CLK3 Kinase Assay
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Scientific Background:
CLK3, also known as CDC-like kinase 3, encodes a serine/threonine type protein kinase with a non-conserved N-terminal domain. A long and short isoform (phclk3 and pclk3/152) result from alternative splicing and coexist in different tissues (1). The CLK3 protein has the molecular functions of ATP binding, nucleotide binding, protein serine/threonine kinase activity, protein-tyrosine kinase activity and transferase activity and the CLK3 protein localize in both the cytoplasm and nuclear compartment. CLK3 is thought to regulate the intranuclear distribution of the serine/arginine-rich (SR) family of splicing factors.


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.
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).

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Table 1. CLK3 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>
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<th>CLK3, ng</th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>13</th>
<th>6.3</th>
<th>3.1</th>
<th>1.6</th>
<th>0.8</th>
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<td>45</td>
<td>33</td>
<td>25</td>
<td>13</td>
<td>8</td>
<td>5</td>
<td>3</td>
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<td>1</td>
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<tr>
<td>% Conversion</td>
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<td>17</td>
<td>12</td>
<td>4</td>
<td>2.2</td>
<td>1.4</td>
<td>0.6</td>
<td>0.2</td>
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</table>

Staurosporine Titration

- 10ng CLK3, 50μM ATP
- 0.1µg/µl MBP, 60 min.

IC50 = 3.88 μM

Figure 3. CLK3 Kinase Assay Development. (A) CLK3 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of CLK3 to determine the potency of the inhibitor (IC50).