

Product Information

**PI4K2A, active, GST tagged, human
PRECISIO® Kinase
recombinant, expressed in Sf9 cells**

Catalog Number **SRP5063**
Storage Temperature -70°C

Synonyms: DKFZp761G1923, PI4KII, PIK42A,
RP11-548K23.6

Product Description

PI4K2A or phosphatidylinositol 4-kinase type 2 alpha is involved in the formation of phosphatidylinositolpolyphosphates (PtdInsPs), which are centrally involved in many biologic processes ranging from cell growth and organization of the actin cytoskeleton to endo- and exocytosis. PI4K2A phosphorylates PtdIns at the D-4 position, which is an essential step in the biosynthesis of PtdInsPs. PI4K2A is a novel regulator of tumor growth by its action on angiogenesis and HIF-1alpha regulation. PI4K2A generates PtdIns4P-rich domains within the Golgi, which regulate targeting of clathrin adaptor AP-1 complexes.¹ PI4K2A is also required for Wnt to regulate LRP6 phosphorylation.²

Recombinant full-length human PI4K2A was co-expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The PI4K2A gene accession number is NM_018425. 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.

Molecular mass: ~79 kDa

Purity: 70–95% (SDS-PAGE, see Figure 1)

Specific Activity: 81–109 nmole/min/mg (see Figure 2)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at -70°C is recommended. After opening, aliquot into smaller quantities and store at -70°C . Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot
70–95% (densitometry)

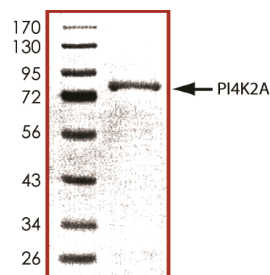
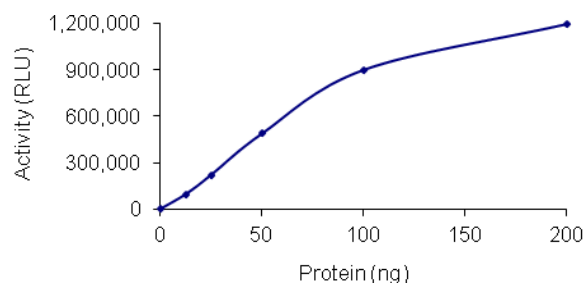


Figure 2.
Specific Activity of Typical Lot
81–109 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl_2 , 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/ μl BSA.

Kinase Solution – Dilute the active PI4K2A (0.1 µg/µl) with Kinase Dilution Buffer to the desired concentration.

Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active PI4K2A kinase for optimal results.

250 µM ATP Stock Solution – Dissolve 55 mg of ATP in 4 ml of Kinase Assay Buffer. Store in 200 µl aliquots at –20 °C.

ADP-Glo™ Kinase Assay Kit (Promega, Cat. No. V9101) – ADP solution, 10 mM, ADP-Glo Reagent, and Kinase Detection Reagent.

Substrate Solution – Phosphatidylinositol (PI) diluted in Kinase Assay Buffer I in the presence of phosphatidylserine (PS) to a final concentration of 125 µM.

Kinase Assay

The PI4K2A assay is performed using the ADP-Glo Kinase Assay kit (Promega, Cat. No. V9101), which quantifies the amount of ADP produced by the PI4K2A 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 PI4K2A, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice.
2. In a pre-cooled 96-well opaque plate, add the following solutions to a volume of 20 µl:
 - 10 µl of diluted active PI4K2A
 - 5 µl of 125 µM stock solution of substrate (sonicate PI/PS for 1 minute prior to use)
 - 5 µl of Kinase Dilution Buffer with 0.1% Triton® X-100
3. Set up a blank control as outlined in step 2, excluding the addition of the substrate. Replace the substrate with an equal volume of Kinase Dilution Buffer.
4. Initiate each reaction with the addition of 5 µl of 250 µM ATP Stock Solution, bringing the final reaction volume to 25 µl. Sonicate the reaction mixture in the 96-well opaque plate for 10 seconds and continue the incubation at 30 °C for 15 minutes.

5. After the 15 minute incubation, stop the reaction by adding 25 µl of the ADP-Glo Reagent. 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 Kinase-Glo® Luminescence Protocol on a GloMax® plate reader (Promega, Cat No. E7031).
8. Determine the corrected activity (RLU) by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity.

Calculations:

1. Specific Activity of ADP (RLU/nmole)

From ADP standard curve, determine RLU/nmole of ADP

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{RLU}}{\text{SR} \times \text{E} \times \text{T}}$$

ΔRLU = RLU of the sample – RLU of the blank (step 3)

T = reaction time (minutes)

E = amount of enzyme (mg)

References

1. Wang, et al., Phosphatidylinositol 4 phosphate regulates targeting of clathrin adaptor AP-1 complexes to the Golgi. *Cell*, **114**, 299-310 (2003).
2. Pan, et al., Wnt3a-mediated formation of phosphatidylinositol 4,5-bisphosphate regulates LRP6 phosphorylation. *Science*, **321**, 1350-1353 (2008).

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