ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

FRK Kinase Assay

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Scientific Background:

FRK (fyn-related kinase) or Rak is a nuclear tyrosine kinase and member of the Src sub-family. Restricted expression of FRK is detected in a broad range of cell lines with highest levels in epithelial cells. Increased expression of FRK has been shown in breast and renal cell carcinoma cell lines. In addition, the retinoblastoma tumor susceptibility gene product pRb associates with FRK in vitro and in vivo (1). Overexpression of FRK in beta-cells from the pancreas increases the susceptibility of these cells to beta-cell-toxic events (hallmark of Type I diabetes) (2).

1. Craven, R J. et al: The nuclear tyrosine kinase Rak associates with the retinoblastoma protein pRb. Cancer Res. 1995 Sep 15;55(18):3969-72.

2. Welsh, M. et al: The tyrosine kinase FRK/RAK participates in cytokine-induced islet cell cytotoxicity. Biochem J. 2004 Aug 15;382(Pt 1):261-8.

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.

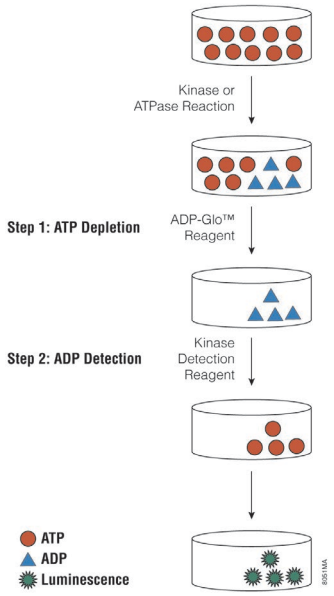

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

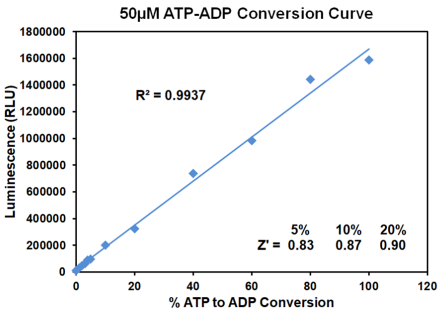

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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.

Enzyme, ng	160	80	40	20	10	5	2.50	0.31	0
Luminescence	678,941	306,283	207,549	101,711	64,846	35,386	20,016	5,554	3,333
S/B	204	92	62	31	19	11	6	2	1
% Conversion	58	26	17	8	5	2	1	0	0

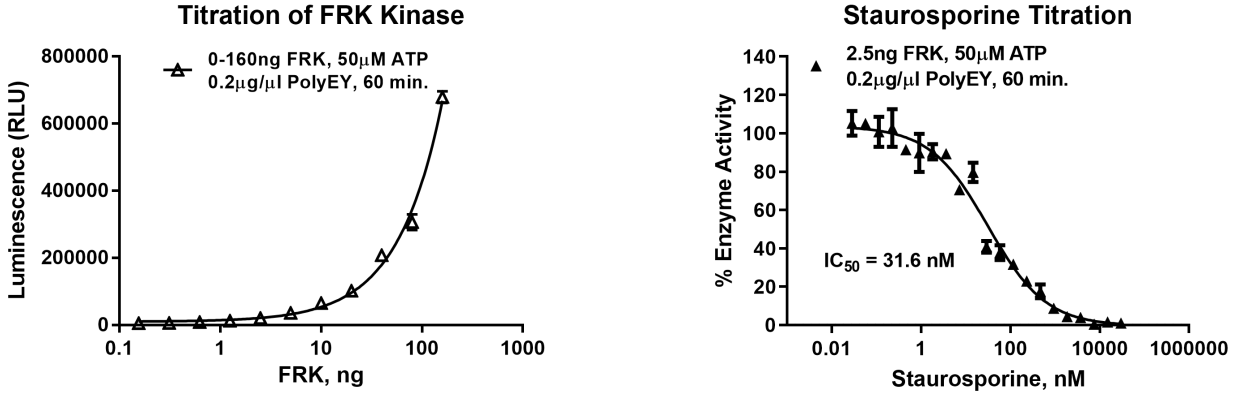

Figure 3. FRK Kinase Assay Development. (A) FRK enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2.5ng of FRK to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
FRK Kinase Enzyme System	10µg	VA7465
	1mg	VA7466
ADP-Glo™ + FRK Kinase Enzyme System	1 Each	VA7467