Version 27-10/24

User's Manual

CDK5 Activity Assay Kit

REF **DEIA-JY24016**

Σ **96T**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

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

General 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® 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.

Reagents And Materials Provided

1. CDK5/p25: 2 µg, -80°C. Avoid multiple freeze/thaw cycles!
2. 5x Kinase assay buffer 1: 1.5 ml, -20°C. Avoid multiple freeze/thaw cycles!
3. ATP (500 µM): 100 µl, -20°C. Avoid multiple freeze/thaw cycles!
4. 10x CDK substrate peptide 1: 500 µl, -20°C. Avoid multiple freeze/thaw cycles!
5. 96-well plate, white: 1, Room Temp.

Materials Required But Not Supplied

1. Kinase-Glo MAX (Promega #V6071)
2. Dithiothreitol (DTT, 1 M; optional)
3. Microplate reader capable of reading luminescence
4. Adjustable micropipettor and sterile tips
5. 30°C incubator

Storage

Up to 6 months when stored as recommended.

Assay Procedure

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)

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
CDK5/p25 (0.75 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

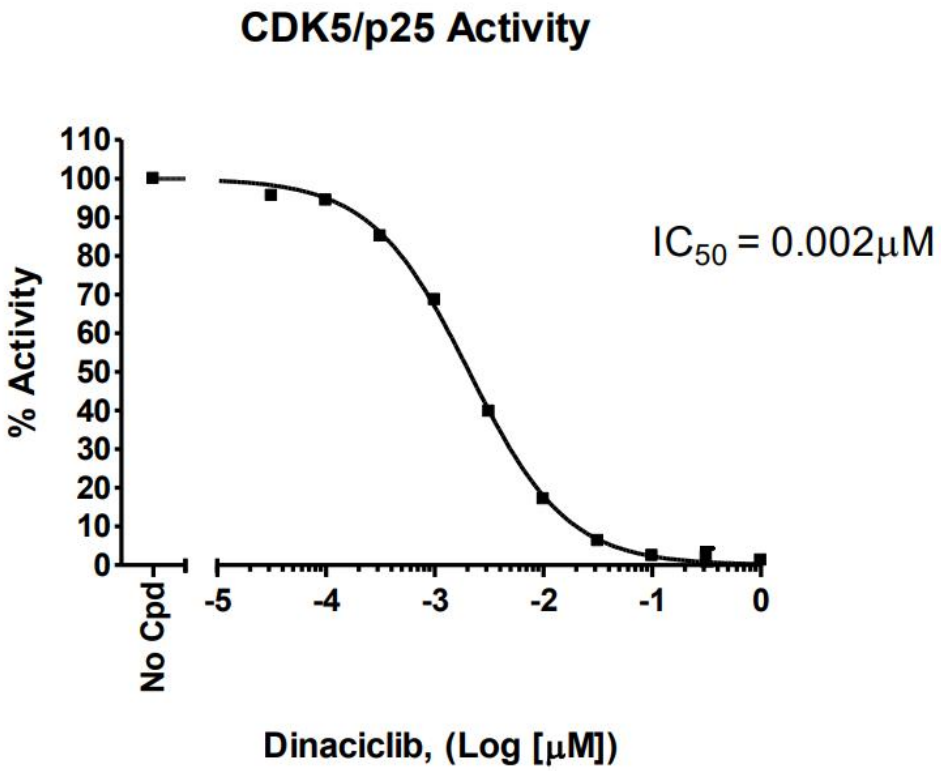
3. Add 5 µl of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control" and "Blank", add 5 µl of the same solution without inhibitor (Inhibitor buffer).
4. Prepare 3 ml of **1x Kinase assay buffer 1** by mixing 600 µl of **5x Kinase assay buffer 1** with 2400 µl water. 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 **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/µl 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.
7. Initiate reaction by adding 20 µl 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 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).

Typical Standard Curve



Inhibition of CDK5/p25 enzyme by Dinaciclib, measured using the CDK5 assay kit. Data shown is lot-specific.