PKAc-gamma (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPP53

Lot No. K138-1
5 µg 0.1 µg/µl

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).

Product Description:

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

Gene Aliases:

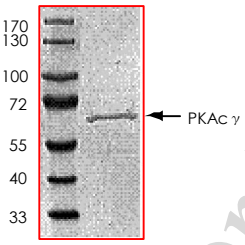
KAPG; PKAr; cAPKr

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.

Purity & Molecular Weight:

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



Storage:

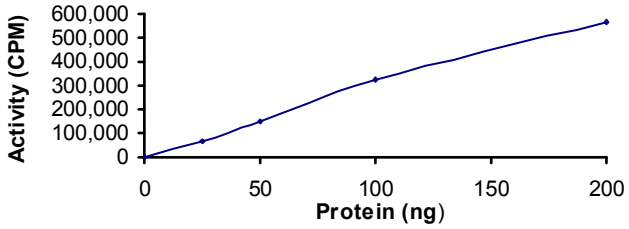
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

Unopened vial at –70 °C, 1 year from date of shipment.

Specific Activity:

The specific activity was determined to be 145 nmol/min/mg as per Activity Assay Protocol.



Activity Assay Protocol:

Assay activity of the kinase in a 25 µL reaction consisting of 5 µL of 5 X Kinase Assay Buffer, 10 µL of 1 mg/ml the Substrate Solution, 5 µL of diluted kinase and 5 µL of 250 µM ATP solution containing [gamma ^{32}P] ATP (0.167 µCi/µL). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20 µL of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

Substrate Solution:

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

5 X Kinase Assay Buffer:

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.

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.

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