CDK5/p35 Kinase Assay

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Scientific Background:

CDK5 is a member of the Cyclin-Dependent Kinase family that is most abundant in the mammalian brain. Active form of CDK5, which has also been called neuronal cdc2-like kinase, is a heterodimer of CDK5 and a 25 kDa protein which is derived proteolytically from a 35 kDa brain and neuron-specific protein and is essential for the kinase activity of CDK5 (1). CDK5 has emerged as a crucial regulator of neuronal migration in the developing central nervous system. CDK5 phosphorylates a diverse list of substrates, implicating it in the regulation of a range of cellular processes - from adhesion and motility, to synaptic plasticity and drug addiction (2).

1. Tang, D. et al: Cyclin-dependent kinase 5 (Cdk5) and neuron-specific Cdk5 activators. Prog Cell Cycle Res. 1996;2:205-16.
2. Dhavan, R. et al: A decade of CDK5. Nat Rev Mol Cell Biol. 2001 Oct;2(10):749-59.

ADP-Glo™ Kinase Assay

Description

ADP-Glo™ Kinase Assay is a luminescent kinase assay that measures ADP formed from a kinase reaction; ADP is converted into ATP, which is converted into light by Ultra-Glo™ Luciferase (Fig. 1). The luminescent signal positively correlates with ADP amount (Fig. 2) and kinase activity (Fig. 3A). The assay is well suited for measuring the effects chemical compounds have on the activity of a broad range of purified kinases—making it ideal for both primary screening as well as kinase selectivity profiling (Fig. 3B). The ADP-Glo™ Kinase Assay can be used to monitor the activity of virtually any ADP-generating enzyme (e.g., kinase or ATPase) using up to 1mM ATP.

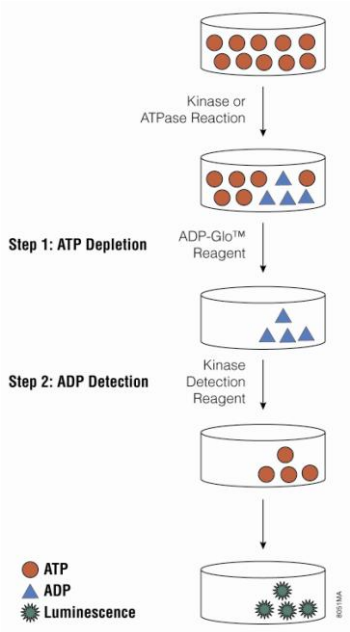

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

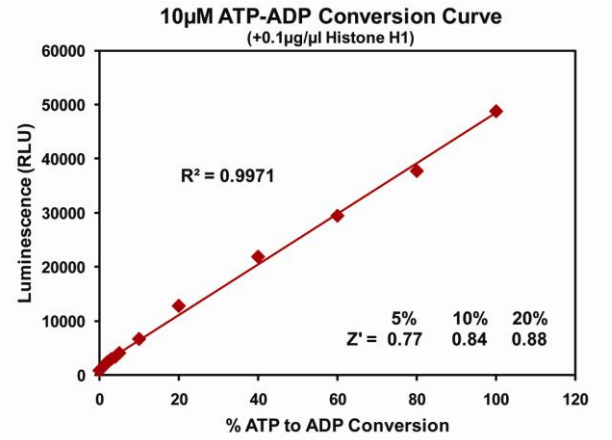

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase Buffer.
- Add to the wells of 384 low volume plate:
  - 1 µl of inhibitor or (5% DMSO)
  - 2 µl of enzyme (defined from table 1)
  - 2 µl of substrate/ATP mix
- Incubate at room temperature for 10 minutes.
- Add 5 µl of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10 µl of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1second).

Table 1. CDK5/p35 Enzyme Titration. Data are shown as relative light units (RLU) that directly correlate to the amount of ADP produced. The correlation between the % of ATP converted to ADP and corresponding signal to background ratio is indicated for each kinase amount.

CDK5/p35, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0
RLU	35447	27011	25799	21564	18373	10913	6919	3816	2507	863
S/B	41	31	30	25	21	13	8	4	3	1
% Conversion	70	52	50	41	34	19	10	4	1	0

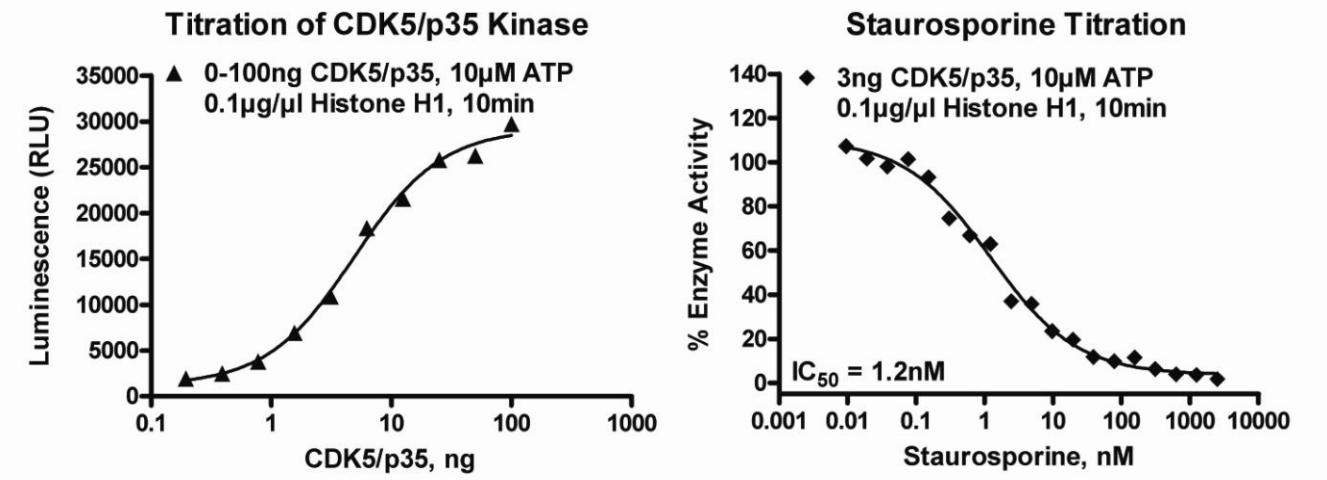

Figure 3. CDK5/p35 Kinase Assay Development. (A) CDK5/p35 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 3ng of CDK5/p35 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CDK5/p35 Kinase Enzyme System	Promega	V3271
ADP-Glo™ + CDK5/p35 Kinase Enzyme System	Promega	V9551

CDK5/p35 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.