TNIK Kinase Assay
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Scientific Background:
TNIK or TRAF2 and NCK interacting kinase is characterized by an N-terminal kinase domain and a C-terminal GCK domain that serves a regulatory function (1). TNIK is mainly expression in brain, heart, and spleen and it is a specific effector of RAP2 which regulate actin cytoskeleton (2). TNIK is autophosphorylated in a manner dependent upon lys54 in the ATP-binding pocket of its kinase domain and plays a main role in cytoskeleton regulation.


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 0.5-1 second).

Table 1. TNIK 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>TNIK, ng</th>
<th>50</th>
<th>25</th>
<th>12.5</th>
<th>6.25</th>
<th>3.13</th>
<th>1.56</th>
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<tbody>
<tr>
<td>RLU</td>
<td>50</td>
<td>25</td>
<td>13</td>
<td>6.3</td>
<td>3.1</td>
<td>1.6</td>
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<tr>
<td>S/B</td>
<td>16903</td>
<td>8133</td>
<td>5452</td>
<td>3215</td>
<td>1644</td>
<td>1261</td>
<td>1083</td>
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<td>% Conversion</td>
<td>16</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>1.5</td>
<td>1.2</td>
<td>1</td>
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</table>

Figure 3. TNIK Kinase Assay Development. (A) TNIK enzyme was titrated using 25μM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 30ng of TNIK to determine the potency of the inhibitor (IC₅₀).

Assay Components and Ordering Information:

<table>
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<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>TNIK Kinase Enzyme System</td>
<td>Promega</td>
<td>V4158</td>
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<tr>
<td>ADP-Glo™ + TNIK Kinase Enzyme System</td>
<td>Promega</td>
<td>V4159</td>
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TNIK Kinase Buffer: 40mM Tris; 7.5; 20mM MgCl₂; 0.1mg/ml BSA; 2.5mM MnCl₂; 50μM DTT.