. *Molec. Endocr.* 4: 465-475, 1990.
2. Reinton, N. et al: The gene encoding the C gamma catalytic subunit of cAMP-dependent protein kinase is a transcribed retroposon. *Genomics* 49: 290-297, 1998.

Specific Activity



The specific activity of PKAc γ 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**.

PKAc γ , Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number 7728-5
Specific Activity 145 nmol/min/mg
Specific Lot

Purity	>90%
Concentration	0.1 $\mu\text{g}/\mu\text{l}$
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 #: 7728-5)

Active PKAc γ (0.1 μ g/ μ l) 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 PKAc γ for optimal results).

Kinase Dilution Buffer III

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

Kinase Assay Buffer I

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 250 μ M [³²P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 μ l of 10mM ATP Stock Solution, 100 μ l [³²P]-ATP (1mCi/100 μ l), 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 200 μ l aliquots at -20°C.

Substrate

CREBtide synthetic peptide substrate (KRREILSRPSYR) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³²P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active PKAc 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 PKAc γ
 - Component 2.** 10 μ l 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 H₂O.
- Step 5.** Initiate the reaction by the addition of 5 μ l [³²P]-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 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 [³²P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μ l [³²P]-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 ³²P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μ g or mg)]*[(Reaction Volume) / (Spot Volume)]

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