

## PP2Cα, Active

Recombinant full-length protein expressed in Sf9 cells

Catalog # P02-20G

Lot # X3646-12

### 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.

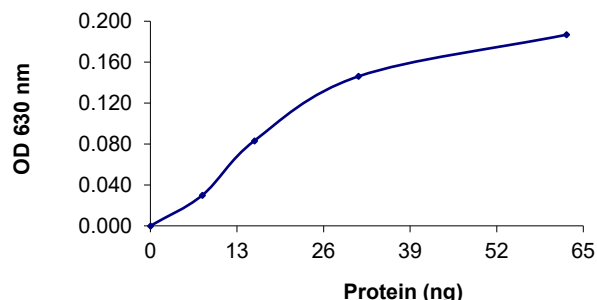
### Scientific Background

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

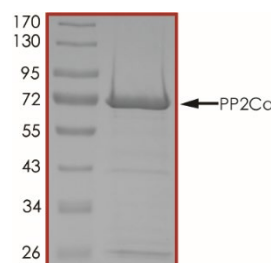
1. Parvari, R. et. al: A recessive contiguous gene deletion of chromosome 2p16 associated with cystinuria and a mitochondrial disease. Am. J. Hum. Genet. 69: 869-875, 2001.
2. Marley, A. E. et. al: The cloning expression and tissue distribution of human PP2C-beta. FEBS Lett. 431: 121-124, 1998.

### Specific Activity



The specific activity of PP2Cα was determined to be **1,418 nmol /min/mg** as per activity assay protocol.

### Purity



The purity of PP2Cα was determined to be **>90%** by densitometry, approx. MW **~70 kDa**.

## PP2Cα, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog #	P02-20G
Specific Activity	1,418 nmol/min/mg
Lot #	X3646-12
Purity	>90%
Concentration	0.1 µg/µl
Stability	1 yr 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 630 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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# SAFETY DATA SHEET

## Article 1 – Product Identification

**Product Name: PP2C $\alpha$ , Active****Catalog # P02-20G**

*This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.*

Manufacturer's Name: SignalChem Biotech Inc.  
Street Address: 110-13120 Vanier Place  
City, Prov. Postal Code: Richmond, BC, V6V 2J2  
Fax: 604-232-4601  
EMERGENCY PHONE: 604-232-4600

## Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** Skin irritation (Category 3); Eye irritation (Category 2B).
- **Hazard Pictograms:** none.
- **Signal words:** Warning.
- **Hazard statements:** Causes mild skin irritation (H316); Causes eye irritation (H320).
- **Precautionary statements:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305 + P351 + P338).
- **Other hazards:** none known.

## Article 3 – Composition/Information on Ingredients

**Chemical Characterization:** Mixtures.

**Description:** This product consists of the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Glycerol	Glycerol	56-81-5	≤25%
NaCl	Sodium chloride	7647-14-5	≤1.753 %
Tris-HCl; Tris (hydroxymethyl) aminomethane hydrochloride	2 - Amino - 2 - (hydroxymethyl) propane - 1, 3 - diol hydrochloride	1185-53-1	<0.8%
Glutathione	Glutathione	70-18-8	0.307%
Protein		No data available	≤0.02%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.0038%
EDTA	Ethylenediaminetetraacetic acid	6381-92-6	0.0037%
PMSF; Phenylmethanesulfonyl fluoride	$\alpha$ -Toluenesulphonyl fluoride	329-98-6	0.002%

## Article 4 – First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breathe in fresh air. If cannot breathe, give artificial respiration and consult a physician.
- **After skin contact:** Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- **After eye contact:** Rinse opened eyes with plenty of water for at least 15 minutes. Consult a physician.
- **After swallowing:** rinse the mouth with plenty of water and consult a physician.

## Article 5 - Fire-fighting Measures

- **Suitable extinguishing media:** Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- **Specific hazards arising from the substance or mixture:** None known.
- **Special protective equipment and precautions for fire-fighters:** Self-contained breathing apparatus if necessary.

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## Article 6 – Accidental Release Measures

- **Personal precautions, protective equipment and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- **Environmental precautions:** Do not allow to enter drains.
- **Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

## Article 7 - Handling and Storage

- **Precautions for safe handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store in a dry and well-ventilated place in -70 °C. Keep container upright and tightly closed.

## Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:**  
Glycerol (CAS-No: 56-81-5)

Values	Control parameters	Regulations
TWA	10 mg/m <sup>3</sup> for mist	British Columbia, Canada
TWA	3 mg/m <sup>3</sup> for respirable mist	British Columbia, Canada
TWA	10 mg/m <sup>3</sup>	Alberta, Canada
TWAEV	10 mg/m <sup>3</sup>	Ontario, Canada
TWAEV	10 mg/m <sup>3</sup>	Quebec, Canada
TWA	10 mg/m <sup>3</sup>	USA

- **Appropriate engineering controls:**  
Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- **Individual protection measures:**  
**Respiratory protection:**  
Use appropriate respirator if there is inadequate ventilation by following the government standards.  
**Hand protection:**  
Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.  
**Eye/face protection:**  
Safety goggles with side-shields approved under appropriate government standards.  
**Skin/body protection:**  
Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

## Article 9 – Physical and Chemical Properties

<b>Appearance:</b> Colorless fluid.	<b>Danger of explosion:</b> Product does not present an explosion hazard.
<b>Odour/Odour Threshold:</b> Not determined.	<b>Explosion limits:</b> Lower: 0.9 Vol %; Upper: 0.0 Vol %.
<b>pH:</b> Not available.	<b>Decomposition temperature:</b> Not available.
<b>Melting point/freezing point:</b> Not determined.	<b>Vapor pressure at 20 °C:</b> 0.1 hPa
<b>Boiling point/Boiling range:</b> 100 °C.	<b>Density:</b> Not determined.
<b>Flash point:</b> > 100 °C.	<b>Relative density:</b> Not determined.
<b>Flammability (solid, gaseous):</b> Not determined.	<b>Vapor density:</b> Not determined.
<b>Ignition temperature:</b> 400 °C.	<b>Evaporation rate:</b> Not determined.
<b>Auto-igniting:</b> Product is not self-igniting.	<b>Solubility in / Miscibility with Water:</b> Fully miscible.

## Article 10 - Stability and Reactivity

- **Reactivity:** Stable under recommended transport and storage conditions.
- **Chemical stability:** Stable under recommended transport and storage conditions.
- **Possible hazardous reactions:** No dangerous reactions known.
- **Conditions to avoid:** Heat and moisture.
- **Incompatible materials:** Strong acids/bases, strong oxidizing/reducing agents.
- **Hazardous decomposition products:** Carbon oxides may formed under fire conditions; no known decomposition information for other decomposition products.

# SAFETY DATA SHEET

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## Article 11 - Toxicological Information

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- **Acute toxicity:** Not available.
- **LD/LC50:** Not available.
- **Skin corrosion/irritation:** Not available.
- **Serious eye damage/eye irritation:** Not available.
- **Respiratory or skin sensitization:** Not available.
- **Germ cell mutagenicity:** Not available.
- **Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- **Reproductive toxicity:** Not available.
- **Teratogenicity:** Not available.
- **Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- **Aspiration hazard:** Not available.
- **Potential health effects:**  
**Inhalation:** May be harmful if inhaled. May cause respiratory tract irritation.  
**Ingestion:** May be harmful if swallowed.  
**Skin:** May be harmful if absorbed through skin. May cause skin irritation.  
**Eyes:** May cause eye irritation.
- **Signs and Symptoms of Exposure:**  
Prolonged or repeated exposure can cause: Nausea, Dizziness.
- **Synergistic effects:** Not available.

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## Article 12 - Ecological Information

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- **Eco-toxicity:** Not applicable.
- **Biodegradability:** Not applicable.
- **Bio-accumulative potential:** Not applicable.
- **Mobility in soil:** Not applicable.
- **PBT and vPvB assessment:** Not applicable.
- **Other adverse effects:** Not applicable.

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## Article 13 - Disposal Considerations

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- **Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- **Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

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## Article 14 - Transport Information

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- **DOT:** Not dangerous goods.
- **IMDG:** Not dangerous goods.
- **IATA:** Not dangerous goods.

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## Article 15 - Regulatory Information

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- **WHMIS Classification:** Non-hazardous.
- **GHS label elements:** Not applicable.
- **Signal word:** Not applicable.
- **Hazard statements:** Not applicable.

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## Article 16 - Other Information

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The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.

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