Description

The CDK6/CyclinD3 Kinase Assay Kit is designed to measure CDK6/CyclinD3 kinase activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified recombinant CDK6/CyclinD3 kinase, kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Background

CDK6 is a serine/threonine protein kinase involved in cell cycle progression and in cellular differentiation. The CDK6/Cyclin D3 complex phosphorylate RB1 to release the transcription factor E2F and drive the transition from G1 to S-phase of the cell cycle. This complex regulates cell proliferation in many cell types and has been validated as a cancer therapeutic target. Palbociclib, a CDK4/CDK6 inhibitor, has shown therapeutic benefit in a small number of patients and is in clinical use for the treatment of $ER^+/HER2^-$ breast cancer, with more clinical trials ongoing.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
40206	CDK6/CyclinD3*	20 µg	-80°C
79334	Kinase assay buffer 1 (5x)	1.5 ml	-20°C
79686	ATP (500 µM)	100 µl	-20°C
78396	Histone H1 (1 mg/ml)	500 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	Catalog #
Kinase-Glo MAX	Promega #V6071
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

Kinase activity is measured using **Kinase-Glo™ Max** (Promega, #V6071). The addition of the reagent results in the generation of a luminescent signal that correlates with the amount of ATP. The reagent is linear to 100 µM 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 assay buffer**, **ATP** and **Histone H1 (1 mg/ml) substrate.**

   Optional: If desired, add DTT to **5x Kinase assay buffer** to make a 10 mM DTT concentration (for example, add 10 µl of 1 M DTT to 1 ml of **5x Kinase assay buffer**).

2. Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 µl of **5x Kinase assay buffer** with 2400 µl water. Three (3) ml of **1x Kinase assay buffer** is sufficient for 100 reactions.

3. Prepare the Test Inhibitor (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 50 µl.

   a) If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x Kinase Assay Buffer, 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use 1x Kinase Assay Buffer (Diluent Solution).

   b) 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 Assay Buffer 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 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).

   The final concentration of DMSO should not exceed 1%.

4. Add 5 µl of Test Inhibitor to each well labeled “Test Inhibitor.” For the “Positive Control" and “Blank,” add Diluent Solution (either distilled water or 10% DMSO in water, as described above).

5. To the wells designated as "Blank," add 20 µl of **1x Kinase assay buffer**.

6. Thaw **CDK6/CyclinD3 kinase** on ice. Briefly spin the tube to recover its full contents. Dilute the protein kinase to 10 ng/µl using **1x Kinase assay buffer**.

   Notes: 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.

7. Add 20 µl of diluted Kinase to the wells designated “Positive Control” and "Test Inhibitor."

8. Preincubate the test inhibitor and CDK6/CyclinD3 for 30 minutes at room temperature.

9. Prepare the Master Mix (25 µl/well): N wells x (6 µl of **5x Kinase assay buffer** + 1 µl of **ATP (500 µM)** + 5 µl of **Histone H1** (1 mg/ml) + 13 µl of distilled water).

10. Initiate the reaction by adding 25 µl of Master Mix to the wells designated “Blank,” Positive Control,” and “Test Inhibitor.”

    Incubate 30°C for 45 minutes.

Component	**Blank**	**Positive Control**	**Test Inhibitor**
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
1x Kinase Buffer	20 µl	-	-
CDK6/CyclinD3 (10 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

11. During the incubation, thaw the Kinase-Glo Max™ reagent. At the end of the 45-minute reaction, add 50 µl of Kinase-Glo Max™ reagent to each well. Cover the plate with aluminum foil and incubate the plate at room temperature for 15 minutes.

12. Immediately read in a luminometer or a microplate reader capable of reading luminescence. The “Blank” value is 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

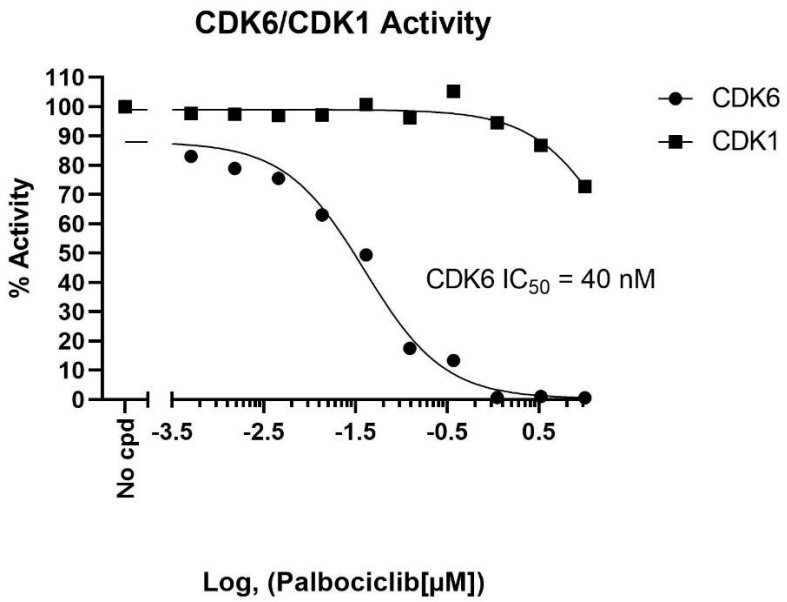

Figure 1: Inhibition of CDK6/CyclinD3 kinase Activity by Palbociclib. The inhibition of CDK6/CyclinD3 kinase activity was measured in the presence of increasing inhibitor concentrations using the CDK6/CyclinD3 Kinase Assay Kit (BPS Bioscience #78395). Similarly, the effect of Palbociclib was measured against CDK1 using the CDK1/CyclinB1 assay kit (BPS Bioscience #40454) to show the specificity of the inhibitor for CDK6. The Blank value was subtracted from all other values. Results are expressed as 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 all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
CDK6/CyclinD3, His-tags	40206	20 µg
CDK6/CyclinD1, His-tag, GST-tag	40097	10 µg
CDK6, His-Tag	100031	20 µg
CDK12/Cyclin K Kinase Assay Kit	78298	96 reactions