Recombinant Human Active PKAcβ Protein, CF

Catalog Number: 4596-KS-010
Lot Number: 1784526

SPECIFICATIONS AND USE

Source	Recombinant human PKAcβ was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST tag. The gene accession number is NM_002731.
Molecular Mass	The approximate molecular weight is 65 kDa.
Purity	The purity was determined to be > 80% by densitometry.
Formulation	Supplied in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.25 mM DTT, 0.1 mM EDTA, 10 mM Glutathione, 0.1 mM PMSF, and 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of PKAcβ was determined to be 430 nmol/min/mg using a CREBtide synthetic peptide substrate.
Storage	This product is stable at ≤ -70 °C for up to one year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

BACKGROUND

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

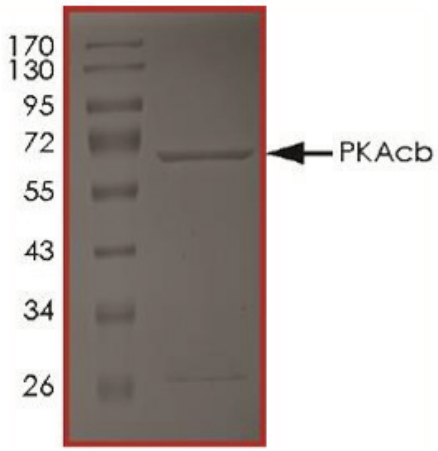

Figure 1: The approximate molecular weight is 65 kDa and the purity is > 80%.

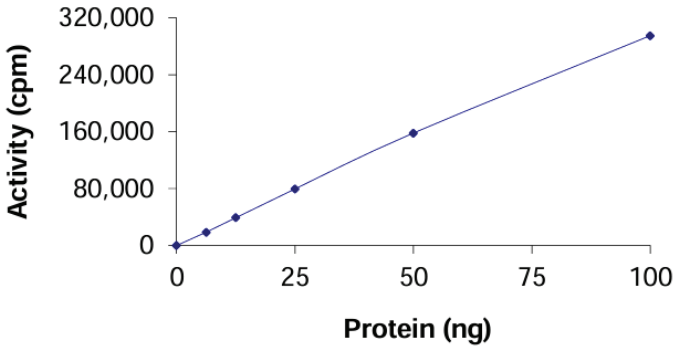

Figure 2: The specific activity of this lot of PKAcβ was determined to be 430 nmol/min/mg as per the Activity Assay Protocol.

REFERENCES

1. Simard, J. *et al.* (1992) Human Genetics **88**:653.

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ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - Active PKAcβ (0.1 µg/µL) diluted with Kinase Dilution Buffer III and assayed as outlined in Figure 2..
Note: *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*

Kinase Assay Buffer I - 25 mM MOPS, pH 7.2, 12.5 mM β-glycerol-phosphate, 25 mM $MgCl_2$, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer I prior to use.

Kinase Dilution Buffer III - Kinase Assay Buffer I diluted at a 1:4 ratio (5X dilution) with 50 ng/µL BSA solution.

10 mM ATP Stock Solution - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I. Store 200 µL aliquots at ≤ -20 °C.

[^{33}P]-ATP Assay Cocktail - Prepare 250 µM [^{33}P]-ATP Assay Cocktail in a designated radioactive work area by combining 150 µL of 10 mM ATP Stock Solution, 100 µL of [^{33}P]-ATP (1 mCi/100 µL), and 5.75 mL of Kinase Assay Buffer I. Store 1 mL aliquots at ≤ -20 °C.

Substrate - CREBtide synthetic peptide substrate (KRREILSRRPSYR) diluted in distilled water to a final concentration of 1 mg/mL.

ASSAY PROCEDURE

1. Thaw the [^{33}P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area.
2. Thaw the Active PKAcβ, Kinase Assay Buffer I, Substrate, and Kinase Dilution Buffer III on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 µL.

Reaction Component	Amount
Diluted Active PKAcβ	10 µL
Stock solution of Substrate (1 mg/mL)	5 µL
Distilled water (2-8 °C)	5 µL

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 or deionized water.
5. Initiate the reaction with the addition of 5 µL [^{33}P]-ATP Assay Cocktail, bringing the final volume up to 25 µL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
6. After the 15 minute incubation, terminate the reaction by spotting 20 µL of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10 mL of phosphoric acid to 990 mL of distilled water to make a 1L solution) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (step 4) for each sample and calculate the Kinase Specific Activity as outlined below.

Calculation of [^{33}P]-ATP Specific Activity (SA) (cpm/pmol)

Specific Activity (SA) = cpm for 5 µL [^{33}P]-ATP / pmole of ATP (in 5 µL of a 250 µM ATP stock solution, i.e. 1250 pmoles)

Calculation of Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in µg or mg)] x [(Reaction volume) / (Spot Volume)]