ADP-Glo™ Kinase Assay Application Notes

TYROSINE KINASE SERIES: FGFR4

FGFR4 Kinase Assay

By Kevin Hsiao, M.S., Juliano Alves, Ph.D., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

FGFR4 is a member of the fibroblast growth factor receptor family which play a role in mitogenesis and differentiation (1). FGFR4 preferentially binds acidic fibroblast growth factor and is overexpressed in gynecological tumor samples, suggesting a role in breast and ovarian tumorigenesis. FGFR4 gene expression is up-regulated in doxorubicin-treated, apoptosis-resistant cancer cell clones (2). Ectopic expression of FGFR4 in cancer cells leads to reduced apoptosis sensitivity on treatment with doxorubicin or cyclophosphamide, whereas knockdown of endogenous FGFR4 expression in breast cancer cell lines has the opposite effect.


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 50µM 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.

Promega Corporation • 2800 Woods Hollow Road • Madison, WI 53711-5399 USA • Telephone 608-274-4330 • Fax 608-277-2601

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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 0.5-1second).

Table 1. FGFR4 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>FGFR4, 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>
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<td>Luminescence</td>
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<td>107229</td>
<td>68142</td>
<td>38438</td>
<td>22356</td>
<td>10785</td>
<td>5845</td>
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<tr>
<td>S/B</td>
<td>205.7</td>
<td>133.9</td>
<td>85.1</td>
<td>48.0</td>
<td>27.9</td>
<td>13.5</td>
<td>7.3</td>
<td>3.5</td>
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<td>1</td>
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<tr>
<td>% Conversion</td>
<td>63.3</td>
<td>41.3</td>
<td>26.3</td>
<td>15.0</td>
<td>8.8</td>
<td>4.4</td>
<td>2.5</td>
<td>1.4</td>
<td>0.9</td>
<td>0</td>
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</table>

Figure 3. FGFR4 Kinase Assay Development. (A) FGFR4 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 FGFR4 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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<td>Promega</td>
<td>V9101</td>
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<tr>
<td>FGFR4 Kinase Enzyme System</td>
<td>Promega</td>
<td>V4062</td>
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<tr>
<td>ADP-Glo™ + FGFR4 Kinase Enzyme System</td>
<td>Promega</td>
<td>V4063</td>
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FGFR4 Kinase Buffer: 40mM Tris,7.5; 20mM MgCl2; 0.1mg/ml BSA; 2.5mM MnCl2; 50µM DTT.