Description

The CDK9/CyclinT Kinase Assay Kit is designed to measure CDK9/CyclinT kinase activity for screening and profiling applications using Kinase-Glo™ Max as a detection reagent. The assay kit comes in a convenient 384-well format, with enough purified recombinant CDK9/CyclinT kinase, kinase substrate, ATP, and kinase assay buffer for 400 enzyme reactions.

Background

Cyclin-dependent kinase 9 (CDK9) is the catalytic subunit of the positive transcription elongation factor b (P-TEFb) complex, which phosphorylates the C-terminal domain of RNA polymerase II, a key player in the production of mature RNA. P-TEFb also includes cyclinT. CDK9 translocates to the nucleus once it gets auto phosphorylated, where it can associate with cyclinT. Hyperactivation of cyclins is known to result in cancer and the development of resistance to therapy, and CDK9-cyclinT is involved in the transcription of oncogenes. The inhibition or degradation of specific CDK-cyclin complexes could prove beneficial in cancer treatment, but the development of specific inhibitors has been difficult. Recently a small molecule degrader specific for CDK9-cyclinT was identified, LL-K9-3, which resulted in lower levels of androgen receptor (AR) and cMyc in 22RV1 cells. LL-K9-3 thus seemed more effective than SNS032, its parental CDK9 inhibitor, and the CDK9 PROTAC Thal-SNS032. Further research into new and more specific degrader molecules will prove beneficial for cancer therapy.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
40307	CDK9/CyclinT, GST-Tag*	2 x 10 µg	-80°C
79334	5x Kinase Buffer 1	2 x 1.5 ml	-20°C
79686	500 µM ATP	200 µl	-20°C
79604	5x CDK Substrate Peptide 2	2 x 1 ml	-20°C
79969	White 384-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
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, that is linear up 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.
- The assay should include "Blank", "Positive Control" and "Test Inhibitor" conditions.

1. Thaw **5x Kinase Assay Buffer 1**, **500 µM ATP** and **5x CDK Substrate Peptide 2.**

   *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 (3 µl of 5x Kinase Assay Buffer 1 + 0.5 µl of 500 µM ATP + 5 µl of 5x CDK Substrate Peptide 2 + 4 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 µl of Test Inhibitor to each well labeled “Test Inhibitor”.

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

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

9. Thaw **CDK9/CyclinT Kinase** on ice. Briefly spin the tube to recover its full content.

10. Dilute the protein kinase (10 µl/well) to 5 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.

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

12. Incubate at 30°C for 45 minutes.

13. Thaw the Kinase-Glo™ Max reagent.

14. At the end of the 45-minute reaction, add 25 µl of Kinase-Glo™ Max reagent to each well.

15. Cover the plate with aluminum foil and incubate the plate at Room Temperature (RT) for 15 minutes.

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

17. 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 CDK9/CyclinT (5 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

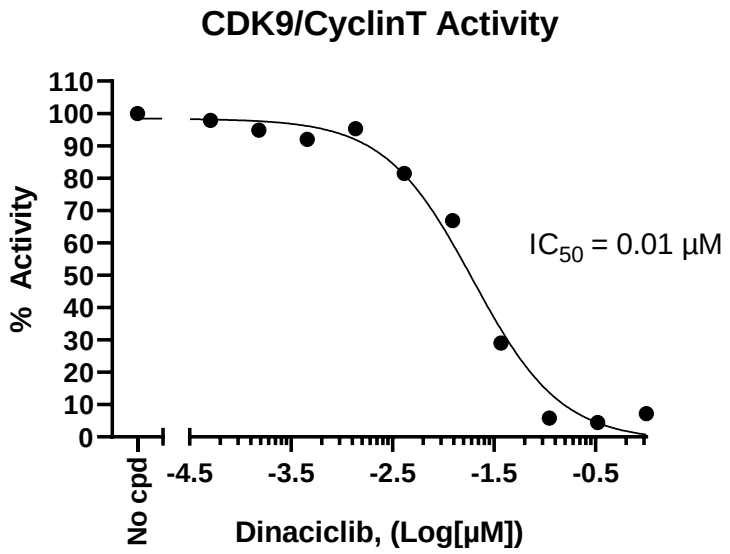

Figure 1: Inhibition of CDK9/CyclinT kinase activity by Dinaciclib.
CDK9/CyclinT kinase activity was measured in the presence of increasing concentrations of Dinaciclib (SelleckChem S2768). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (kinase 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

Napolitano G, *et al.*, 2003 *J Cell Physiol.* 197(1):1-7.
Li J., *et al.,* 2022 *J Med Chem.* 65(16):11034-11057.

Related Products

Products	*Catalog #*	*Size*
CDK9/CyclinK, GST-Tag Recombinant	40106	10 µg
PROTAC® Optimization Kit for CDK Kinase-Cereblon Binding	79924	96 reactions
Chemi-Verse™ CDK3/CyclinE1 Kinase Assay Kit	78884	96 reactions
Chemi-Verse™ CDK17/CyclinY Kinase Assay Kit	78885	96 reactions