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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
P16-10BG -05	5 µg
P16-10BG -10	10 µg

PIP5K1B, Active

Recombinant full-length protein expressed in Sf9 cells

Catalog # P16-102BG

Lot # Q2477-10

Product Description

Recombinant full-length human PIP5K1B was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_003558.

Gene Aliases

MSS4; STM7

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.

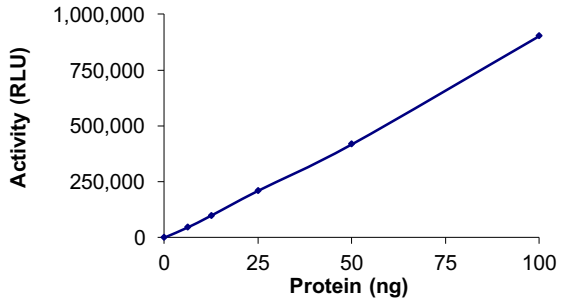
Scientific Background

PIP5K1B (phosphatidylinositol-4-phosphate 5-kinase, type I, beta) is a member of the phosphatidylinositol-4-phosphate 5-kinase family. PIP5K1B gene contains 17 exons and spans more than 300 kb. The seventeenth exon was found by RT-PCR, and are derived from the 3-prime untranslated region of the PRKACG gene which is located on 9q13 approximately 3 kb downstream of the STM7.I 3-prime untranslated region (1). The overexpression of PIP5K1B in COS-7 cells induces an increase in short actin fibers and a decrease in actin stress fibers(2).

References

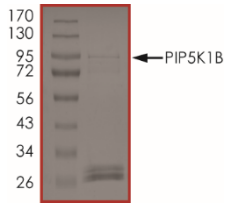
1. Pook, M. A.et.al: Exon-intron structure of a 2.7-kb transcript of the STM7 gene with phosphatidylinositol-4-phosphate 5-kinase activity. Genomics 42: 170-172, 1997.
2. Ishihara, H.et.al: Type I phosphatidylinositol-4-phosphate 5-kinases: cloning of the third isoform and deletion/substitution analysis of members of this novel lipid kinase family. J. Biol. Chem. 273: 8741-8748, 1998.

Specific Activity



The specific activity of PIP5K1B was determined to be **3,400 nmol /min/mg** as per activity assay protocol.

Purity



The purity of PIP5K1B was determined to be **>70%** by densitometry, approx. MW **96 kDa**.

PIP5K1B, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog #	P16-102BG
Specific Activity	3,400 nmol/min/mg
Lot #	Q2477-10
Purity	>70%
Concentration	0.05 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 Kinase (Catalog #: P16-102BG)

Active PIP5K1B (0.05μg/μl) diluted with Kinase Dilution Buffer IX (Catalog #: K29-09) 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 PIP5K1B for optimal results).

Kinase Dilution Buffer IX (Catalog #: K29-09)

Kinase Assay Buffer III (Catalog #: K03-09) diluted at a 1:4 ratio (5X dilution) with cold water. Add freshly 50μM DTT to the aliquot prior to use.

Kinase Assay Buffer III (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM MgCl2 and 0.5 mg/ml BSA.

ADP-Glo™ Kinase Assay Kit (Promega, Cat # V9101)

ADP solution, 10 mM
ADP-Glo™ Reagent
Kinase Detection Reagent

250 μM ATP Assay Solution

Prepare ATP assay solution by dissolving 0.55mg of ATP in 4ml of Kinase Assay Buffer III (Catalog #: K03-09). Store 200μl aliquots at –20°C.

Substrate (Catalog #: P427-59)

PI(4)P:PS substrate solution contains 250μM of PI(4)P and 1000μM of PS in Lipid Dilution Buffer (Catalog #: L21-09).

Assay Protocol

The PIP5K1B assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the PIP5K1B reaction. The ADP- Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

Step 1. Thaw the Active PIP5K1B, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

Step 2. In a pre-cooled 96-well opaque plate, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active PIP5K1B (Catalog #P16-102BG)
- **Component 2.** 5μl of PI(4)P:PS substrate solution (sonicate for 1 minute prior to use)
- **Component 3.** 5μl of Kinase Dilution Buffer IX with final 0.02% Triton X-100

Step 3. Set up the blank control as outlined in step 2, excluding the addition of the substrate. Replace the substrate with an equal volume of Kinase Dilution Buffer IX.

Step 4. Initiate the reaction by the addition of 5μl of 250 μM ATP Assay Solution thereby bringing the final volume up to 25μl. Sonicate the reaction mixture in the 96-well opaque plate for 10 seconds and continue the incubation at 30°C for 40 minutes.

Step 5. After the 40 minute incubation period, terminate the reaction and deplete the remaining ATP by adding 25μl of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minute at ambient temperature.

Step 6. Then add 50μl of the Kinase Detection Reagent to the 96-well plate and incubate the reaction mixture for another 30 minute at ambient temperature.

Step 7. Read the 96-well reaction plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031).

Step 8. Determine the corrected activity (RLU) by removing the blank control value (see Step 3) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected RLU from reaction / [(SA of ADP in RLU/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)

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