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## **Data Sheet**

### **CDK5 Assay Kit**

**Catalog #79600**

**DESCRIPTION:** CDK5 is an unconventional member of the CDK family. It is expressed in post-mitotic neurons, and its activity is regulated by neuron-specific activators p35/p25 and p39 rather than the cyclins. It has been suggested that CDK5 plays several roles in the nervous system, including Tau aggregation and tangle formation. The *CDK5 Assay Kit* is designed to measure CDK5 activity for screening and profiling applications, using Kinase-Glo<sup>®</sup> MAX as a detection reagent. The *CDK5 Assay Kit* comes in a convenient 96-well format, with enough purified recombinant CDK5/p25 enzyme, CDK substrate peptide, ATP and kinase assay buffer for 100 enzyme reactions.

#### **COMPONENTS:**

Catalog #	Reagent	Amount	Storage	
40105	CDK5/p25	2 µg	-80°C	<b>Avoid multiple freeze/thaw cycles!</b>
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)

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### 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  $\mu$ l of 1 M DTT to 1 ml **5x Kinase assay buffer 1**)
- 2) Prepare the master mixture (25  $\mu$ l per well): N wells x (6  $\mu$ l **5x Kinase assay buffer 1** + 1  $\mu$ l **ATP (500  $\mu$ M)** + 5  $\mu$ l **10x CDK substrate peptide 1** + 13  $\mu$ l distilled water). Add 25  $\mu$ l to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer 1	6 $\mu$ l	6 $\mu$ l	6 $\mu$ l
ATP (500 $\mu$ M)	1 $\mu$ l	1 $\mu$ l	1 $\mu$ l
10X CDK substrate peptide 1	5 $\mu$ l	5 $\mu$ l	5 $\mu$ l
Water	13 $\mu$ l	13 $\mu$ l	13 $\mu$ l
Test Inhibitor	-	5 $\mu$ l	-
Inhibitor Buffer (no inhibitor)	5 $\mu$ l	-	5 $\mu$ l
1x Kinase buffer 1	-	-	20 $\mu$ l
CDK5/p25 (0.75 ng/ $\mu$ l)	20 $\mu$ l	20 $\mu$ l	-
<b>Total</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>

- 3) Add 5  $\mu$ l of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control" and "Blank", add 5  $\mu$ l of the same solution without inhibitor (Inhibitor buffer).
- 4) Prepare 3 ml of **1x Kinase assay buffer 1** by mixing 600  $\mu$ l of **5x Kinase assay buffer 1** with 2400  $\mu$ l water. 3 ml of **1x Kinase assay buffer 1** is sufficient for 100 reactions.
- 5) To the wells designated as "Blank", add 20  $\mu$ l of **1x Kinase assay buffer 1**.
- 6) Thaw **CDK5/p25 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **CDK5/p25** required for the assay and dilute enzyme to ~0.75 ng/ $\mu$ l with **1x Kinase assay buffer 1**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: CDK5/p25 enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

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- 7) Initiate reaction by adding 20  $\mu$ l of **diluted CDK5/p25** enzyme to the wells designated "Positive Control" and "Test Inhibitor Control". Incubate at 30°C for 45 minutes.
- 8) Thaw Kinase-Glo Max reagent.
- 9) After the 45-minute reaction, add 50  $\mu$ l 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).

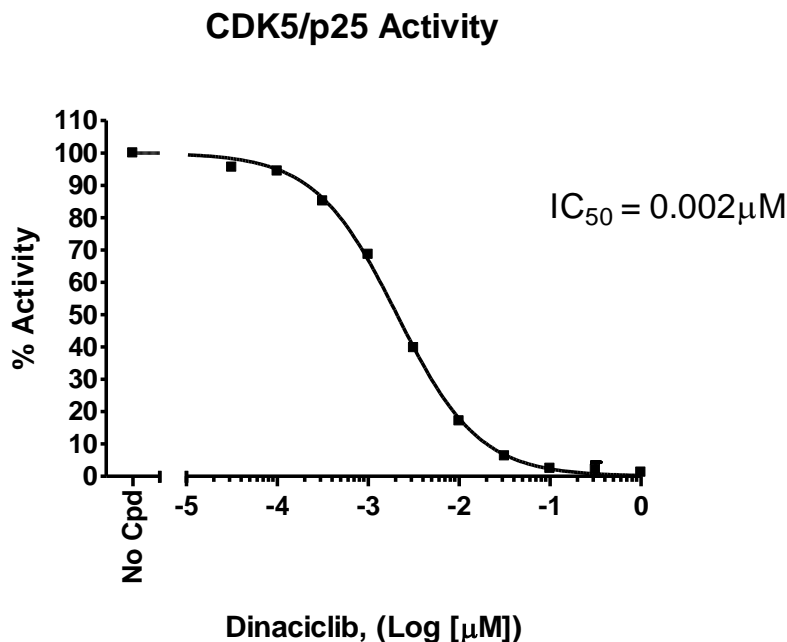
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**Example of Assay Results:**



Inhibition of CDK5/p25 enzyme by Dinaciclib, measured using the CDK5 assay kit (Cat. #79600). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.*

**RELATED PRODUCTS:**

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
CDK5/p25, GST-tag	40105	10 µg
CDK5/p35, GST-tag	40095	10 µg
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
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

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