ADP-Glo™ Kinase Assay Application Note
Tyrosine Kinase Series

C-KIT (T670E) Kinase Assay
By Juliano Alves, Laurie Engel, Said A. Goueli, and Hicham Zegzouti, Promega Corporation

Scientific Background:

C-KIT (T670E) is the human homolog of the proto-oncogene c-kit which was first identified as the cellular homolog of the feline sarcoma viral oncogene v-kit. C-KIT (T670E) is a type 3 transmembrane receptor for MGF (mast cell growth factor, also known as stem cell factor). C-KIT (T670E) function in hematopoiesis, melanogenesis, and gametogenesis (1) and mutations in this gene are associated with gastrointestinal stromal tumors, mast cell disease, acute myelogenous leukemia, and piebaldism. C-KIT (T670E) signaling is involved in human skin pigmentation and that this signaling pathway is regulated by sKIT (2). It is also help in regulating primordial germ cell growth.


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.
ADP-Glo™ Kinase Assay Application Note
Tyrosine Kinase Series

The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

Short Protocol

• Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction 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 indicated time (See Figure 3).

- 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. 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>Enzyme, ng</th>
<th>300</th>
<th>150</th>
<th>75</th>
<th>37.50</th>
<th>18.75</th>
<th>9.38</th>
<th>2.34</th>
<th>1.17</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminescence</td>
<td>565,796</td>
<td>288,237</td>
<td>182,023</td>
<td>102,272</td>
<td>55,297</td>
<td>24,736</td>
<td>6,358</td>
<td>4,465</td>
<td>2,674</td>
</tr>
<tr>
<td>S/B</td>
<td>212</td>
<td>108</td>
<td>68</td>
<td>38</td>
<td>21</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>% Conversion</td>
<td>43</td>
<td>21</td>
<td>13</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 3. c-KIT (T670E) Kinase Assay Development. (A) c-KIT (T670E) enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 18ng of c-KIT (T670E) to determine the potency of the inhibitor (IC50).

Ordering Information:

<table>
<thead>
<tr>
<th>Products</th>
<th>Size</th>
<th>Cat. #</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-KIT (T670E) Kinase Enzyme System</td>
<td>10µg</td>
<td>VA7411</td>
</tr>
<tr>
<td></td>
<td>1mg</td>
<td>VA7412</td>
</tr>
<tr>
<td>ADP-Glo™ + c-KIT (T670E) Kinase Enzyme System</td>
<td>1 Each</td>
<td>VA7413</td>
</tr>
</tbody>
</table>