6405 Mira Mesa Blvd Ste. 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Data Sheet

CDK4 Assay Kit

Catalog # 79674

DESCRIPTION: Cyclin-dependent kinase 4 (CDK4) assembles with the 'D' type of Cyclins, e.g. cyclins D1, D2, and D3, and play a pivotal role in the cell cycle entering the S phase. Dysregulation of the activity of CDK4 is related to many different cancers, indicating CDK4 inhibitors as promising anticancer treatments. In fact, multiple CDK4 inhibitors, including Palbociclib, were approved by the FDA recently. The *CDK4 Assay Kit* is designed to measure CDK4/CyclinD3 activity for screening and profiling applications, using Kinase-Glo® Max as a detection reagent. The *CDK4 Assay Kit* comes in a convenient 96-well format, with enough purified recombinant CDK4/CyclinD3 enzyme, CDK4 substrate peptide, ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40104	CDK4/CyclinD3	20 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	
79675	10x CDK4 substrate peptide	500 µl	-20°C	
79696	96-well plate, white	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Kinase-Glo® Max Luminescence Kinase Assay (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)

6405 Mira Mesa Blvd Ste. 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

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

1) Thaw **5x Kinase assay buffer 1**, **ATP** and **10x CDK4 substrate peptide.** (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**)

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 CDK4 substrate peptide** + 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 CDK4 substrate peptide	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
CDK4/CyclinD3 (10 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 **CDK4/CyclinD3 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **CDK4/CyclinD3** required for the assay and dilute enzyme to ~10 ng/µl with **1x Kinase assay buffer 1**. Store remaining undiluted enzyme in aliquots at -80°C. *Note:* **CDK4/CyclinD3 *enzyme*** *is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6405 Mira Mesa Blvd Ste. 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

7) Initiate reaction by adding 20 µl of **diluted CDK4/CyclinD3** enzyme to the wells designated “Positive Control” and "Test Inhibitor Control". Incubate at 30˚C for 60 minutes.

8) Thaw Kinase-Glo® Max Luminescence Kinase Assay reagent.

9) After the 60 minutes 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 10 ~ 15 minutes.

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

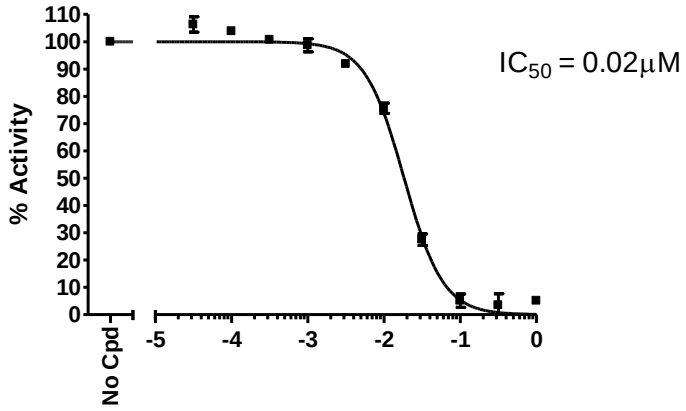
Reading Chemiluminescence:
Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

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).

6405 Mira Mesa Blvd Ste. 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Example of Assay Results:

CDK4/CyclinD3 Activity



Inhibition of CDK4/CyclinD3 enzyme by Palbociclib, measured using the CDK4 assay kit (Cat. #79674). *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
CDK1 Assay Kit	79597	96 rxns.
CDK2 Assay Kit	79599	96 rxns.
CDK5 Assay Kit	79600	96 rxns.
CDK7 Assay Kit	79603	96 rxns.
CDK9 Assay Kit	79628	96 rxns.