Creative Diagnostics®

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User's Manual

CDK5/p35 Kinase Activity Assay Kit

REF **DEIA-JY24017**

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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

The CDK5/p35 Kinase Assay Kit is designed to measure CDK5 (cyclin dependent kinase 5)/p35 kinase activity for screening and profiling applications using ADP-Gl as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified recombinant CDK5/p35 complex, substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

General Description

CDK5 (cyclin dependent kinase 5) is a serine/threonine protein kinase of the CDK family of proteins. It is expressed mainly in the brain, where it is linked to neuronal development and differentiation. CDK5 is an unconventional CDK protein, as its partner are the non-cyclin proteins p35 (early 35 kDa protein) and p39. P35 and p39 are myristoylated and recruit CDK5 to the cell membrane. Recent findings identified new roles for CDK5 in the nervous system, such as participating in neurite outgrowth, synaptic plasticity and homeostasis and circadian rhythm. Accumulation of p25, a fragment of p35, in neurons creates dysregulation of CDK5 function, and can result in Alzheimer's disease (AD), Parkinson's disease (PD), ALS (amyothropic lateral sclerosis) and Huntington's disease (HD). CDK5 also participates in T cell activation, and overexpression of this protein has been found in cancer samples. It acts on increasing brain tumor stem cell numbers and can lead to upregulation of PD-L1 (programmed death ligand-1) and increase cancer cell immune evasion. Inhibition of CDK5 via small molecules and peptides is a promising area of research that can benefit cancer with neurodegenerative disorders and cancer.

Reagents And Materials Provided

1. CDK5/p35, GST-Tag*: 2 µg, -80°C
2. 5x Kinase Buffer: 1, 1.5 ml, -20°C
3. 500 µM ATP: 50 µl, -20°C
4. Histone H1: (1 mg/ml), 500 µl, -20°C
5. White 96-well plate: 1, Room Temperature

*The concentration of the protein is lot-specific and will be indicated on the tube.

Materials Required But Not Supplied

1. ADP-Glo™ Kinase Assay Promega #V6930

Assay Principle

The ADP-Glo™ Kinase Assay (Promega #V6930) quantifies the amount of ADP produced by a kinase upon phosphorylation of a substrate. First, addition of the ADP-Glo™ reagent terminates the reaction and quenches the remaining ATP. Second, the addition of the Kinase Detection reagent converts the produced ADP to ATP. The newly generated ATP is quantified by a luciferase reaction. The luminescent signal

correlates with the amount of ADP generated by the kinase and is linear to 1 mM ATP. The final concentration of DMSO in the assay should not exceed 1%.

2. DTT (Dithiothreitol), 1M, optional
3. Microplate reader capable of reading luminescence
4. Adjustable micropipettor and sterile tips
5. 30°C incubator

Storage

This assay kit will perform optimally for up to 6 monthsfrom date of receipt when the materials are stored as directed

Assay Procedure

• All samples and controls should be tested in duplicate.

• The assay should include "Blank", "Positive Control" and "Test Inhibitor" conditions.

• We recommend maintaining the diluted protein on ice during use.

• We recommend using Dinaciclib as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC50 value shown in the validation data below.

1. Thaw **5x Kinase Assay Buffer 1, 500 µM ATP,** and Histone H1 (1 mg/ml).

Optional: If desired, make **5x Kinase Assay Buffer 1** with 10 mM DTT.

2. Prepare 3 ml of **1x Kinase Assay Buffer 1** by mixing 600 µl of 5x Kinase Assay Buffer 1 with 2,400 µl of distilled water.

Note: Three (3 ml) of **1x Kinase Assay Buffer** 1 is sufficient for 100 reactions.

3. Prepare a Master Mix (12.5 µl/well): N wells x (6 µl of 5x Kinase Assay Buffer 1 + 0.5 µl of 500 µM ATP + 5 µl of Histone H1 (1 mg/ml) + 1 µl of distilled water).
4. Add 12.5 µl of Master Mix to every well.
5. Prepare the **Test Inhibitor** (2.5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

5.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in 1x Kinase Assay Buffer 1, 10-fold more concentrated than the desired final concentrations.

For the positive and negative controls, use 1x Kinase Assay Buffer 1 (Diluent Solution).

OR

5.2 If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x Kinase Assay Buffer 1 to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Kinase Assay Buffer 1 to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Kinase Assay Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

6. Add 2.5 µl of Test Inhibitor to each well labeled "Test Inhibitor".
7. Add 2.5 µl of Diluent Solution to the "Positive Control" and "Blank" wells.
8. Add 10 µl of 1x Kinase Assay Buffer 1 to the "Blank" wells.
9. Thaw CDK5/p35 Kinase on ice. Briefly spin the tube to recover its full content.
10. Dilute the protein kinase (10 µl/well) to 2 ng/µl with 1x Kinase Assay Buffer 1.
11. Initiate the reaction by adding 10 µl of diluted kinase to the wells designated "Positive Control" and "Test Inhibitor"

Component	Blank	Positive Control	Test Inhibitor
Master Mix	12.5 µl	12.5 µl	12.5 µl
Test Inhibitor	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	-
1x Kinase Assay Buffer 1	10 µl	-	-
Diluted CDK5/p35 (2 ng/µl)	-	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**

12. Incubate at 30°C for 45 minutes.
13. Thaw the ADP-Glo™ reagent.
14. At the end of the 45-minute reaction, add 25 µl of ADP-Glo™ reagent to each well.
15. Cover the plate with aluminum foil and incubate at Room Temperature (RT) for 45 minutes.
16. Thaw the Kinase Detection Reagent.
17. Add 50 µl of Kinase Detection reagent to each well.
18. Cover the plate with aluminum foil and incubate at RT for another 45 minutes.
19. Immediately read in a luminometer or a microplate reader capable of reading luminescence.
20. The "Blank" value is subtracted from all other readings.

Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, 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: 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

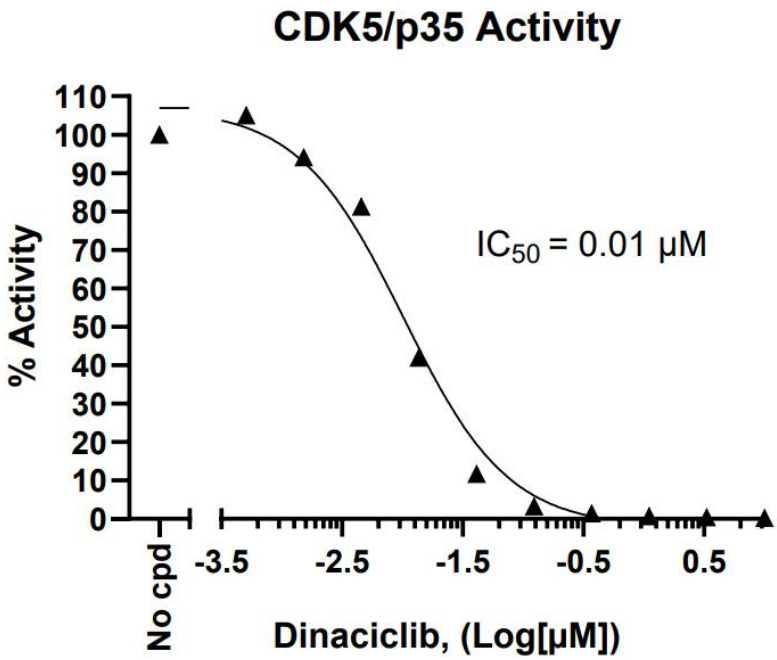

Figure 1: Inhibition of CDK5/p35 kinase activity by Dinaciclib. CDK5/p35 kinase activity was measured in the presence of increasing concentrations of Dinaciclib (SelleckChem S2768). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of inhibitor, set at 100%).

Precautions

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.