6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

CDK7 Assay Kit

Catalog #79603

DESCRIPTION: Cyclin-dependent kinase 7 (CDK7) assembled with Cyclin H and MAT1 comprises an important sub-component of the transcription factor TFIIH. It is known to have dual roles, which include transcription regulation via phosphorylating the C-terminal domain (CTD) of RNA polymerase II as well as controlling cell cycle progression as a CDK activating kinase (CAK). The *CDK7 Assay Kit* is designed to measure CDK7/Cyclin H/MAT1 activity for screening and profiling applications, using ADP-Glo® as a detection reagent. The *CDK7 Assay Kit* comes in a convenient 96-well format, with enough purified recombinant CDK7/Cyclin H/MAT1 enzyme, CDK substrate peptide, ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40098	CDK7/Cyclin H/MAT1	10 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	
79604	CDK substrate peptide 2 (1 mg/ml)	125 µl	-20°C	
79696	96-well plate, white	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
ADP-Glo® Kinase Assay (Promega #V6930)
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)

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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

1) Thaw **5x Kinase assay buffer 1**, **ATP** and **10x CDK substrate peptide 2.** (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 (12.5 µl per well): N wells x (3 µl **5x Kinase assay buffer 1** + 0.5 µl **ATP (500 µM)** + 1.25 µl **CDK substrate peptide 2 (1 mg/ml)** + 7.75 µl distilled water). Add 12.5 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer 1	3 µl	3 µl	3 µl
ATP (500 µM)	0.5 µl	0.5 µl	0.5 µl
CDK substrate peptide 2 (1 mg/ml)	1.25 µl	1.25 µl	1.25 µl
Water	7.75 µl	7.75 µl	7.75 µl
Test Inhibitor	–	2.5 µl	–
10% DMSO in water (Inhibitor buffer)	2.5 µl	–	2.5 µl
1x Kinase buffer 1	–	–	10 µl
CDK7/Cyclin H/MAT1 (10 ng/µl)	10 µl	10 µl	–
Total	25 µl	25 µl	25 µl

3) Add 2.5 µl of Inhibitor solution of each well labeled as “Test Inhibitor”. For the “Positive Control" and “Blank”, add 2.5 µl of 10% DMSO in water (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 10 µl of **1x Kinase assay buffer 1.**

6) Thaw **CDK7/Cyclin H/MAT1 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **CDK7/Cyclin H/MAT1** 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:* **CDK7/Cyclin H/MAT1 *enzyme*** *is sensitive to freeze/thaw cycles.*

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

7) Initiate reaction by adding 10 µl of **diluted CDK7/Cyclin H/MAT1** enzyme to the wells designated “Positive Control” and "Test Inhibitor Control". Incubate at 30˚C for 60 minutes.

8) Thaw ADP-Glo reagent.

9) After the 60 minute reaction, add 25 µl of ADP-Glo reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 45 minutes.

10) Thaw Kinase-Detection reagent

11) After the 45 minutes incubation, add 50 µl of Kinase Detection reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for another 45 minutes.

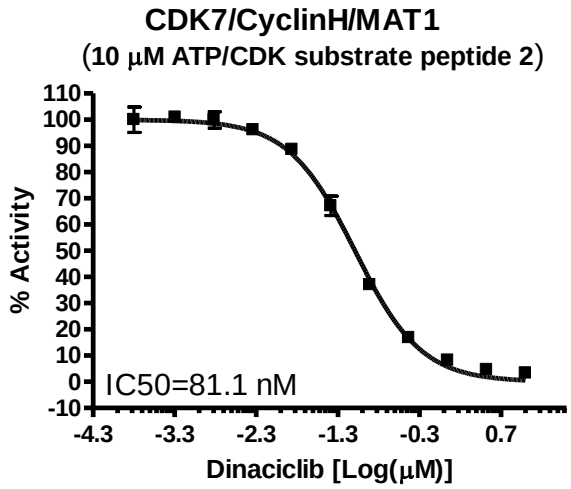
12) Measure luminescence using the microplate reader. “Blank” value is subtracted from all readings.

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).

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Example of Assay Results:



Inhibition of CDK7/Cyclin H/MAT1 enzyme by Dinaciclib, measured using the CDK7 assay kit (Cat. #79603). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@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