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Specialists in Signaling Proteins
1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
P27-10CG-05	5 µg
P27-10CG-10	10 µg

PI3K (p110α/p55γ), Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P27-10CG

Lot # P1688-6

Product Description

Recombinant full-length human PI3K (p110α/p55γ) was co-expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The p110α gene accession numbers is NM_006218; p55γ is BC021622.

Gene Aliases

p110a: PI3K, p110-alpha
p55g: PIK3R3, p55, p55-GAMMA, FLJ41892

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM 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.

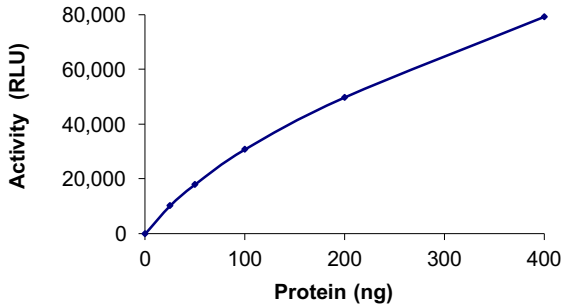
Scientific Background

PI3K (p110alpha/p55gamma) or Phosphatidylinositol 3-kinase is a lipid kinase that phosphorylates the inositol ring of phosphatidylinositol at the 3-prime position which serve as second messengers in growth signaling pathways. PI3K comprises a 110 kD catalytic subunit and a regulatory subunit of either 85, 55, or 50 kD. Phosphatidylinositol 3-kinase plays an important role in the metabolic actions of insulin, and a mutation in the PI3K has been associated with insulin resistance and plays an important role in glucose homeostasis in vivo (1). PI3K also plays an essential role in the development and induction of mast cells in normal and pathogenic immune responses (2).

References

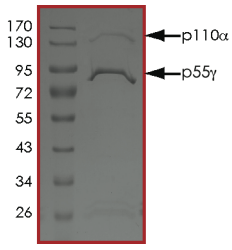
1. Terauchi, Y. et.al: Increased insulin sensitivity and hypoglycaemia in mice lacking the p85-alpha subunit of phosphoinositide 3-kinase. Nature Genet. 21: 230-235, 1999.
2. Fukao, T. et.al: Selective loss of gastrointestinal mast cells and impaired immunity in PI3K-deficient mice. Nature Immun. 3: 295-304, 2002.

Specific Activity



The specific activity of PI3K (p110α/p55γ) was determined to be **8 nmol /min/mg** as per activity assay protocol.

Purity



The purity of PI3K(p110α/p55γ) was determined to be **>70%** by densitometry, p110α was approx. MW **140 kDa** and p55γ was approx. MW **82 kDa**

PI3K (p110α/p55γ), Active

Full-length recombinant protein expressed in Sf9 cells

Catalog #	P27-10CG
Specific Activity	8 nmol/min/mg
Lot #	P1688-6
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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ADP-Glo™ Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: P27-10CG)

Active PI3K (p110α/p55γ) (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 PI3K (p110α/p55γ) 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 final 50ng/μl BSA solution.

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

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 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 4,5-bisphophate [PI(4,5)P2]) diluted in Kinase Assay Buffer to a final concentration of 125 μM.

Assay Protocol

The PI3K assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the PI3K 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 PI3K (p110α/p55γ), 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 PI3K (p110α/p55γ)(Catalog #P27-10CG)
- **Component 2.** 5μl of 125 μM stock solution of substrate (sonicate PI(4,5)P2 for 1 minute prior to use)
- **Component 3.** 5μl Kinase Dilution Buffer III

Step 3. Set up the blank control as outlined in step 3, 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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