

Product: PKAcg, Active

Catalog #: 02-2040

Amount: 5 µg

Product Description

Recombinant full-length human PKAcg was expressed by baculovirus in Sf9 insect cells using a 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.

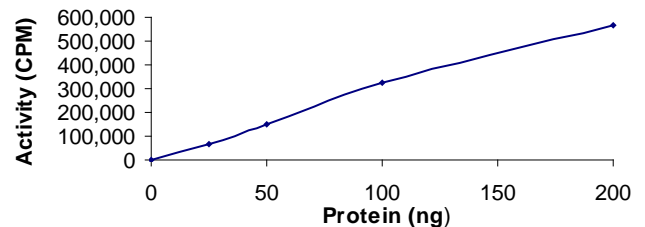
Scientific Background

PKA C-gamma (PKAcg) 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

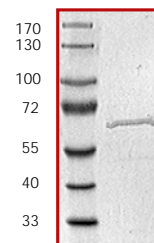
1. Beebe, S. J. et al: Molecular cloning of a tissue-specific protein kinase (C gamma) from human testis--representing a third isoform for the catalytic subunit of cAMP-dependent protein kinase. *Molec. Endocr.* 4: 465-475, 1990.
2. Reinton, N. et al: The gene encoding the C gamma catalytic subunit of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

Specific Activity



The specific activity of PKAcg 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**.

PKAcg, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	02-2040
Quantity	5µg
Specific Activity	145 nmol/min/mg
Specific Lot Number	K138-1
Purity	>60%
Format	5µg in 50µl
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.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: 02-2040)

Active PKA cg (0.1µg/µl) diluted with Kinase Dilution Buffer (Catalog #: K23-09) 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 PKA cg for optimal results).

Kinase Dilution Buffer (Catalog #: K23-09)

Kinase Assay Buffer (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

Kinase Assay Buffer (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM NaCl, 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 (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 (Catalog #: K01-09). Store 200µl aliquots at -20°C.

Substrate (Cat#: C50-58)

CREBtide synthetic peptide substrate (KRREILSRPSYR) diluted in distilled water 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 PKAcg, Kinase Assay Buffer, Substrate and Enzyme 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 PKAcg (Catalog #02-2040)
 - 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 H₂O.
- 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.
- 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 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 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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