PI3K(p110α/p85α) Kinase Assay

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Scientific Background:

Phosphatidylinositol 3-kinase is composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The catalytic subunit uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns(4,5)P2. This gene has been found to be oncogenic and has been implicated in cervical cancers.


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 25μM 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.

Protocol

- Prepare PI3K Reaction Buffer/Lipid Substrate mixture.
- Dilute PI3K Enzyme into prepared PI3K Reaction Buffer/Lipid Substrate mixture (amount defined from table 1).
- Add to the wells of 384 low volume plate:
  - 0.5 µl of inhibitor or vehicle
  - 4 µl of enzyme/Lipid mixture
  - 0.5 µl of 250µM ATP in water
- Incubate at room temperature for 60 minutes.
- Add 5 µl of ADP-Glo™ Reagent (with 10mM MgCl2)
- 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. PI3K(p110α/p85α) 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>PI3K (p110α/p85α), ng</th>
<th>32</th>
<th>16</th>
<th>8</th>
<th>4</th>
<th>2</th>
<th>1</th>
<th>0.5</th>
<th>0.25</th>
<th>0.125</th>
<th>0</th>
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<tbody>
<tr>
<td>RLU</td>
<td>121</td>
<td>137</td>
<td>148</td>
<td>151</td>
<td>154</td>
<td>157</td>
<td>161</td>
<td>165</td>
<td>170</td>
<td>174</td>
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<tr>
<td>S/B</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
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<tr>
<td>% Conversion</td>
<td>90</td>
<td>71</td>
<td>48</td>
<td>23</td>
<td>11</td>
<td>4.4</td>
<td>1.6</td>
<td>0.4</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

Figure 3. PI3K(p110α/p85α) Kinase Assay Development. (A) PI3K(p110α/p85α) enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) AS605240 dose response was created using 5ng of PI3K(p110α/p85α) 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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<tr>
<td>ADP-Glo™ Kinase Assay with PI:3PS</td>
<td>1,000 Assays</td>
<td>V1781</td>
</tr>
<tr>
<td>ADP-Glo™ Kinase Assay with PIP2:3PS</td>
<td>1,000 Assays</td>
<td>V1791</td>
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<tr>
<td>PI3K(p110α/p85α), 20µg</td>
<td>200µl</td>
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<tr>
<td>PI3K-Glo™ Class I Profiling Kit</td>
<td>1 each</td>
<td>V1690</td>
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</table>

PI3K Kinase Buffer: 50mM HEPES, pH 7.5; 50mM NaCl; 3mM MgCl2; 0.025mg/ml BSA.