

Description

The Chemi-Verse™ PAK4 Kinase Assay Kit is designed to measure PAK4 (p21-activated kinase 4) serine/threonine kinase activity 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 PAK4 kinase, kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Background

PAK4 (p-12-activated kinase 4) is a member of the PAK family of serine/threonine kinases. The PAK family is highly evolutionary conserved and regulates cell survival, apoptosis, cytoskeleton dynamics, and other critical cellular functions. PAK4 belongs to group II, which can be activated by Cdc42 (cell division control protein 42) only as opposed to group I, which depends on either Rac1 or Cdc42. It is ubiquitously expressed, participating in cytoskeleton remodeling, cell motility, apoptosis, and metastasis. PAK4 is regulated by HGF (hepatocyte growth factor), PKA (protein kinase A), CDK5RAP3 (CDK5 regulatory subunit associated protein 3) and SH3RF2 (SH3 domain containing ring finger 2), and in turn phosphorylates proteins like p120-catenin, SMAD2 and β -catenin. Upregulation of PAK4 has been found in multiple cancers, such as ovarian, breast and gastric cancer. Inhibition of PAK4 leads to milder cancer cell migration and invasion, and to regulation of the immune response to cancer, such as T cell infiltration. Interestingly, it was observed that the cell line HCT116, a KRAS-driven cell line, is highly sensitive to PAK4 inhibition. The development of specific inhibitors targeting PAK4, to be used alone or as combination therapy, will greatly benefit the cancer therapy field.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
	PAK4*	1 μ g	-80°C
79334	5x Kinase Buffer 1	1.5 ml	-20°C
79686	500 μ M ATP	50 μ l	-20°C
	PAKtide, 1 mg/ml	500 μ l	-20°C
79696	White 96-well plate	1	Room Temperature

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

Materials Required but Not Supplied

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%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include “Blank”, “Positive Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

1. Thaw **5x Kinase Assay Buffer 1**, **500 μM ATP**, and **PAKtide (1 mg/ml)**.

*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 a **Master Mix** (12.5 μl/well): N wells x (6 μl of 5x Kinase Assay Buffer 1 + 0.5 μl of 500 μM ATP + 5 μl of PAKtide (1 mg/ml) + 1 μl of distilled water).
4. Add 12.5 μl of Master Mix to every well.
5. 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.

5.1 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

5.2 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%.

6. Add 2.5 μ l of Test Inhibitor to each well labeled "Test Inhibitor".
7. Add 2.5 μ l of Diluent Solution to the "Positive Control" and "Blank" wells.
8. Add 10 μ l of 1x Kinase Assay Buffer 1 to the "Blank" wells.
9. Thaw **PAK4 kinase** on ice. Briefly spin the tube to recover its full content.
10. Dilute the protein kinase (10 μ l/well) to 1 ng/ μ l with 1x Kinase Assay Buffer 1.
11. Initiate the reaction by adding 10 μ l of diluted kinase to the wells designated "Positive Control" and "Test Inhibitor".
12. Incubate at 30°C for 45 minutes.
13. Thaw the ADP-Glo™ reagent.
14. At the end of the 45-minute reaction, add 25 μ l of ADP-Glo™ reagent to each well.
15. Cover the plate with aluminum foil and incubate at Room Temperature (RT) for 45 minutes.
16. Thaw the Kinase Detection Reagent.
17. Add 50 μ l of Kinase Detection reagent to each well.
18. Cover the plate with aluminum foil and incubate at RT for another 45 minutes.
19. Immediately read in a luminometer or a microplate reader capable of reading luminescence.
20. The "Blank" value should be subtracted from all other readings.

Component	Blank	Positive Control	Test Inhibitor
Master Mix	12.5 μ l	12.5 μ l	12.5 μ l
Test Inhibitor	-	-	2.5 μ l
Diluent Solution	2.5 μ l	2.5 μ l	-
1x Kinase Assay Buffer 1	10 μ l	-	-
Diluted PAK4 (1 ng/ μ l)	-	10 μ l	10 μ l
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

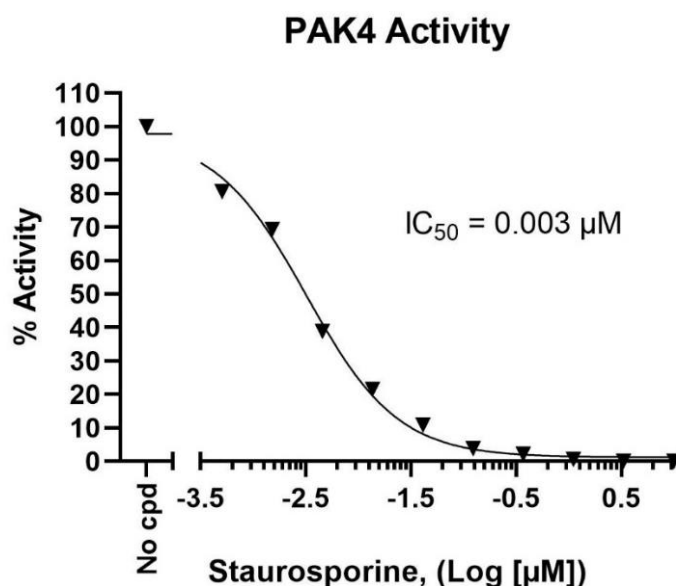


Figure 1: Inhibition of PAK4 kinase activity by Staurosporine.

PAK4 kinase activity was measured in the presence of increasing concentrations of Staurosporine (SelleckChem #S1421). 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

1. Wang H., *et al.*, 2021 *Expert Opin Ther Pat.* 31(11): 977-987.
2. Li Y., *et al.*, *Front Pharmacol.* 13:956220.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Aurora A Protein Kinase, His-tag (HEK293-derived) Recombinant	100112	10 µg
Aurora A Protein Kinase, His-tag (Sf9-derived) Recombinant	40004	10 µg
Chemi-Verse™ Aurora Kinase A Assay Kit	82094	96 reactions
PAK7, GST-tag Recombinant	40148	10 µg
PAK5 Recombinant	40077	10 µg
Chemi-Verse™ PAK6 Assay Kit	82182	96 reactions
Chemi-Verse™ PAK7 Assay Kit	82183	96 reactions