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Data Sheet
PI3K γ (p110 γ /PIK3R5) Assay Kit
Catalog #79803
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 PI3K γ (p110 γ /PIK3R5) Assay Kit is designed to measure PI3K γ activity for screening and profiling applications, using ADP-Glo[®] Kinase Assay as a detection reagent. The PI3K γ (p110 γ /PIK3R5) Assay Kit comes in a convenient 96-well format, with enough purified recombinant PI3K γ enzyme, PI3K lipid substrate, ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40626	PI3K γ (p110 γ /PIK3R5)	5 μ g	-80°C	Avoid multiple freeze/thaw cycles!
79334	5x Kinase assay buffer	1.5 ml	-20°C	
79686	ATP (500 μ M)	100 μ l	-20°C	
40560	PI3K lipid substrate (Packaged separately, Do Not Freeze!)	500 μ l	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 4°C 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 **PI3Ky** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Calculate the amount of **PI3Ky** 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: PI3Ky 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 8-11 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 **PI3Ky**). 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 μ l	5 μ l	5 μ l
Test Inhibitor	-	5 μ l	-
Inhibitor buffer (step 5)	5 μ l	-	5 μ l
Diluted ATP (12.5 μ M)	5 μ l	5 μ l	5 μ l
2.5x Kinase buffer	-	-	10 μ l
PI3K γ (~4 ng/ μ l)	10 μ l	10 μ l	-
Total	25 μl	25 μl	25 μl

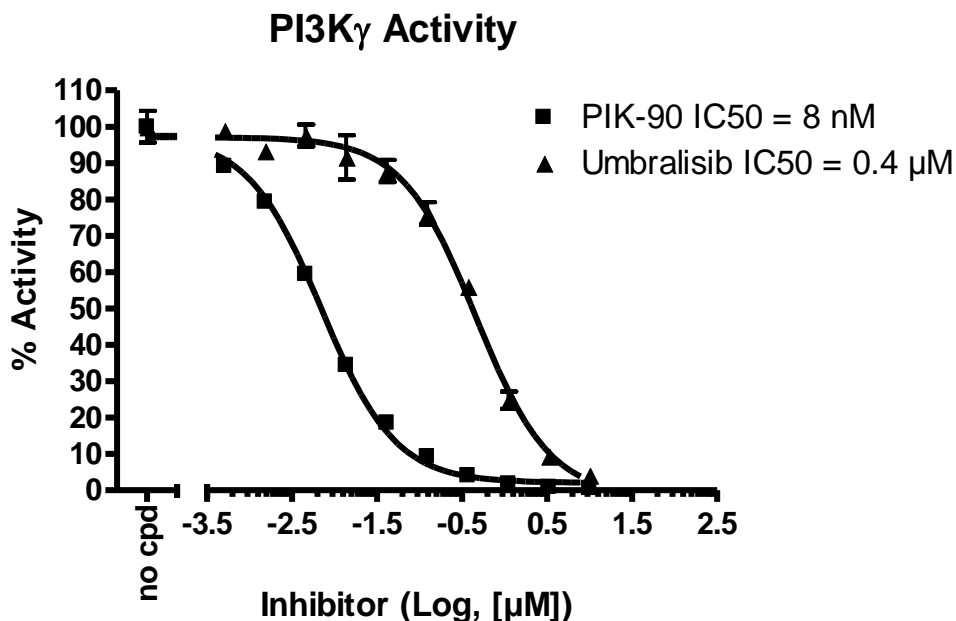
- 8) Add 5 μ l PI3K lipid substrate to all wells.
- 9) Add 5 μ l of 5x Inhibitor solution of each well labeled as "Test Inhibitor." For the "Positive Control" and "Blank," add 5 μ l of the same solution without inhibitor (Inhibitor buffer, e.g. 2.5% DMSO(aq)).
- 10) Add 5 μ l diluted ATP to all wells.
- 11) To the wells designated as "Blank," add 10 μ l of 2.5x Kinase assay buffer.
- 12) Initiate reaction by adding 10 μ l of **diluted PI3K γ 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 μ l 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 μ l 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 PI3K γ enzyme, measured using the *PI3K γ (p120 γ / PIK3R5) assay kit* (BPS Bioscience #79803). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
PI-3 Kinase Lipid Substrate	40560	1 mg
PI3 kinase (p110 γ /PIK3R5), His, GST-tag	40626	20 μg
ATP (500 μM)	79686	200 μl
PI3 kinase (p110 α /p85 α), FLAG-tag	40620	20 μg
PI3 kinase [p110 α (E545K)/p85 α], FLAG-tag	40640	20 μg
PI3 kinase [p110 α (H1047R)/p85 α], FLAG-tag	40641	20 μg
PI3 kinase (p110 α /p85 α), GST-tag	40621	20 μg
PI3 Kinase (p110 α /p55 γ), His-Tag	40645	10 μg
PI3 kinase (p110 β /p85 α), FLAG-tag	40622	20 μg
PI3 Kinase [p110 α (N345K)/p85 α], His-Tag	40646	10 μg
PI3 Kinase [p110 α (E545K)/p85 α], His-Tag	40644	10 μg
PI3 kinase (p110 δ /p85 α), GST-tag	40628	20 μg
PI3 kinase (p120 γ), His-tag	40625	20 μg

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