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Product Information

PI3K (p110β/p85α), Active, His-tagged, human
Precisio™ Kinase
recombinant, expressed in *Sf*9 cells

Catalog Number **I1036**
Lot Number 109K0802
Storage Temperature –70 °C

Synonyms:
p110β: PIK3CB, PIK3C1, PI3Kbeta, MGC133043, DKFZp779K1237
p85α: PIK3R1, GRB1

Product Description
PI3K contains a 110 kDa catalytic subunit and a 85 kDa regulatory subunit. A number of isoforms of the 110 kDa catalytic subunit and the 85 kDa regulatory subunit exist in cells. The p110β catalytic subunit (PIK3CB) plays a role in regulating the formation and stability of α2B-β3 integrin adhesion bonds, which are necessary for shear force-induced platelet activation.[1] In animal models of prostate tumor formation induced by the tumor suppressor PTEN loss, ablation of p110β impedes tumorigenesis with a concomitant diminution of AKT phosphorylation.[2]

This recombinant product was expressed by baculovirus in *Sf*9 insect cells using an N-terminal His-tag. The gene accession numbers are NM 006219 for p110β and NM 181523 for p85α. It is supplied in 50 mM sodium phosphate, pH 7.0, with 300 mM NaCl, 0.2 mM DTT, 0.1 mM PMSF, and 25% glycerol.

Molecular mass:
p110β ~111 kDa
p85α ~86 kDa

Purity: ≥70% (SDS-PAGE, see Figure 1)

Specific Activity: 13–19 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 Lot Number 109K0802
>80% (densitometry)

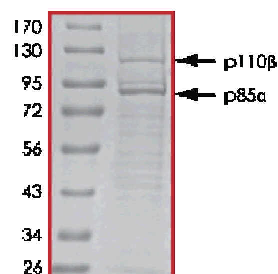
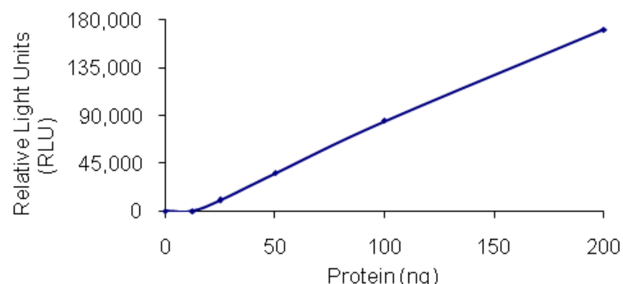


Figure 2.
Specific Activity of Lot Number 109K0802:
16.3 nmole/min/mg



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 solution.

Kinase Solution – Dilute the Active PI3K (0.1 µg/µl) with Kinase Dilution Buffer to the desired concentration. Note: The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended that the researcher perform a serial dilution of Active PI3K kinase for optimal results.

ATP Stock Solution – Dissolve 0.55 mg of ATP in 4 ml of Kinase Assay Buffer. Store in 200 µl aliquots at –20 °C.

Substrate Solution – Dilute phosphatidylinositol 4,5-diphosphate ($PI(4,5)P_2$) in Kinase Assay Buffer at a final concentration of 125 µM.

Kinase Assay

This assay is performed using the ADP-Glo Kinase Assay kit (Promega), 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. The Kinase Detection Reagent is then added to convert ADP to ATP and to measure the newly synthesized ATP using the luciferase/luciferin reaction.

1. Thaw the Active PI3K, 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 Kinase Solution
   - 5 µl of Substrate Solution
   - 5 µl of Kinase Assay Buffer
3. Set up a blank control as outlined in step 2, substituting 5 µl of Kinase Assay Buffer for the Substrate Solution.
4. Initiate each reaction with the addition of 5 µl of the 250 µM ATP solution, bringing the final reaction volume to 25 µl. Incubate the reaction mixture in the plate 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 plate and incubate for 40 minutes at ambient temperature.
6. Add 50 µl of the Kinase Detection Reagent to the plate and incubate the reaction mixture for 30 minutes at ambient temperature.
7. Read the 96-well reaction plate using the KinaseGlo Luminescence Protocol.
8. Determine the corrected activity by removing the blank control value for each sample and calculate the kinase specific activity as outlined below.

Calculations:

1. Specific activity of ADP (RLU/pmol)

$$SR = \frac{\text{RLU of ADP}}{\text{pmol of ADP}}$$

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

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

RLU = Relative Light Units as determined by a luminometer

SR = specific activity of the ADP (RLU/pmol) from the ADP standard curve

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

T = reaction time (minutes)

E = amount of enzyme (mg)

References

1. Jackson, S.P. et al., PI 3-kinase p110-beta: a new target for antithrombotic therapy. Nature Med., **11**, 507-514 (2005).
2. Jia, S. et al., Essential roles of PI(3)K-p110-beta in cell growth, metabolism and tumorigenesis. Nature, **454**, 776-779 (2008).

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