ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

CDK7/CyclinH1/MNAT1 Kinase Assay

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Scientific Background:

CDK7 gene is a member of the cyclin-dependent protein kinase family that is important regulators of cell cycle progression (1). CDK7 forms a trimeric complex with cyclin H and MAT1, which functions as a CDK-activating kinase (CAK). CDK7 is an essential component of the transcription factor TFIIH that is involved in transcription initiation and DNA repair. CDK7 is thought to serve as a direct link between the regulation of transcription and the cell cycle (2).

1. Fisher, R. P.; A novel cyclin associates with MO15/CDK7 to form the CDK-activating kinase. Cell 78: 713-724, 1994.

2. Larochelle, S. Requirements for Cdk7 in the assembly of Cdk1/cyclin B and activation of Cdk2 revealed by chemical genetics in human cells. Molec. Cell 25: 839-850, 2007.

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.

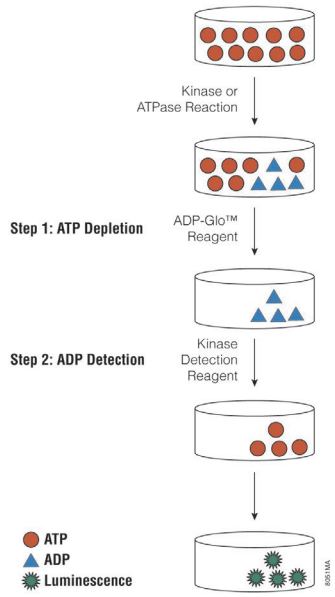

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

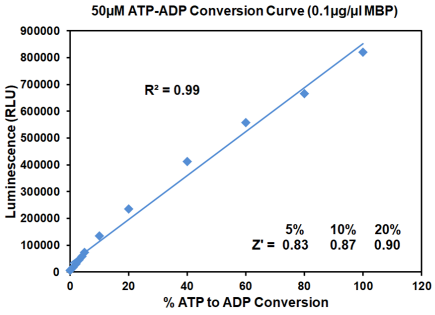

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction 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 indicated time (See Figure 3).
- 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 0.5-1 second).

Table 1. 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.

Enzyme, ng	300	150	75	37.50	18.75	9.38	4.69	0
Luminescence	356,754	131,034	72,643	32,701	18,186	8,861	5,408	2,575
S/B	139	51	28	13	7	3	2	1
% Conversion	48	15	7	1	0	0	0	0

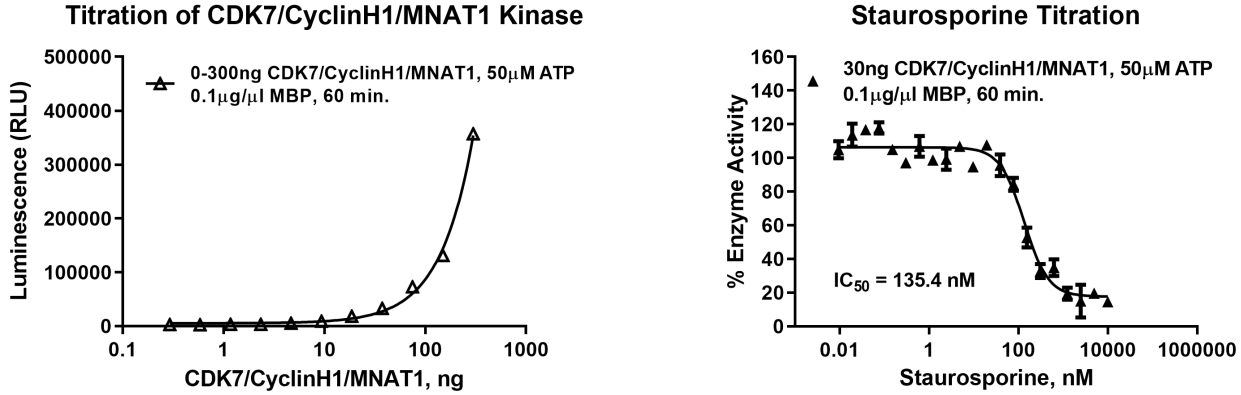

Figure 3. CDK7/CyclinH1/MNAT1 Kinase Assay Development. (A) CDK7/CyclinH1/MNAT1 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 30ng of CDK7/CyclinH1/MNAT1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
CDK7/CyclinH1/MNAT1 Kinase Enzyme System	10µg	VA7402
	1mg	VA7403
ADP-Glo™ + CDK7/CyclinH1/MNAT1 Kinase Enzyme System	1 Each	VA7404