FLT3 (D835Y) Kinase Assay

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Scientific Background:

FLT3 is a receptor tyrosine kinase that has been shown to play a role in proliferation and survival of hematopoietic progenitor cells as well as differentiation of early B lymphoid progenitors (1). FLT3 consists of an extracellular domain composed of five immunoglobulin-like domains, one transmembrane region, and a cytoplasmic kinase domain split into two parts by a kinase-insert domain. FLT3 is the most frequently mutated gene in cases of acute myelogenous leukemia (AML). About 30% to 35% of patients have either internal tandem duplications (ITDs) in the juxtamembrane domain or mutations in the activating loop of FLT3 (2). The consequence of either FLT3-ITD or activating loop mutations is the constitutive activation of the tyrosine kinase activity.


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 Tyrosine 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 0.5-1sec).

Table 1. FLT3 (D835Y) 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>FLT3 (D835Y), ng</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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<tr>
<td>Luminescence</td>
<td>190571</td>
<td>145174</td>
<td>83466</td>
<td>61566</td>
<td>41865</td>
<td>21216</td>
<td>9627</td>
<td>4257</td>
<td>2752</td>
<td>1437</td>
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<tr>
<td>S/B</td>
<td>133</td>
<td>101</td>
<td>58</td>
<td>43</td>
<td>29</td>
<td>15</td>
<td>7</td>
<td>3</td>
<td>2</td>
<td>1</td>
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<tr>
<td>% Conversion</td>
<td>70</td>
<td>49</td>
<td>25</td>
<td>17</td>
<td>11</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>0.4</td>
<td>0</td>
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</table>

Figure 3. FLT3 (D835Y) Kinase Assay Development. (A) FLT3 (D835Y) 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 FLT3 (D835Y) 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</td>
<td>Promega</td>
<td>V9101</td>
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<td>FLT3 (D835Y) Kinase Enzyme System</td>
<td>Promega</td>
<td>V4514</td>
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<tr>
<td>ADP-Glo™ + FLT3 (D835Y) Kinase Enzyme System</td>
<td>Promega</td>
<td>V4515</td>
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<td>FLT3 (D835Y) Kinase Buffer: 40mM Tris, pH 7.5; 20mM MgCl₂; 0.1mg/ml BSA; 50µM DTT.</td>
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