PKAc-beta (Human), Active

Full-length recombinant protein expressed in Sf9 cells

Cat# CY-SPP52

Lot No. U051-1
5 µg 0.1 µg/µl

Background:

The catalytic subunit C-beta of PKA (PKAcβ) is a member of the Ser/Thr protein kinase family (the PKA catalytic subunit consist of three gene products: C-alpha, C-beta, and C-gamma) and has been assigned to human chromosome region 1p36.1 (1). PKAcβ is derived from a gene distinct from C-alpha and shows tissue-specific expression. At the amino acid level C-alpha and C-beta showed 93% homology.

Product Description:

Recombinant full-length human PKAcβ was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is NM_002731.

Gene Aliases:

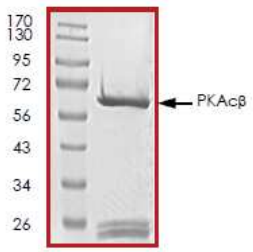
PKAb; cAPKb

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:

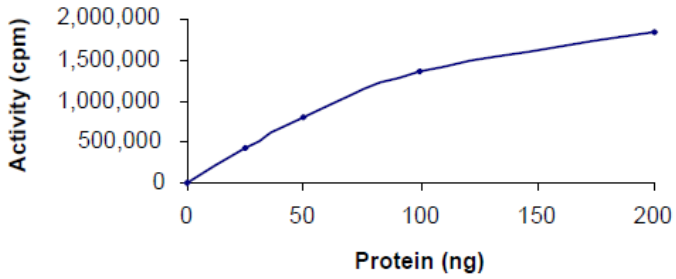
Store product at –70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Stability:

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

Specific Activity:

The specific activity was determined to be 342 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 $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

References:

1.Simard, J. et al: Assignment of the gene encoding the catalytic subunit C-beta of cAMP-dependent protein kinase to the p36 band on chromosome 1. Hum. Genet. 88: 653-657, 1992.

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