

## PP2Calpha, Active

Recombinant full-length protein expressed in Sf9 cells

**Catalog # P02-20G**

Lot # S335-3

### Product Description

Recombinant full-length human PP2Cα was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM\\_021003](#).

### Gene Aliases

PP2C-ALPHA, PPM1A, PP2CA, MGC9201, FLJ42306

### Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

### Storage and Stability

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.

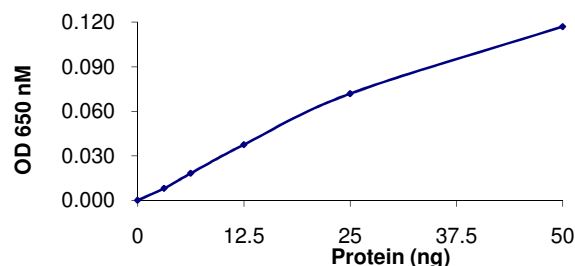
### Scientific Background

PP2Cα is a member of the family of Ser/Thr protein phosphatases and is composed of a catalytic subunit and a constant regulatory subunit. PP2C alpha is known to be negative regulators of cell stress response pathways. PP2C alpha has been reported to negatively regulate TGFβ signaling pathway and cell cycle. PP2C alpha has been shown to dephosphorylate cyclin-dependent kinases (CDKs) and it may be involved in cell cycle control (1). Overexpression of PP2C alpha is reported to cause cell-growth arrest or cell death. PP2C alpha is highly expressed in heart and skeletal muscle (2).

### References

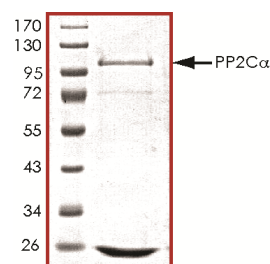
1. Parvari, R. et. al: A recessive contiguous gene deletion of chromosome 2p16 associated with cystinuria and a mitochondrial disease. Am. J. Hum. Genet. 69: 869-875, 2001.
2. Marley, A. E. et. al: The cloning expression and tissue distribution of human PP2C-beta.FEBS Lett. 431: 121-124, 1998.

### Specific Activity



The specific activity of PP2Cα was determined to be **1,200 nmol /min/mg** as per activity assay protocol.

### Purity



The purity of PP2Cα was determined to be **>70%** by densitometry, approx. MW **105kDa**.

## PP2Calpha, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog Number	P02-20G
Specific Activity	1,200 nmol/min/mg
Specific Lot Number	S335-3
Purity	>70%
Concentration	0.1 µg/µl
Stability	1yr At -70°C from date of shipment
Storage & Shipping	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. Product shipped on dry ice.

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# Activity Assay Protocol

## Reaction Components

### Active Phosphatase (Catalog #: P02-20G)

Active PP2C $\alpha$  (0.1 $\mu$ g/ $\mu$ l) diluted with Phosphatase Dilution Buffer II (Catalog #: P22-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active PP2C $\alpha$  for optimal results).

### Phosphatase Dilution Buffer II (Catalog #: P22-09)

Phosphatase Assay Buffer II (Catalog #: P02-09) diluted at a 1:4 ratio (5X dilution) with freshly prepared solution containing 0.2% 2-mercaptoethanol and 65ng/ $\mu$ l BSA.

### Phosphatase Assay Buffer II (Catalog #: P02-09)

Buffer components: 250 mM Imidazole, pH 7.2

### Substrate (Catalog #: T69-58)

Thr-phosphopeptide synthetic substrate (KRT(p)IRR) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

### Detection Solution

BIOMOL GREEN reagent phosphatase detection kit (BioMol Catalog #: AK-111).

## Assay Protocol

- Step 1.** Prepare a fresh batch of Phosphatase Dilution Buffer and keep on ice.
- Step 2.** Prepare phosphate standard curve following the instruction of BIOMOL GREEN reagent phosphatase detection kit. Briefly, prepare 1:1 serial dilutions of phosphate standard solutions with Phosphatase Dilution Buffer in a volume of 25 $\mu$ l. Also, use 25 $\mu$ l Phosphatase Dilution Buffer as a blank. The range of phosphate amount should be 0~4 nmol.
- Step 3.** Thaw the Active PP2C $\alpha$  on ice. Prepare serial dilutions of PP2C $\alpha$  using Phosphatase Dilution Buffer.
- Step 4.** In a pre-cooled microfuge tube, add the following reaction components in total volume of 25 $\mu$ l:

- Component 1.** 10 $\mu$ l of diluted Active PP2C $\alpha$  (Catalog #P02-20G)
- Component 2.** 5 $\mu$ l of Substrate Assay solution (Catalog #T69-58)
- Component 3.** 10 $\mu$ l Phosphatase Dilution Buffer II (Catalog #P22-09)

- Step 5.** Set up the blank control as outlined in step 4, excluding the addition of the Active Phosphatase. Replace the Active Phosphatase with an equal volume of Phosphatase Dilution Buffer (Catalog # P22-09).
- Step 6.** Start the reaction by incubating the mixture in a water bath at 37°C for 15 minutes.
- Step 7.** Add 100 $\mu$ l BIOMOL GREEN Reagent to each reaction including control tubes.
- Step 8.** Add 100 $\mu$ l BIOMOL GREEN Reagent to each phosphate standard solution including the blank (step 2).
- Step 9.** Incubate at room temperature for 30 minutes to allow development of the green color
- Step 10.** Measure the absorbance of the reaction solution in a spectrophotometer at 650 nm.
- Step 11.** Plot the free phosphate standard curve. Determine absorbance (y) for each sample (where y = absorbance of sample – background absorbance) and calculate the corresponding nmol phosphate released (x) during the assay using the equation  $y = A \cdot x + B$  or  $x = [y - B] / A$  (the A and B values are determined from the slope of the line from the standard curve).
- Step 12.** Calculate the phosphatase specific activity (SA):

### Phosphatase Specific Activity (SA) (nmol/min/mg)

$$SA = \text{Corresponding phosphate released} \cdot 1000 / [(\text{Reaction time in min}) \cdot (\text{Enzyme amount in } \mu\text{g})]$$

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