

WIP1, Active

Recombinant full-length protein expressed in Sf9 cells

Catalog # P04-20G

Lot # X3480-2

Product Description

Recombinant full-length human WIP1 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [BC016480](#).

Gene Aliases

PPM1D; JDVS; IDDGIP; PP2C-DELTA

Formulation

Recombinant protein stored in 20mM MOPS, pH 7.5, 300mM NaCl, 10mM glutathione, 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.

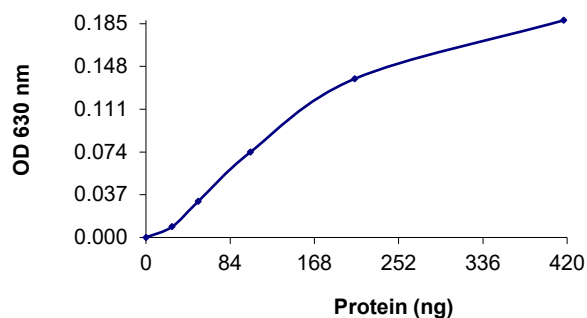
Scientific Background

Wild-type p53-induced phosphatase 1 (WIP1) is a member of the PP2C family of Ser/Thr protein, and is highly expressed in testis and ovary (1). The expression of WIP1 is induced in responses to the environmental stresses (2). WIP1 dephosphorylates CHK2 at Thr-68 in as a negative regulator in the response of DNA damage (2). WIP1 suppresses the DNA double strand break repair by dephosphorylating the phosphorylated Ser-139 in a histone variant gamma-H2AX. (3)

References

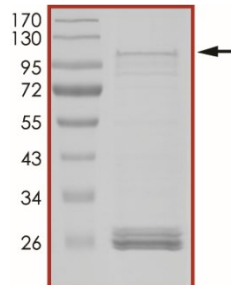
1. Fiscella M. et. al: Wip1, a novel human protein phosphatase that is induced in response to ionizing radiation in a p53-dependent manner. PNAS. 1997 Jun 10; 94(12):6048-53.
2. Yoda A. et. al: Intrinsic kinase activity and SQ/TQ domain of Chk2 kinase as well as N-terminal domain of Wip1 phosphatase are required for regulation of Chk2 by Wip1. J Biol Chem. 2006 Aug 25; 281(34):24847-62.
3. Moon S. H. et. al: Wild-type p53-induced phosphatase 1 dephosphorylates histone variant gamma-H2AX and suppresses DNA double strand break repair. J Biol Chem. 2010 Apr 23; 285(17):12935-47.

Specific Activity



The specific activity of WIP1 was determined to be **151 nmol /min/mg** as per activity assay protocol.

Purity



The purity of WIP1 was determined to be **>70%** by densitometry, approx. MW **110kDa**.

WIP1, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog #	P04-20G
Specific Activity	151 nmol/min/mg
Lot #	X3480-2
Purity	>70%
Concentration	0.05 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 #: P04-20G)

Active WIP1 (0.05µg/µl) diluted with Phosphatase Dilution Buffer III (Catalog #: P23-09) 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 WIP1 for optimal results).

Phosphatase Dilution Buffer III (1x)

(Catalog #: P23-09)

Phosphatase Assay Buffer III (Catalog #: P03-09) diluted at a 1:4 ratio (5X dilution) with cold water. Add to final concentration of 0.4mM DTT and 65ng/µl BSA prior to use

Phosphatase Assay Buffer III (5X)

(Catalog #: P03-09)

Buffer components: 100mM Tris-HCl pH 7.5, 50mM MgCl₂ and 0.1% Tween-20. Add to final concentration of 2mM DTT and 300ng/µl BSA prior to use.

Substrate (Catalog #: T72-58)

Thr-phosphopeptide synthetic substrate-3 (RRAT(p)VA) diluted in 20mM Tris-HCl, pH 7.5 solution 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 III and keep on ice.
- Step 2.** Prepare a 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 WIP1 on ice. Prepare serial dilutions of WIP1 using Phosphatase Dilution Buffer III.
- Step 4.** In a pre-cooled microfuge tube, add the following reaction components to a total volume of 25µl:

Component 1. 5µl Phosphatase Dilution Buffer III (Catalog #P23-09)

Component 2. 5µl of 150 mM MgCl₂ solution

Component 3. 5µl of Substrate solution

Component 4. 10µl of diluted Active WIP1 (Catalog #P04-20G)

- 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 III (Catalog # P23-09).
- 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 the control.
- 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: WIP1, Active

Catalog # P04-20G

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	≤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:** 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 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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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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