**PIPK1B, Active**

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_0033558.

**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**

PIPK1B (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 STM7.1 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**

Activity Assay Protocol

Reaction Components

**Active Kinase** (Catalog #: P16-10BG)

Active PIP5K1B (0.05μg/μl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) 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 III** (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/μl BSA solution.

**Kinase Assay Buffer I** (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl2, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**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 I (Catalog #: K01-09). Store 200μl aliquots at −20°C.

**Substrate**

Phosphatidylinositol (3,4) bis-phosphate [PI(3,4)P2] diluted in Kinase Assay Buffer I to a final concentration of 125 μM.

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. 5μl of diluted Active PIP5K1B (Catalog #P16-10BG)
- Component 2. 10μl of 125 μM stock solution of substrate (sonicate PI(3,4)P2 for 1 minute prior to use)
- Component 3. 5μl of Kinase Dilution Buffer III with 0.1% Triton-X100

**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 III.

**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 15 minutes.

**Step 5.** After the 15 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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