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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
P16-20BH-05	5 µg
P16-20BH-10	10 µg

PP2Aα/PPP2R1A Complex, Active

Full-length human recombinant protein expressed in Sf9 cells

Catalog # P16-20BH

Lot # C1888-4

Product Description

Recombinant full length human PP2Aα and human PPP2R1A were expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The PP2Aα gene accession number is NM_002715; PPP2R1A is NM_014225.

Gene Aliases

PP2Aα: RP-C; PP2Ac; PP2CA; PPP2CA
PPP2R1A: MRD36; PP2A-Aalpha; PP2AAALPHA; PR65A

Formulation

Recombinant protein stored in 50mM MOPS, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, 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.

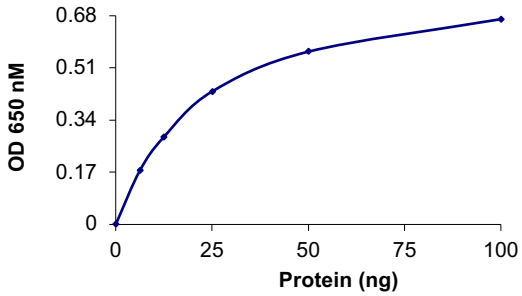
Scientific Background

PP2Aα is also known as Protein phosphatase 2A active subunit. PP2A is one of the four major Ser/Thr phosphatases and is a human tumor suppressor that inhibits cellular transformation. PP2A alpha gene encodes a specific phosphotyrosyl phosphatase activator of the dimeric form of protein phosphatase-2A (1). Protein phosphatase 2A holoenzyme is a heterotrimeric proteins composed of a structural subunit A, a catalytic subunit C, and a regulatory subunit B. PTP2A alpha can alter the substrate specificity of PP2A leading to enhanced phosphotyrosine phosphatase activity and decreased phosphoserine phosphatase activity (2).

References

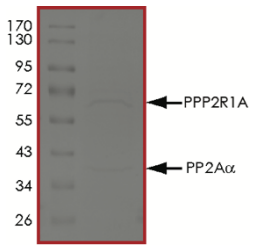
1. Van Hoof, C. et.al: Structure and chromosomal localization of the human gene of the phosphotyrosyl phosphatase activator (PTPA) of protein phosphatase 2A. Genomics 28: 261-272, 1995.
2. Chao, Y. et.al: Structure and mechanism of the phosphotyrosyl phosphatase activator. Molec. Cell 23: 535-546, 2006.

Specific Activity



The specific activity of PP2Aα/PPP2R1A protein Complex was determined to be **~4,000 nmol phosphate released /min/mg** as per activity assay protocol.

Purity



The purity of PP2Aα/PPP2R1A complex was determined to be **>75%** by densitometry. The PP2Aα was approx. MW **38kDa** and PPP2R1A was approx. MW **65kDa.**

PP2Aα/PPP2R1A Complex, Active

Full-length human recombinant protein expressed in Sf9 cells

Catalog #	P16-20BH
Specific Activity	4,000 nmol/min/mg
Lot #	C1888-4
Purity	>75%
Concentration	0.1µg/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 #: P16-20BH)

Active PP2Aα/PPP2R1A Complex (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 PP2Aα/PPP2R1A Complex 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-mercaptolethanol 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 H_2O 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 PP2Aα/PPP2R1A complex on ice. Prepare serial dilutions of PP2Aα/PPP2R1A complex 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 PP2Aα/PPP2R1A complex (Catalog #P16-20BH)

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 = Corresponding phosphate released *1000/ [(Reaction time in min)*(Enzyme amount in μg)]

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MATERIAL SAFETY DATA SHEET

Article 1 - Product Identification and Use

Product Name: PP2Aα/PPP2R1A Complex, Active **Catalog # P16-20BH**

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 Pharmaceuticals 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 - Hazardous Ingredients

NOT AVAILABLE. We are not aware of any hazards associated with this product or its ingredients, but the chemical, physical, and toxicological properties of this product have not been investigated thoroughly. Observe normal laboratory precautions.

Article 3 - Physical Data

This product consists of purified protein in MOPS buffer shipped on dry ice. The physical properties of this product have not been investigated thoroughly.

Article 4 - Fire and Explosion Hazard

NOT APPLICABLE

Article 5 - Reactivity Data

NOT APPLICABLE

Article 6 – Toxicologically Data

May be harmful by inhalation, ingestion, or skin absorption. The toxicological properties of this product have not been investigated thoroughly. Exercise due caution.

Article 7 - Preventative Measures

Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.

*****MULTIPLE COMPONENT SPILL OR LEAK PROCEDURES*****

- Wear protective equipment.
- Absorb on sand or vermiculite and place in closed containers for disposal.
- Observe all federal, state and local environmental regulations.

Article 8 - First Aid Measures

- If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
- In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. If a rash or other irritation develops, call a physician.
- If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.
- In case of eye contact, flush with copious amounts of water for at least 15 minutes while separating the eyelids with fingers. Call a physician.

Article 9 – Preparation

Prepared by: Jun Yan Phone#: 1-866-954-6273

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 Catalogue for additional terms and conditions of sale.