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siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
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- Expressversand

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Chemi-Verse™ PI3 Kinase P110α(H1047L)/P85α Kinase Assay Kit

#82120
96 reactions

Description

The Chemi-Verse™ PI3 Kinase P110α(H1047L)/P85α Kinase Assay Kit is designed to measure the kinase activity of the complex of PI3 (phosphoinositide 3) subunit p110α with an H1047L mutation and p85α for screening and profiling applications using ADP-Glo™ as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified recombinant PI3 Kinase p110α(H1047L)/p85α kinase, kinase substrate, ATP and kinase assay buffer for 100 enzyme reactions.

Background

PI3 (phosphoinositide 3) kinase, or phosphatidylinositol 3 kinases, are a family of proteins that can be subdivided into four classes: I, II, III and IV. Class I are involved in converting PI (4, 5) P2 (phosphatidylinositol (4, 5)-biphosphate) into PI (3, 4, 5) P3 (phosphatidylinositol (3, 4, 5)-triphosphate) when activated by tyrosine kinase receptors and G-protein coupled receptors. They are heterodimeric proteins with a regulatory and a catalytic subunit. The heterodimer between p110 (catalytic subunit) and p85 (regulatory subunit) belongs to class IA. P110 and p85 have three variants each. p110α is ubiquitously expressed, and p85α is the most abundant variant of p85. Class I PI3K participates in cell signaling, mostly via the activation of PKB (protein kinase B) and the PI3K/AKT/mTOR pathway. Dysfunction of these kinases impacts cell growth and differentiation, and mutations in p110α have been linked to cancer. At least three isoform-specific inhibitors are approved by FDA for the treatment of lymphoma and leukemia. Further studies will help identify more selective inhibitors with a good tolerance that can bypass the development of drug resistance.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
101914	PI3 Kinase p110α (H1047L) /p85α FLAG-Tag*	>1 µg	-80°C
79334	5x Kinase Buffer 1	1.5 ml	-20°C
79686	500 µM ATP	50 µl	-20°C
40560	PI3K Lipid Substrate **(Packaged separately, Do Not Freeze!)**	500 µl	4°C
79696	White 96-well plate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Name	Ordering Information
ADP-Glo™ Kinase Assay	Promega #V6930
DTT (Dithiothreitol), 1M, optional	
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	
30°C incubator	

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Principle

The **ADP-Glo™ Kinase Assay (Promega #V6930)** quantifies the amount of ADP produced by a kinase upon phosphorylation of a substrate. First, addition of the ADP-Glo™ reagent terminates the reaction and quenches the remaining ATP. Second, the addition of the Kinase Detection reagent converts the produced ADP to ATP. The newly generated ATP is quantified by a luciferase reaction. The luminescent signal correlates with the amount of ADP generated by the kinase and is linear to 1 mM ATP.

Contraindications

- The final concentration of DMSO in the assay should not exceed 1%.
- The PI3K Lipid Substrate is shipped separately on ice. Please store it at 4°C upon arrival **(DO NOT FREEZE the PI3K Lipid Substrate).**

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Blank", "Positive Control" and "Test Inhibitor" conditions.

1. Thaw **5x Kinase Assay Buffer 1** and **500 µM ATP.**

   *Optional: If desired, make **5x Kinase Assay Buffer 1** with 10 mM DTT.*

2. Prepare 3 ml of **1x Kinase Assay Buffer 1** by mixing 600 µl of **5x Kinase Assay Buffer 1** with 2,400 µl of distilled water.

   *Note: Three (3 ml) of **1x Kinase Assay Buffer 1** is sufficient for 100 reactions.*

3. Prepare 125 µM ATP solution by diluting 500 µM ATP 4-fold in distilled water.

   Note: 50 µl of 125 µM ATP is enough for a 96-well plate.

4. Prepare a **Master Mix** (12.5 µl/well): N wells x (6 µl of 5x Kinase Assay Buffer 1 + 0.5 µl of 125 µM ATP + 5 µl of PI3K Lipid Substrate + 1 µl of distilled water).

5. Add 12.5 µl of Master Mix to every well.

6. Prepare the **Test Inhibitor** (2.5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

6.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in 1x Kinase Assay Buffer 1, 10-fold more concentrated than the desired final concentrations.

For the positive and negative controls, use 1x Kinase Assay Buffer 1 (Diluent Solution).

OR

6.2. If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x Kinase Assay Buffer 1 to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Kinase Assay Buffer 1 to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Kinase Assay Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

7. Add 2.5 µl of Test Inhibitor to each well labeled “Test Inhibitor”.

8. Add 2.5 µl of Diluent Solution to the “Positive Control" and “Blank” wells.

9. Add 10 µl of 1x Kinase Assay Buffer 1 to the "Blank" wells.

10. Thaw **PI3 Kinase p110α (H1047L)/p85α Kinase** on ice. Briefly spin the tube to recover its full content.

11. Dilute the protein kinase (10 µl/well) to 1 ng/µl with **1x Kinase Assay Buffer 1**.

    Note: The concentration of protein is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly. This kinase is particularly sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use the thawed protein and do not re-use the diluted kinase.

12. Initiate the reaction by adding 10 µl of diluted kinase to the wells designated “Positive Control” and "Test Inhibitor".

13. Incubate at 30°C for 45 minutes.

14. Thaw the ADP-Glo™ reagent.

15. At the end of the 45-minute reaction, add 25 µl of ADP-Glo™ reagent to each well.

16. Cover the plate with aluminum foil and incubate at Room Temperature (RT) for 45 minutes.

17. Thaw the Kinase Detection Reagent.

18. Add 50 µl of Kinase Detection reagent to each well.

19. Cover the plate with aluminum foil and incubate at RT for another 45 minutes.

20. Immediately read in a luminometer or a microplate reader capable of reading luminescence.

21. The "Blank" value should be subtracted from all other readings.

Component	Blank	Positive Control	Test Inhibitor
Master Mix	12.5 µl	12.5 µl	12.5 µl
Test Inhibitor	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	-
1x Kinase Assay Buffer 1	10 µl	-	-
Diluted PI3 Kinase p110α(H1047L)/p85α (1 ng/µl)	-	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**

Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example Results

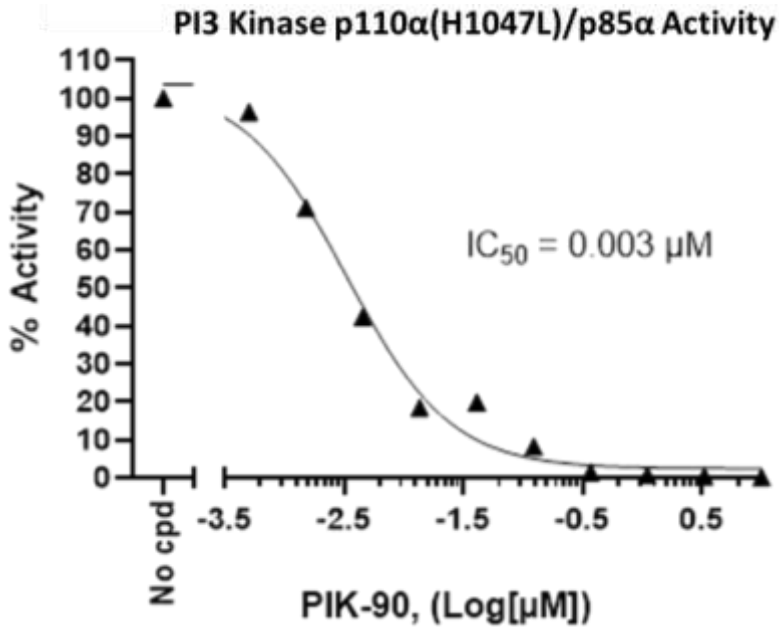

Figure 1: Inhibition of PI3 kinase p110α (H1047L)/p85α kinase activity by PIK-90.
The inhibition of PI3 Kinase p110α (H1047L)/p85α kinase activity was measured in the presence of increasing concentrations of PIK-90 (Selleckchem S1187). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of inhibitor, set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References
Vanhaesebroeck B., *et al.*, 2021 *Nature Reviews Drug Discovery* 20: 741-769.

Related Products

Products	*Catalog #*	*Size*
PI3 kinase (p110α/p85α) Recombinant	40621	20 µg
PI3 kinase p110α(N345K)/p85α Recombinant	40646	10 µg
PI3 kinase [p110α(E545K)/p85α], His-tag Recombinant	40644	10 µg
PI3 kinase p110α(E545K)/p85α, Recombinant	40640	20 µg