ADP-Glo™ Kinase Assay Application Notes
SER-THR KINASE SERIES: CDK6/CyclinD3

CDK6/CyclinD3 Kinase Assay
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Scientific Background:

CDK6 is a member of the cyclin-dependent family of protein kinases that are important regulators of cell cycle progression. CDK6 activity is regulated by the D-type cyclins and members of the INK4 family of CDK inhibitors (1). The CDK6 kinase activity is detected in mid-G1 phase of the cell cycle and is responsible for the phosphorylation and regulation of the activity of tumor suppressor protein Rb. Although CDK6 and CDK4 can both phosphorylate multiple residues in the Rb protein, they do so with different residue selectivities in vitro; CDK6 phosphorylates Thr821 while CDK4 phosphorylates Thr826 on Rb protein (2).


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 250µM 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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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 60 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. CDK6/CyclinD3 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.

<table>
<thead>
<tr>
<th>CDK6/CyclinD3, ng</th>
<th>200</th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>13</th>
<th>6.3</th>
<th>3.1</th>
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<tr>
<td>RLU</td>
<td>885779</td>
<td>296450</td>
<td>169291</td>
<td>72676</td>
<td>27640</td>
<td>12436</td>
<td>7223</td>
<td>4709</td>
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<tr>
<td>S/B</td>
<td>188</td>
<td>63</td>
<td>36</td>
<td>15</td>
<td>6</td>
<td>2.6</td>
<td>1.5</td>
<td>1</td>
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<tr>
<td>% Conversion</td>
<td>82</td>
<td>27</td>
<td>15</td>
<td>5</td>
<td>1.2</td>
<td>0.5</td>
<td>0.3</td>
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</table>

Figure 3. CDK6/CyclinD3 Kinase Assay Development. (A) CDK6/CyclinD3 enzyme was titrated using 250μM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 20ng of CDK6/CyclinD3 to determine the potency of the inhibitor (IC₅₀).

Assay Components and Ordering Information:

<table>
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<tr>
<th>Products</th>
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<th>Cat.#</th>
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<tr>
<td>ADP-Glo™ Kinase Assay</td>
<td>Promega</td>
<td>V9101</td>
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<tr>
<td>CDK6/CyclinD3 Kinase Enzyme System</td>
<td>Promega</td>
<td>V4510</td>
</tr>
<tr>
<td>ADP-Glo™ + CDK6/CyclinD3 Kinase Enzyme System</td>
<td>Promega</td>
<td>V4511</td>
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<tr>
<td>CDK6/CyclinD3 Kinase Buffer: 40mM Tris,7.5; 20mM MgCl₂; 0.1mg/ml BSA; 50μM DTT.</td>
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