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**Data Sheet**  
***PI3K $\delta$*  (p110 $\delta$ /p85 $\alpha$ ) Assay Kit**  
Catalog #79799  
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 $\delta$*  (p110 $\delta$ /p85 $\alpha$ ) Assay Kit is designed to measure PI3K $\delta$  (PI3K-delta) activity for screening and profiling applications, using ADP-Glo<sup>®</sup> Kinase Assay as a detection reagent. The *PI3K $\delta$*  (p110 $\delta$ /p85 $\alpha$ ) Assay Kit comes in a convenient 96-well format, with enough purified recombinant PI3K $\delta$  enzyme, PI3K lipid substrate, ATP and kinase assay buffer for 100 enzyme reactions.

**COMPONENTS:**

Catalog #	Reagent	Amount	Storage	
40628	PI3K $\delta$ (p110 $\delta$ /p85 $\alpha$ )	15 $\mu$ g	-80°C	<b>Avoid multiple freeze/thaw cycles!</b>
79334	5x Kinase assay buffer	1.5 ml	-20°C	
79686	ATP (500 $\mu$ M)	100 $\mu$ l	-20°C	
40560	PI3K lipid substrate <b>(Packaged separately, Do Not Freeze!)</b>	500 $\mu$ l	4°C	
79696	96-well plate, white	1	Room Temp.	

**MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:**

ADP-Glo<sup>®</sup> 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  $\mu$ l **5x Kinase assay buffer** and 750  $\mu$ l distilled water.)
- 3) Prepare **12.5  $\mu$ M ATP** solution by diluting **ATP (500  $\mu$ M)** in distilled water. (e.g. ~ 1 mL of 12.5  $\mu$ M ATP is enough for a 96-well reaction. Mix 25  $\mu$ l 500  $\mu$ M ATP provided and 975  $\mu$ l distilled water.)
- 4) Prepare 5X concentrated inhibitor in an aqueous-based solution. (*Note: Final DMSO concentration must be  $\leq$ 0.5%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor at 10  $\mu$ M final concentration, prepare the inhibitor at 1 mM in 100% DMSO. Then dilute 1 mM inhibitor in water to 25  $\mu$ M, which contains 2.5% of DMSO. Next, add 5  $\mu$ l of the 25  $\mu$ M inhibitor solution (2.5% DMSO) to the assay to make a 0.5% DMSO concentration in the final 25  $\mu$ l 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 **PI3K $\delta$**  on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Calculate the amount of **PI3K $\delta$**  required for the assay and dilute enzyme to ~ 13 ng/ $\mu$ l with **2.5x Kinase assay buffer** prepared in step 2. Store remaining undiluted enzyme in aliquots at -80°C. *Note: PI3K $\delta$  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  $\mu$ l PI3K lipid substrate first, 5  $\mu$ l inhibitor or inhibitor buffer second, 5  $\mu$ l **12.5  $\mu$ M ATP** third and 10  $\mu$ l of **diluted PI3K $\delta$** ). 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 $\mu$ l	5 $\mu$ l	5 $\mu$ l
Test Inhibitor	-	5 $\mu$ l	-
Inhibitor buffer (step 5)	5 $\mu$ l	-	5 $\mu$ l
Diluted ATP (12.5 $\mu$ M)	5 $\mu$ l	5 $\mu$ l	5 $\mu$ l
2.5x Kinase buffer	-	-	10 $\mu$ l
PI3K $\delta$ (~13 ng/ $\mu$ l)	10 $\mu$ l	10 $\mu$ l	-
<b>Total</b>	<b>25 <math>\mu</math>l</b>	<b>25 <math>\mu</math>l</b>	<b>25 <math>\mu</math>l</b>

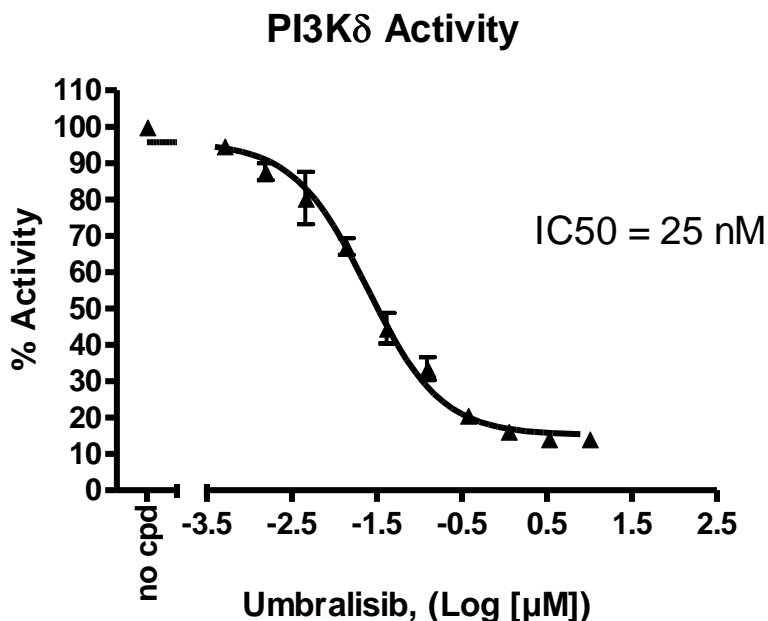
- 8) Add 5  $\mu$ l PI3K lipid substrate to all wells.
- 9) Add 5  $\mu$ l of 5x Inhibitor solution of each well labeled as "Test Inhibitor." For the "Positive Control" and "Blank," add 5  $\mu$ l of the same solution without inhibitor (Inhibitor buffer, e.g. 2.5% DMSO(aq)).
- 10) Add 5  $\mu$ l diluted ATP to all wells.
- 11) To the wells designated as "Blank," add 10  $\mu$ l of 2.5x Kinase assay buffer.
- 12) Initiate reaction by adding 10  $\mu$ l of **diluted PI3K $\delta$  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  $\mu$ 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  $\mu$ 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 $\delta$  enzyme by Umbralisib (TGR-1202), measured using the PI3K $\delta$  (p110 $\delta$ /p85 $\alpha$ ) assay kit (BPS Bioscience #79799). 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 $\delta$ /p85 $\alpha$ ), GST-tag	40628	20 $\mu$ g
ATP (500 $\mu$ M)	79686	200 $\mu$ l
PI3K $\alpha$ (p110 $\alpha$ /p85) Assay Kit	79781	96 rxns.
PI3 kinase (p110 $\alpha$ /p85 $\alpha$ ), FLAG-tag	40620	20 $\mu$ g
PI3 kinase [p110 $\alpha$ (E545K)/p85 $\alpha$ ], FLAG-tag	40640	20 $\mu$ g
PI3 kinase [p110 $\alpha$ (H1047R)/p85 $\alpha$ ], FLAG-tag	40641	20 $\mu$ g
PI3 kinase (p110 $\alpha$ /p85 $\alpha$ ), GST-tag	40621	20 $\mu$ g
PI3 Kinase (p110 $\alpha$ /p55 $\gamma$ ), His-Tag	40645	10 $\mu$ g
PI3 kinase (p110 $\beta$ /p85 $\alpha$ ), FLAG-tag	40622	20 $\mu$ g
PI3 Kinase [p110 $\alpha$ (N345K)/p85 $\alpha$ ], His-Tag	40646	10 $\mu$ g
PI3 Kinase [p110 $\alpha$ (E545K)/p85 $\alpha$ ], His-Tag	40644	10 $\mu$ g
PI3 kinase (p110 $\gamma$ /PIK3R5), His, GST-tag	40626	20 $\mu$ g
PI3 kinase (p120 $\gamma$ ), His-tag	40625	20 $\mu$ g

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