Product Information

PRECISIO® Kinase, Recombinant

PIP5K3 (PIKFYVE), Active

SRP0730

Product Description

Recombinant human PIP5K3 (1400-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_015040.

Gene Aliases

PIKFYVE; CFD; FAB1; PIP5K; FLJ37746; KIAA0981; MGC40423

Formulation

Recombinant protein stored in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM Glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, and 25% Glycerol.

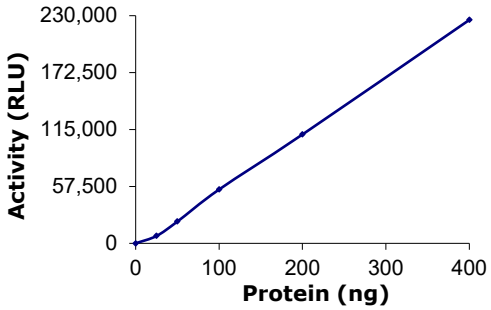
Storage and Stability

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. Product shipped on dry ice.

Scientific Background

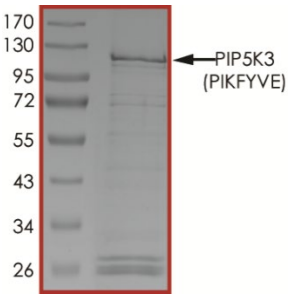
PIP5K3 (also known as phosphatidylinositol-3-phosphate 5-kinase type III or PIPKIII) encodes an enzyme that phosphorylates the D-5 position in PtdIns and phospha-tidylinositol-3-phosphate (PtdIns3P) to make PtdIns5P and PtdIns (3,5)biphosphate. PIP5K3 belongs to a large family of lipid kinases that alter the phosphorylation status of intracellular phosphatidylinositol but also have protein kinase activity.[1] PIP5K3 regulate cytoskeletal functions, membrane trafficking, and receptor signaling by recruiting protein complexes to cell- and endosomal-membranes. The inhibition of PIP5K3 renders the late endosome/lysosome compartment refractory to fusion with autophagosomes and multivesicular bodies.[2]

Specific Activity



The specific activity of PIP5K3 was determined to be 114 nmol/min/mg as per activity assay protocol.

Purity



The purity of PIP5K3 was determined to be > 85% by densitometry, approx. MW 110 kDa.

ADP-Glo9™ Activity Assay Protocol

Reaction Components

- **Active Kinase (P17-112BG)**
  Active PIP5K3 (0.05 µg/µL) diluted with 1x Lipid Kinase Buffer (L01-09) 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 PIP5K3 for optimal results.
- **Lipid Kinase Buffer (5x) (L01-09)**
  250 mM HEPES pH 7.5, 250 mM NaCl, 10 mM $MgCl_2$, 2.5 mM EGTA, 125 µg/mL BSA and 0.2% Triton® X-100.
  Dilute to 1x and add 0.05 mM fresh DTT prior to use.
- **Lipid Dilution Buffer (1x) (L21-09)**
  25 mM HEPES pH 7.5 and 0.5 mM EGTA
- **ADP-GloTM Kinase Assay Kit**
  (Promega, V9101)
    - ATP solution, 10 mM
    - ADP solution, 10 mM
    - ADP-Glo™ Reagent
    - Kinase Detection Reagent
    - 1 M $MgCl_2$ Solution – Required for ADP-Glo™ reaction
- **250 µM ATP Assay Solution**
  Prepare the ATP assay solution by diluting ATP solution, 10 mM (Promega, V9101) to 250 µM in 1x Lipid Assay Buffer (prepared from Lipid Kinase Buffer 5x; L01-09).
- **Substrate (P426-59)**
  PI(3)P:PS substrate solution contains 250 µM of PI and 2000 µM of PS in Lipid Dilution Buffer (L21-09).

Assay Protocol

The PIP5K3 assay is performed using the ADP-Glo™ Kinase Assay Kit (Promega, V9101) which quantifies the amount of ADP produced by the PIP5K3 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.

1. Thaw the Active PIP5K3, Lipid Kinase Buffer, Substrate and Lipid Dilution Buffer on ice.
2. In a pre-cooled 96-well opaque plate, add the following reaction components bringing the initial reaction volume up to 20 µL:
    - Component 1. 10 µL of diluted Active PIP5K3 (P17-112BG)
    - Component 2. 5 µL of PI(3)P:PS substrate (sonicate for 1 minute prior to use)
    - Component 3. 5 µL of 1x Lipid Kinase Buffer
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 Lipid Dilution Buffer.
4. Initiate the reaction by the addition of 5 µL of 250 µM ATP Assay Solution thereby bringing the final volume up to 25 µL. Shake the reaction mixture in the 96-well opaque plate for 2 minutes and continue the incubation at 30 °C for 40 minutes.
5. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 25 µL of ADP-Glo™ Reagent with 10 mM $MgCl_2$. Shake the 96-well plate and then incubate the reaction mixture for another 40 minutes at ambient temperature.
6. Then add 50 µL of the Kinase Detection Reagent to the 96-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.
7. Read the 96-well reaction plate using the KinaseGlo® Luminescence Protocol on a GloMax® Plate Reader (Promega, E7031).
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)]

References

1. Shisheva, A..et.al: Cloning, characterization, and expression of a novel Zn(2+)-binding FYVE finger-containing phosphor-inositide kinase in insulin-sensitive cells. Molec. Cell. Biol. 19: 623-634, 1999.
2. de Lartigue, J.et.al: PIKfyve regulation of endosome-linked pathways. Traffic 10: 883-893, 2009.

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