Description

The breast tumor kinase (BRK) Kinase Assay Kit is designed to measure BRK tyrosine 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 BRK kinase, kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Background

Breast Tumor Kinase (BRK, also known as Tyrosine-protein kinase-6 or PKT6) is a non-receptor protein with tyrosine kinase activity. BRK is involved in epithelial differentiation and apoptosis. It interacts with multiple proteins and is involved in several signaling pathways, like activating STAT3 and STAT5B to promote cell proliferation. It is involved in cancer progression, migration and evasion, namely in breast cancer. Targeting BRK kinase activity via small molecule inhibitors may be a viable therapeutic approach for breast cancer.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
40403	BRK, GST-Tag*	10 µg	-80°C
79334	5x Kinase Buffer 1	1.5 ml	-20°C
79686	500 µM ATP	50 µl	-20°C
40217	PTK Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml)	50 µl	-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), 1 M, 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, the addition of the ADP-Glo™ reagent terminates the reaction and quenches the remaining ATP present. 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 up 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.

1. Thaw **5x Kinase Buffer 1**, **500 µM ATP,** and **PTK Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml).**

   *Optional: If desired, add DTT to **5x Kinase Buffer 1** to make a solution containing 10 mM DTT.*

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

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

3. Prepare a **Master Mix** (12.5 µl/well): N wells x (6 µl of **5x Kinase Buffer 1** + 0.5 µl of **500 µM ATP** + 0.5 µl of PTK Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml) + 5.5 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 Buffer 1**, 10-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use **1x Kinase 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 DMSO, then dilute the inhibitor 10-fold in **1x Kinase 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 to 10-fold the desired final concentrations using 10% DMSO in 1x Kinase Assay Buffer, in order to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in water (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 µl of **Test Inhibitor** to each well labeled “Test Inhibitor.”

7. Add 2.5 µl of **Diluent Solution** on the “Blank” and “Positive Control" wells.

8. Add 10 µl of **1x Kinase Buffer 1** to the “Blank” wells.

9. Thaw **BRK kinase** on ice. Briefly spin the tube to recover its full content.

10. Dilute the protein kinase (10 µl/well) to 10 ng/µl using **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.

    Note: 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.

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

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		
BRK (10 ng/µl)	-	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**

12. Incubate at 30°C for 45 minutes.

13. During the incubation, thaw the ADP-Glo™ reagent. At the end of the 45-minute reaction, add 25 µl of ADP-Glo™ reagent to each well. Cover the plate with aluminum foil and incubate at Room Temperature for 45 minutes.

14. Thaw the Kinase Detection Reagent. At the end of the 45-minute incubation, add 50 µl of Kinase Detection reagent to each well. Cover the plate with aluminum foil and incubate at Room Temperature for another 45 minutes.

15. Immediately read in a luminometer or a microplate reader capable of reading luminescence. The “Blank” value should be subtracted from all other readings.

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

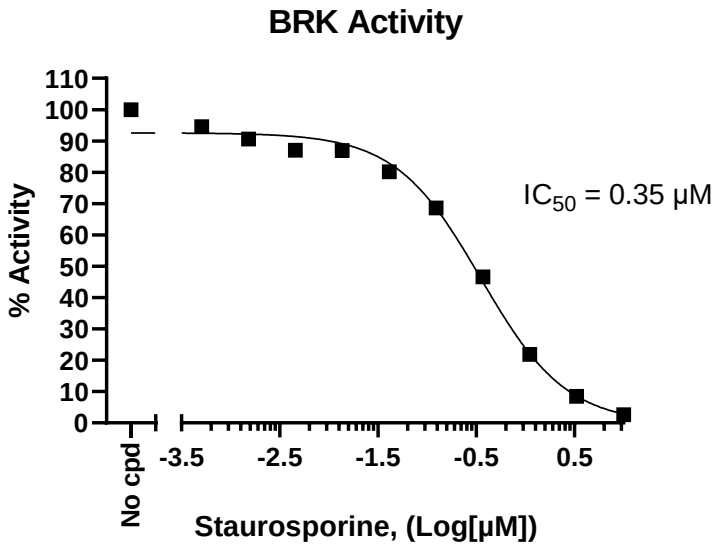

Figure 1: Inhibition of BRK kinase Activity by Staurosporine.
The inhibition of BRK kinase activity was measured in the presence of increasing concentrations of Staurosporine. The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of inhibitor, set at 100%).

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 further questions, please email support@bpsbioscience.com

References
Liu C., *et al.*, 2021, Pharmacological targeting PTK6 inhibits the JAK2/STAT3 sustained stemness and reverses chemoresistance of colorectal cancer., *J Exp Clin Cancer Res* 20: 297.

Related Products

Products	*Catalog #*	*Size*
BRK, GST-Tag Recombinant	40403	10 µg
FAK Kinase Assay Kit	40722	96 reactions
JAK3 (Janus Kinase 3) Assay Kit	79521	96 reactions
JAK2 (Janus Kinase 2) Assay Kit	79520	96 reactions