ERK1 Kinase Assay

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Scientific Background:

ERK1 is a protein serine/threonine kinase that is a member of the extracellular signal-regulated kinases (ERKs) which are activated in response to numerous growth factors and cytokines (1). Activation of ERK1 requires both tyrosine and threonine phosphorylation that is mediated by MEK. ERK1 is ubiquitously distributed in tissues with the highest expression in heart, brain and spinal cord. Activated ERK1 translocates into the nucleus where it phosphorylates various transcription factors (e.g., Elk-1, c-Myc, c-Jun, c-Fos, and C/EBP beta).


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).

**Table 1. ERK1 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>ERK1, ng</th>
<th>200</th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>12.5</th>
<th>6.3</th>
<th>3.1</th>
<th>1.6</th>
<th>0.8</th>
<th>0.4</th>
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<tbody>
<tr>
<td>RLU</td>
<td>366887</td>
<td>202326</td>
<td>134418</td>
<td>96021</td>
<td>69008</td>
<td>48367</td>
<td>36279</td>
<td>24015</td>
<td>15792</td>
<td>8928</td>
<td>3608</td>
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<tr>
<td>S/B</td>
<td>102</td>
<td>56</td>
<td>37</td>
<td>27</td>
<td>19</td>
<td>13</td>
<td>10</td>
<td>7</td>
<td>4.4</td>
<td>2.5</td>
<td>1</td>
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<tr>
<td>% Conversion</td>
<td>114</td>
<td>61</td>
<td>40</td>
<td>27</td>
<td>19</td>
<td>12</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

**Figure 3. ERK1 Kinase Assay Development.** (A) ERK1 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) K252a dose response was created using 4ng of ERK1 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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<td>ERK1 Kinase Enzyme System</td>
<td>Promega</td>
<td>V1951</td>
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<tr>
<td>ADP-Glo™ + ERK1 Kinase Enzyme System</td>
<td>Promega</td>
<td>V9281</td>
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ERK1 Kinase Buffer: 40mM Tris,7.5; 20mM MgCl2; 0.1mg/ml BSA; 50µM DTT.