ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

FGR Kinase Assay

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Scientific Background:

FGR is a protooncogene that is a unique member of the tyrosine kinase gene family. Certain lymphomas (but not sarcomas or carcinomas) express FGR-related messenger RNA. This transcript is detected in Burkitt's lymphoma cell lines naturally infected with Epstein-Barr virus (EBV), but not in EBV-negative Burkitt's lymphoma cells (1). FGR expression is limited to normal peripheral blood granulocytes, monocytes, and alveolar macrophages, all of which contain 50 to 100 copies of c-fgr mRNA per cell (2).

1. Cheah, MS. et al: fgr proto-oncogene mRNA induced in B lymphocytes by Epstein-Barr virus infection. Nature. 1986 Jan 16-22;319(6050):238-40..

2. Willman, CL. et al: Differential expression and regulation of the c-src and c-fgr protooncogenes in myelomonocytic cells. Proc Natl Acad Sci U S A. 1987 Jul;84(13):4480-4..

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.

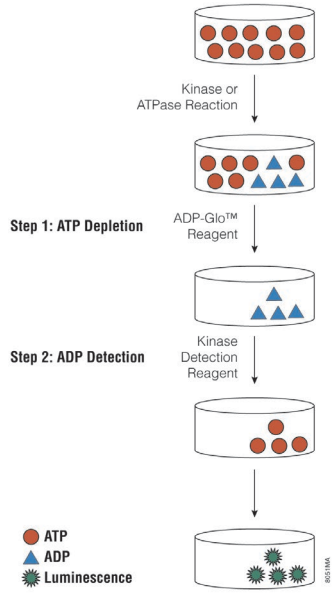

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

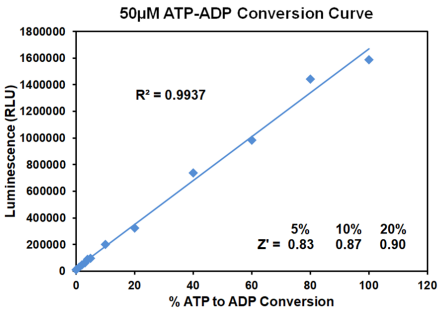

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	200	100	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0
Luminescence	948,225	771,137	734,145	669,785	430,312	251,925	156,495	88,863	55,535	26,711	24,698	6,600
S/B	144	117	111	101	65	38	24	13	8	4	4	1
% Conversion	68	55	52	47	30	17	10	5	3	0	0	0

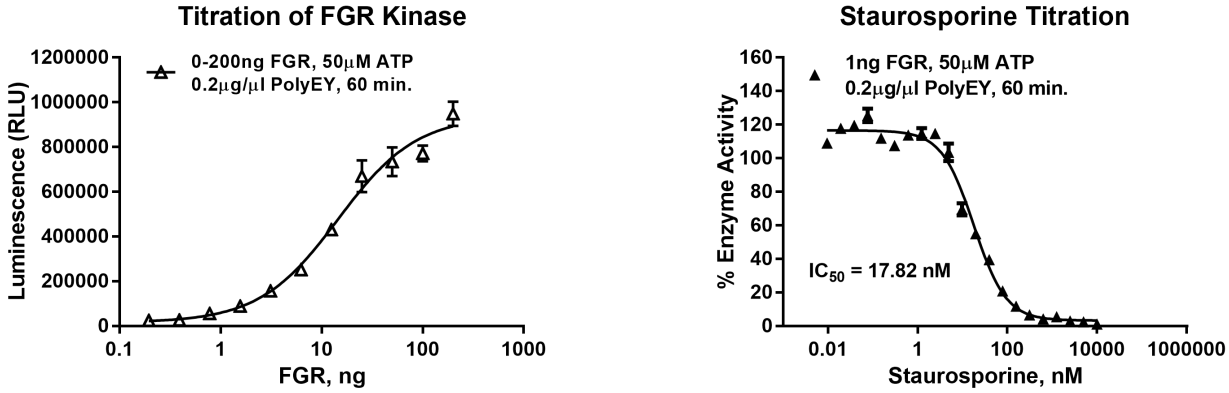

Figure 3. FGR Kinase Assay Development. (A) FGR enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1ng of FGR to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
FGR Kinase Enzyme System	10µg	VA7462
	1mg	VA7463
ADP-Glo™ + FGR Kinase Enzyme System	1 Each	VA7464