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Product Information

PI4KB, active, GST tagged, human
PRECISIO® Kinase
recombinant, expressed in *Sf*9 cells

Catalog Number **SRP5065**
Storage Temperature –70 °C

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

Product Description

PI4KB is a member of the phosphatidylinositol 4-kinase family (PI4K) and is the first committed step in the production of the second messenger inositol-1,4,5-trisphosphate (PIP_3). PI4KB regulates the golgi disintegration/reorganization during mitosis possibly via the phosphorylation of phosphatidylinositol.[1] PI4KB is a type III enzyme that is sensitive to wortmannin and is responsible for regulating the synthesis of agonist-sensitive pools of polyphosphoinositides. PI4KB is critical for glucose-induced insulin secretion due to its capacity to regulate the release of secretory granules.[2] PI4KB inhibits the insulin-stimulated translocation of glucose transporter-4 in mouse adipocytes through its interaction with neuronal calcium sensor-1.

Full-length recombinant human PI4KB was co-expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST tag. The PI4KB gene accession number is BC000029. 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: ~120 kDa

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

Specific Activity: 27–37 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)

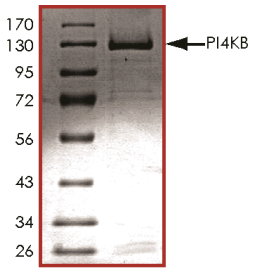
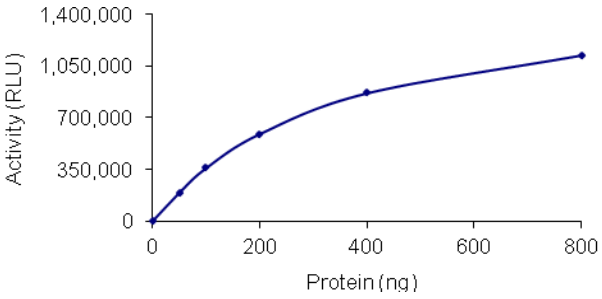


Figure 2.
Specific Activity of Typical Lot
27–37 nmole/min/mg



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 PI4KB (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 PI4KB 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 phosphatidyl-serine (PS) to a final concentration of 125 μM.

Kinase Assay

The PI4KB assay is performed using the ADP-Glo™ Kinase Assay kit (Promega, Cat. No. V9101), which quantifies the amount of ADP produced by the PI4KB 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 PI4KB, 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 PI4KB
   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. Preplace 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 temerpature
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)

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

ΔRLU = RLU of the sample – RLU of the blank (step 3)
T = reaction time (minutes)
E = amount of enzyme (mg)

References

1. Balla, A. et al., Characterization of type II phosphatidylinositol 4-kinase isoforms reveals association of the enzymes with endosomal vesicular compartments. J. Biol. Chem., **277**, 20041-20050 (2002).
2. Griffioen, M. et al., Identification of phosphatidyl-inositol 4-kinase type II beta as HLA class II-restricted target in graft versus leukemia reactivity. Proc. Nat. Acad. Sci., **105**, 3837-3842 (2008).

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