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Data Sheet

PI3Kβ (p110β/p85α) Assay Kit

Catalog #79802
Size : 96 reactions

Background: PI3Ks (Phosphatidylinositol 3-kinases) are lipid kinases that phosphorylate PIP2 (phosphatidylinositol 4,5-bisphosphate) to produce PIP3 (phosphatidylinositol 3,4,5-trisphosphate), which plays important roles in fundamental cellular activities such as cell growth, survival, migration, and metabolism. In human cancers, gain-of-function mutations of PI3Ks are found frequently, suggesting that PI3Ks are closely involved in tumorigenesis and that PI3K targeting inhibitors may be promising anticancer drug candidates.

Description: The *PI3Kβ (p110β/p85α) Assay Kit* is designed to measure PI3Kβ activity for screening and profiling applications, using ADP-Glo® Kinase Assay as a detection reagent. The *PI3Kβ (p110β/p85α) Assay Kit* comes in a convenient 96-well format, with enough purified recombinant PI3Kβ enzyme, PI3K lipid substrate, ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40622	PI3Kβ (p110β/p85α)	5 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer	1.5 ml	-20°C	
79686	ATP (500 µM)	100 µl	-20°C	
40560	PI3K lipid substrate (Packaged separately, Do Not Freeze!)	500 µl	+4°C	
79696	96-well plate, white	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
ADP-Glo® Kinase Assay (Promega #V6930)
Microplate reader capable of reading luminescence
Adjustable micropipettor and sterile tips
30°C incubator

APPLICATIONS: Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months when stored as recommended.

CONTRAINDICATION: Avoid >0.5% DMSO. *Higher DMSO levels can significantly decrease the enzyme activity.*

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REFERENCE:
Zhao W., *et al. Acta Pharmaceutica Sinica B,* **7(1):** 27-37 (2017)

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Thaw **5x Kinase assay buffer,** and **ATP.** The **PI3K lipid substrate** is shipped separately on ice. Please store it at 4ºC upon arrival (DO NOT FREEZE the PI3K lipid substrate).

2) Prepare **2.5x Kinase assay buffer** by diluting **5x Kinase assay buffer** in distilled water at 1:1 ratio. (*e.g.* ~ 1.5 mL of 2.5x Kinase assay buffer is enough for a 96-well plate. Mix 750 µl **5x Kinase assay buffer** and 750 µl distilled water.)

3) Prepare 12.5 µM ATP solution by diluting **ATP** (500 µM) in distilled water. (*e.g.* ~ 1 mL of 12.5 µM ATP is enough for a 96-well reaction. Mix 25 µl 500 µM ATP provided and 975 µl distilled water.)

4) Prepare 5X concentrated inhibitor in an aqueous-based solution. (*Note: Final DMSO concentration must be ≤0.5%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor at 10 µM final concentration, prepare the inhibitor at 1 mM in 100% DMSO. Then dilute 1 mM inhibitor in water to 25 µM, which contains 2.5% of DMSO. Next, add 5 µl of the 25 µM inhibitor solution (2.5% DMSO) to the assay to make a 0.5% DMSO concentration in the final 25 µl reaction mixture.)*

5) For the inhibitor buffer, prepare the same solution as above, but without the test inhibitor (e.g. *2.5% DMSO in water). The DMSO concentration should be the same as in the 5X inhibitor solution above.*

6) Thaw **PI3Kβ** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Calculate the amount of **PI3Kβ** required for the assay and dilute enzyme to ~ 4 ng/µl with 2.5x Kinase assay buffer prepared in step 2. Store remaining undiluted enzyme in aliquots at -80°C. *Note:* PI3Kβ *enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7) Add each reaction component as in the following table, in the order indicated for steps 8-12 below (i.e. add 5 µl **PI3K lipid substrate** first, 5 µl inhibitor or inhibitor buffer second, 5 µl 12.5 µM **ATP** third and 10 µl of diluted **PI3Kβ**). The volume of each component is very small so we recommend shaking the plate for 1 minute between the steps to be sure all components are thoroughly mixed.

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	Positive Control	Test Inhibitor	Blank
PI3K lipid substrate	5 µl	5 µl	5 µl
Test Inhibitor	–	5 µl	–
Inhibitor buffer (step 5)	5 µl	–	5 µl
Diluted ATP (12.5 µM)	5 µl	5 µl	5 µl
2.5x Kinase buffer	–	–	10 µl
PI3Kβ (~4 ng/µl)	10 µl	10 µl	–
Total	25 µl	25 µl	25 µl

8) Add 5 µl PI3K lipid substrate to all wells.

9) Add 5 µl of 5x Inhibitor solution of each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 5 µl of the same solution without inhibitor (Inhibitor buffer, *e.g.* 2.5% DMSO(aq)).

10) Add 5 µl diluted ATP to all wells.

11) To the wells designated as "Blank," add 10 µl of 2.5x Kinase assay buffer.

12) Initiate reaction by adding 10 µl of **diluted PI3Kβ enzyme** to the wells designated “Positive Control” and "Test Inhibitor." Carefully shake the plate well and incubate it at 30˚C for 40 minutes.

13) Thaw ADP-Glo reagent.

14) After the 40 minutes reaction, add 25 µl of ADP-Glo reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 45 minutes.

15) Thaw Kinase Detection reagent.

16) After the 45 minutes incubation, add 50 µl of Kinase Detection reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for another 30 minutes.

17) Measure luminescence using the microplate reader. “Blank” value should be subtracted from all wells.

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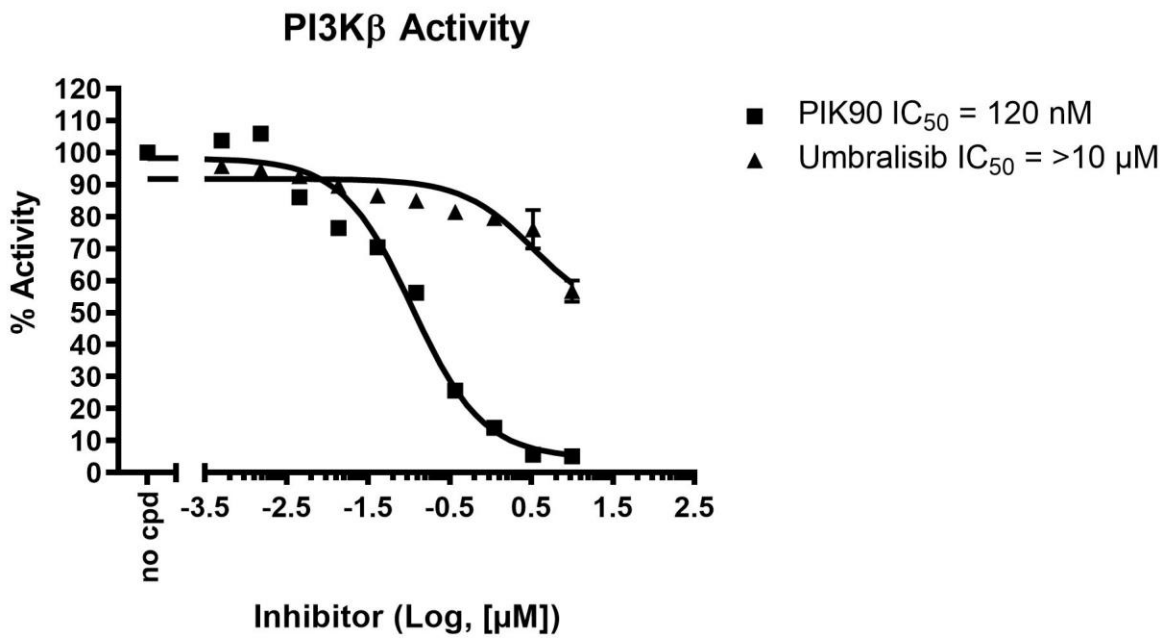
Example of Assay Results:



Inhibition of PI3Kβ, measured using the *PI3Kβ (p110β/ p85α) assay kit* (BPS Bioscience #79802). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	**Catalog #**	**Size**
PI-3 Kinase Lipid Substrate	40560	1 mg
ATP (500 μM)	79686	200 μl
PI3 kinase (p110β/p85α), FLAG-tag	40622	20 μg
PI3 kinase (p110α/p85α), FLAG-tag	40620	20 μg
PI3 kinase [p110α(E545K)/p85α], FLAG-tag	40640	20 μg
PI3 kinase [p110α(H1047R)/p85α], FLAG-tag	40641	20 μg
PI3 kinase (p110α/p85α), GST-tag	40621	20 μg
PI3 Kinase (p110α/p55γ), His-Tag	40645	10 μg
PI3 Kinase [p110α (N345K)/p85α], His-Tag	40646	10 μg
PI3 Kinase [p110α (E545K)/p85α], His-Tag	40644	10 μg
PI3 kinase (p110γ/PIK3R5), His, GST-tag	40626	20 μg
PI3 kinase (p110δ/p85α), GST-tag	40628	20 μg
PI3 kinase (p120γ), His-tag	40625	20 μg