Recombinant Human Active PKA Cα

Certificate of Analysis

Catalog Number: 4268-KS
Lot Number: 1575174

Specifications and Use

Source	♦ Recombinant human PKA Cα was expressed by baculovirus in *Sf* 9 insect cells using an N-terminal GST tag. The gene accession number is NM_002730.
Molecular Mass	♦ The approximate molecular weight is 69 kDa (see Figure 1 below).
Purity	♦ The purity was determined to be > 90% by densitometry (see Figure 1 below).
Formulation	♦ Supplied in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.25 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.
Size	♦ 10 µg.
Concentration	♦ 0.1 µg/µL.
Activity	♦ The specific activity of PKA Cα was determined to be 2100 nmol/min/mg using a synthetic peptide substrate (see Activity Assay Protocol).
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.**

PKA Cα

The catalytic subunit C-alpha of PKA (PKA Cα) is a member of the Ser/Thr protein kinase family and has been assigned to chromosome region 19p13.1 (1). Null mutation in PKA Cα 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).

References

1. Tasken, K. *et al*. (1996) Genomics **36**:535.
2. Skalhegg, B.S. *et al*. (2002) Molec. Endocr. **16**:630.

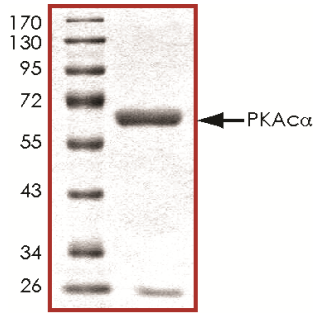

Figure 1: The approximate molecular weight is 69 kDa and the purity is > 90%.

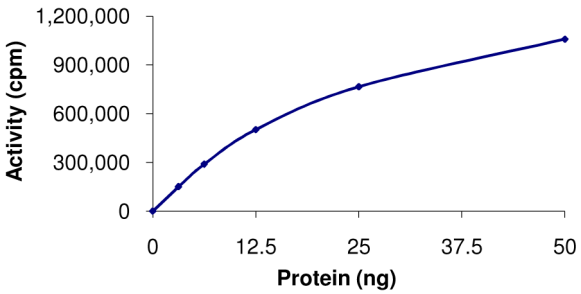

Figure 2: The specific activity of this lot of PKA Cα was determined to be 2100 nmol/min/mg as per the Activity Assay Protocol (on reverse).

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Activity Assay Protocol

Solutions Required

- **Active Kinase** - Active PKA Cα (0.1 µg/µL) diluted with Kinase Dilution Buffer VII. **Note:** *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*
- **Kinase Assay Buffer I, pH 7.2** - 25 mM MOPS, 12.5 mM β-glycerolphosphate, 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 VII, pH 7.2** - Kinase Assay Buffer I diluted at a 1:4 ratio (5-fold dilution) with 50 ng/µL BSA and 5% glycerol solution.
- **10 mM ATP Stock Solution** - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer. 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 1.0 mL aliquots at ≤ -20 °C.
- **Substrate** - CREBtide synthetic peptide substrate (KRREILSRRPSYR) diluted in distilled or deionized water to a final concentration of 1.0 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 PKA Cα, Kinase Assay Buffer, Substrate, and Kinase Dilution Buffer 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 PKA Cα	10 µL
Substrate (1.0 mg/mL stock solution)	5 µL
Distilled Water (4 °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 (add 10 mL of phosphoric acid to 990 mL of distilled or deionized water) with constant gentle stirring. It is recommended that the strips be washed a total of three times 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 (see 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/minutes/µg or nmol/minutes/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)]

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