ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

FER Kinase Assay

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Scientific Background:

FER is a member of the FPS/FES family of non-transmembrane receptor tyrosine kinases. FER plays a role in regulating cytoskeletal rearrangements and inside out signaling that accompany receptor ligand, cell matrix and cell-cell interactions (1). Genetic analysis using transgenic mouse models implicate FER in the regulation of inflammation and innate immunity. FER-deficient mice displayed enhanced recruitment of leukocytes in response to local LPS challenge. FER is required for cell-cycle progression in malignant cells. Decreasing the level of FER using RNAi impeded the proliferation of prostate and breast carcinoma cells and led to their arrest at the G0/G1 phase (2).

1. Greer, P.: Closing in on the biological functions of Fps/Fes and Fer. Nat Rev Mol Cell Biol. 2002 Apr;3(4):278-89.

2. Pasder, O. et al: Downregulation of Fer induces PP1 activation and cell-cycle arrest in malignant cells. Oncogene. 2006 Jul 13;25(30):4194-206.

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.

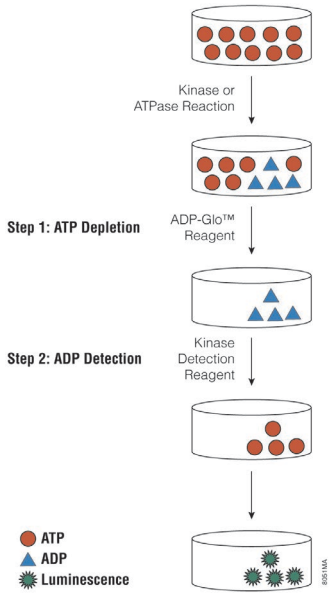

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

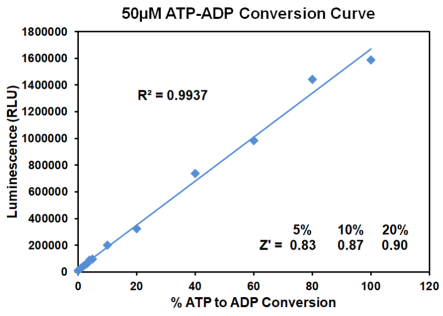

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	1.25	0.63	0.31	0.16	0
Luminescence	924,947	819,432	832,481	672,147	546,397	406,494	276,834	174,465	115,778	60,212	35,793	5,133
S/B	180	160	162	131	106	79	54	34	23	12	7	1
% Conversion	66	58	59	48	38	28	19	11	7	3	1	0

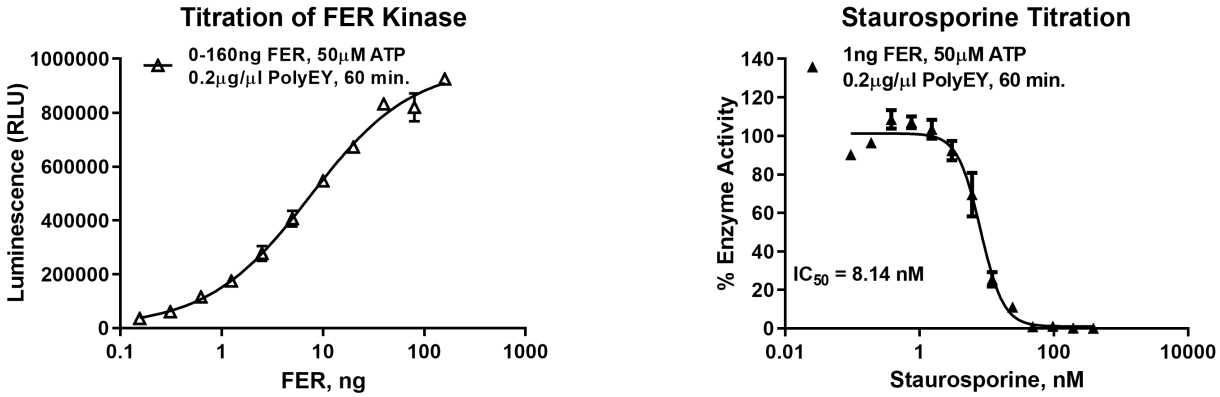

Figure 3. FER Kinase Assay Development. (A) FER enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1ng of FER to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
FER Kinase Enzyme System	10µg	VA7453
	1mg	VA7454
ADP-Glo™ + FER Kinase Enzyme System	1 Each	VA7455