PKAc-alpha (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPP51

Lot No. B207-1
5 µg 0.1 µg/µl

Background:

The catalytic subunit C-alpha of PKA (PKAc α) is a member of the Ser/Thr protein kinase family and has been assigned to chromosome region 19p13.1 (1). Null mutation in PKAc α leads to early postnatal lethality in the majority of C-alpha knockout mice. Surprisingly, a small percentage of C-alpha knockout mice, although runted, survived to adulthood. In these animals, compensatory increases in C-beta levels occurred in brain whereas many tissues, including skeletal muscle, heart, and sperm, contained less than 10% of the normal PKA activity (2).

Product Description:

Recombinant full-length human PKAc α was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_002730.

Gene Aliases:

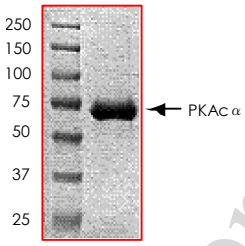
PKAa; cAPKa

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 >75% by densitometry. Approx. MW 69kDa.



Storage:

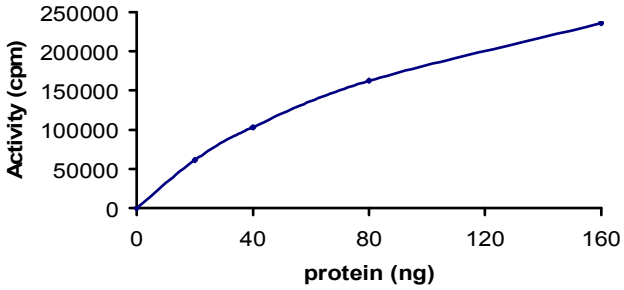
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

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

Specific Activity:

The specific activity was determined to be 141 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, 12.5mM β-glycerol-phosphate, 25mM $MgCl_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

1.Tasken, K. et al: The gene encoding the catalytic subunit C-alpha of cAMP-dependent protein kinase (locus PRKACA) localizes to human chromosome region 19p13.1. Genomics 36: 535-538, 1996.

2.Skalhegg, BS. Et al: Mutation of the C-alpha subunit of PKA leads to growth retardation and sperm dysfunction. Molec. Endocr. 16: 630-639, 2002.

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