

PP1A, Active

Recombinant full-length protein expressed in E.coli cells

Catalog # P13-20GH

Lot # 13262-8

Product Description

Recombinant full-length human PP1A was expressed in E.coli cells using the N-terminal GST, His tags. The PP1A gene accession number is [NM_002708](#).

Gene Aliases

PP-1A, PPP1A, PPP1CA, MGC1674, MGC15877

Formulation

Recombinant protein stored in 20mM MOPS, pH 7.5, 300mM NaCl, 10mM glutathione, 0.1mM PMSF, 0.25mM DTT, and 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.

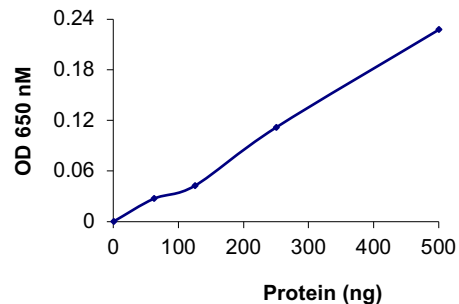
Scientific Background

PP1A is a serine/threonine specific protein phosphatase family which is involved in the regulation of a variety of cellular processes, such as cell division, glycogen metabolism, muscle contractility, protein synthesis, and HIV-1 viral transcription. PP1A is one of the three catalytic subunits of protein phosphatase 1 (PP1) (1). PP1A functions as a suppressor of learning and memory and as a potential mediator of cognitive decline during aging (2). PP1A is also play a main role in splice site selection and is an important regulator of cardiac function.

References

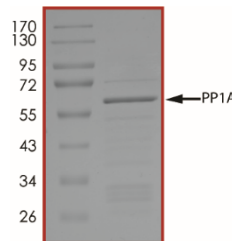
1. Cohen, P.et.al: Protein phosphatases come of age. J. Biol. Chem. 264: 21435-21438, 1989.
2. Genoux, D. et.al: Protein phosphatase 1 is a molecular constraint on learning and memory. Nature 418: 970-975, 2002.

Specific Activity



The specific activity of PP1A was determined to be **197 nmol /min/mg** as per activity assay protocol.

Purity



The purity of PP1A was determined to be **>70%** by densitometry, approx. MW **64 kDa**.

PP1A, Active

Recombinant full-length human protein expressed in E.coli cells

Catalog #	P13-20GH
Specific Activity	197 nmol/min/mg
Lot #	13262-8
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 #: P13-20GH)

Active PP1A (0.1µg/µl) 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 PP1A 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/µl 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 H₂O 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 25µl. Also, use 25µl Phosphatase Dilution Buffer as a blank. The range of phosphate amount should be 0~4 nmol.
- Step 3.** Thaw the Active PP1A on ice. Prepare serial dilutions of PP1A using Phosphatase Dilution Buffer.
- Step 4.** In a pre-cooled microfuge tube, add the following reaction components in total volume of 25µl:

Component 1. 10µl of diluted Active PP1A (Catalog #P13-20GH)

Component 2. 5µl of Substrate Assay solution (Catalog #T69-58)

Component 3. 10µl 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 100µl BIOMOL GREEN Reagent to each reaction including control tubes.
- Step 8.** Add 100µl 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 $y = A*x + B$ 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)

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

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SAFETY DATA SHEET

Article 1 - Product Identification

Product Name: PP1A, Active

Catalog # P13-20GH

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	≤30%
NaCl	Sodium chloride	7647-14-5	≤1.753%
MOPS	3-(N-morpholino) propanesulfonic acid	1132-61-2	0.418%
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%
PMSF; Phenylmethanesulfonyl fluoride	α-Toluenesulphonyl fluoride	329-98-6	0.002%

Article 4 - First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breath in fresh air. If cannot breath, 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 ³ for mist	British Columbia, Canada
TWA	3 mg/m ³ for respirable mist	British Columbia, Canada
TWA	10 mg/m ³	Alberta, Canada
TWAEV	10 mg/m ³	Ontario, Canada
TWAEV	10 mg/m ³	Quebec, Canada
TWA	10 mg/m ³	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

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

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SAFETY DATA SHEET

Article 11 - Toxicological Information

- **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.

Article 12 - Ecological Information

- **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.

Article 13 - Disposal Considerations

- **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.

Article 14 - Transport Information

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

Article 15 - Regulatory Information

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

Article 16 - Other Information

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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