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Data Sheet

CDK1 Assay Kit

Catalog # 79597

DESCRIPTION: Cyclin-dependent kinases (CDKs) are key regulators of the cell cycle. CDKs are active only when bound to their regulator proteins, cyclins. CDK activity is tightly controlled for successful cell division. Since abnormal cell division represents cancer pathology, controlling CDK activity has been shown as a promising therapeutic strategy. In particular, CDK1 plays an important role in mitotic progression. The *CDK1 Assay Kit* is designed to measure CDK1/CyclinB1 activity for screening and profiling applications, using Kinase-Glo® MAX as a detection reagent. The *CDK1 Assay Kit* comes in a convenient 96-well format, with enough purified recombinant CDK1/CyclinB1 enzyme, CDK substrate peptide, ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40454	CDK1/CyclinB1	2 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer 1	1.5 ml	-20°C	
79686	ATP (500 µM)	100 µl	-20°C	
79598	10x CDK substrate peptide 1	500 µl	-20°C	
79696	96-well plate, white	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Kinase-Glo MAX (Promega #V6071)
Dithiothreitol (DTT, 1 M; optional)
Microplate reader capable of reading luminescence
Adjustable micropipettor and sterile tips
30°C incubator

APPLICATIONS: Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months when stored as recommended.

REFERENCE: Uzma A. et. al., *Nature Review Drug Discovery* **14:**130-146 (2015)

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Thaw **5x Kinase assay buffer 1**, **ATP** and **10x CDK substrate peptide 1.** (Optional: If desired, add DTT to **5x Kinase assay buffer 1** to make a 10 mM concentration; *e.g.* add 10 µl of 1 M DTT to 1 ml 5x **Kinase assay buffer 1**)

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2) Prepare the master mixture (25 µl per well): N wells x (6 µl **5x Kinase assay buffer 1** + 1 µl **ATP (500 µM)** + 5 µl **10x CDK substrate peptide 1** + 13 µl distilled water). Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer 1	6 µl	6 µl	6 µl
ATP (500 µM)	1 µl	1 µl	1 µl
10X CDK substrate peptide 1	5 µl	5 µl	5 µl
Water	13 µl	13 µl	13 µl
Test Inhibitor	–	5 µl	–
Inhibitor Buffer (no inhibitor)	5 µl	–	5 µl
1x Kinase buffer 1	–	–	20 µl
CDK1/CyclinB1 (1.0 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

3) Add 5 µl of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control" and "Blank", add 5 µl of the same solution without inhibitor (Inhibitor buffer).

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

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

6) Thaw **CDK1/CyclinB1 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **CDK1/CyclinB1** required for the assay and dilute enzyme to ~1.0 ng/µl with **1x Kinase assay buffer 1**. Store remaining undiluted enzyme in aliquots at -80°C. *Note*: ***CDK1/CyclinB1 enzyme*** *is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7) Initiate reaction by adding 20 µl of **diluted CDK1/CyclinB1** enzyme to the wells designated "Positive Control" and "Test Inhibitor Control". Incubate at 30°C for 45 minutes.

8) Thaw Kinase-Glo Max reagent.

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9) After the 45 minute reaction, add 50 µl of Kinase-Glo Max reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes.

10) Measure luminescence using the microplate reader. “Blank” value is subtracted from all readings.

Reading Chemiluminescence:
Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, 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 are: 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).

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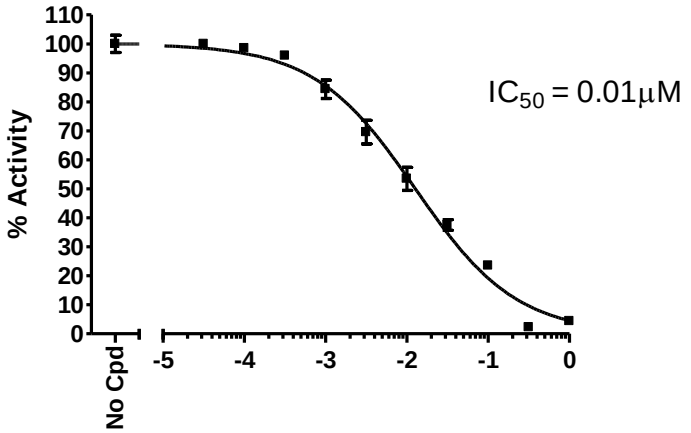
Example of Assay Results:

CDK1/CyclinB1 Activity



Inhibition of CDK1/CyclinB1 enzyme by Dinaciclib, measured using the CDK1 assay kit (Cat. #79597). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
CDK1/CyclinB1, GST-tag	40454	10 µg
CDK1/CyclinA2, GST-tag	40100	10 µg
CDK2/CyclinA2, GST-tag	40101	10 µg
CDK2/CyclinE1, GST-tag	40102	10 µg
CDK2 (no tag)/CyclinA2, His-GST-tags	41101	10 µg
CDK3/CyclinE1, GST-tag	40103	10 µg
CDK4, FLAG-Tag	100052	20 µg
CDK4/CyclinD3, GST-His-Tag	40104	10 µg
CDK4(EE,T172A)/Cyclin D1, His-tag	40094	20 µg
CDK5/p25, GST-tag	40105	10 µg
CDK5/p35, GST-tag	40095	10 µg
CDK6/CyclinD1, His-tag, GST-tag	40097	10 µg
CDK6/CyclinD3, His-tags	40206	20 µg
CDK7/CyclinH1/MNAT1, His-tags	40098	10 µg
CDK9/CyclinK, GST-tag	40106	10 µg
CDK9/CyclinT1, GST-tag	40307	10 µg