Recombinant Human Active PKCδ

Certificate of Analysis

Catalog Number: 4585-KS
Lot Number: 1467200

Specifications and Use

Source	♦ Recombinant human PKCδ was expressed by baculovirus in *Sf* 9 insect cells using an N-terminal GST tag. The gene accession number is NM_006254.
Molecular Mass	♦ The approximate molecular weight is 104 kDa (see Figure 1 below).
Purity	♦ The purity was determined to be > 75% by densitometry (see Figure 1 below).
Formulation	♦ Supplied in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.25 mM DTT, 10 mM glutathione, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.
Size	♦ 10 µg.
Concentration	♦ 0.1 µg/µL.
Activity	♦ The specific activity of PKCδ was determined to be 255 nmol/min/mg using a synthetic peptide substrate (see Activity Assay Protocol).
Storage	♦ This product is stable at ≤ -70 °C for up to 1 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.**

PKCδ

Protein Kinase C delta (PKCδ) is a member of the protein kinase C (PKC) family of serine-threonine kinases. It is a 104 kDa protein kinase that shows strict dependence on the presence of phospholipids but shows no activation by Ca^{2+} (1). Phosphatidylinositol is the most potent activator of PKCδ. Northern blot analysis indicates that PKCδ is widely distributed in almost all the tissues and is a major isoform of PKC expressed in hemopoietic cells (2). PKCδ is involved in fundamental cellular functions regulated by diacylglycerols and mimicked by phorbol esters.

References

1. Leibersperger, H. *et al.* (1991) J. Biol. Chem. **266**:14778.
2. Mischak, H. *et al.* (1991) Biochemistry **30**:7925.

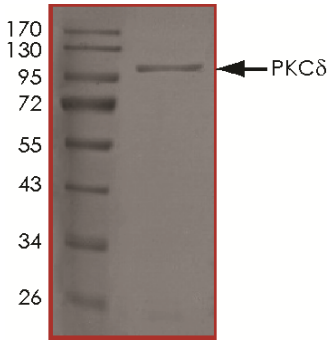

Figure 1: The approximate molecular weight is 104 kDa and the purity is > 75%.

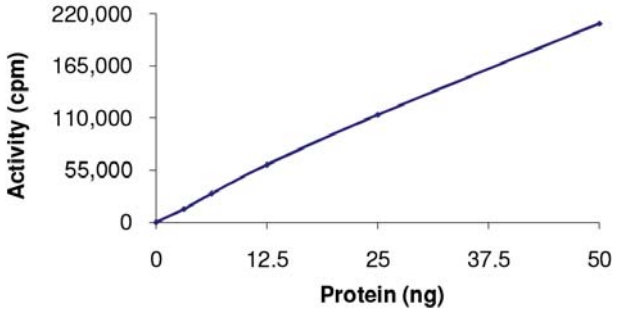

Figure 2: The specific activity of this lot of PKCδ was determined to be 255 nmol/min/mg as per the Activity Assay Protocol (on reverse).

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Activity Assay Protocol

Solutions Required

- **Active Kinase** - PKCδ (0.1 μg/μL) diluted with Kinase Dilution Buffer III. **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 $MgCl_2$, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer prior to use.
- **Kinase Dilution Buffer III, pH 7.2** - Kinase Assay Buffer I diluted at a 1:4 ratio (5-fold dilution) with 50 ng/mL 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 1.0 mL aliquots at ≤ -20 °C.
- **PKC Lipid Activator** - 0.5 mg/mL phosphatidylserine and 0.05 mg/mL diacylglycerol in 20 mM MOPS, pH 7.2, containing 1 mM $CaCl_2$. Sonicate the lipid for 1 minute prior to use.
- **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 PKCδ, Kinase Assay Buffer I, 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 PKCδ	10 μL
Substrate (1.0 mg/mL stock solution)	5.0 μL
PKC Lipid Activator	2.5 μL
Distilled water (4 °C)	2.5 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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