FMS Kinase Assay

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Scientific Background:

FMS is a proto-oncogene that encodes the tyrosine kinase transmembrane receptor for colony stimulating factor 1 (CSF1). FMS is homodimeric that contains a so-called kinase insert domain and is a member of the CSF1/PDGF receptor family of tyrosine-protein kinases. FMS mediates most if not all of the biological effects of CSF1 which control the production, differentiation, and function of cell of the monocyte/macrophage lineage (1). Mutations in FMS have been associated with providing sustained signals for cell growth and a predisposition to myeloid malignancy (2).

1. Sherr, C J.: Regulation of mononuclear phagocyte proliferation by colony-stimulating factor-1. Int J Cell Cloning. 1990 Jan;8 Suppl 1:46-60.

2. Follows, G A. et al: c-FMS chromatin structure and expression in normal and leukaemic myelopoiesis. Oncogene. 2005 May 19;24(22):3643-51.

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.

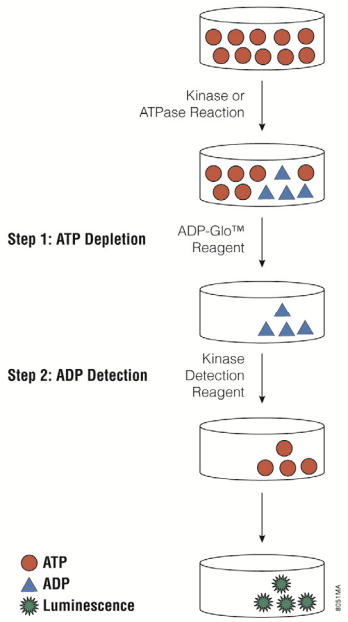


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

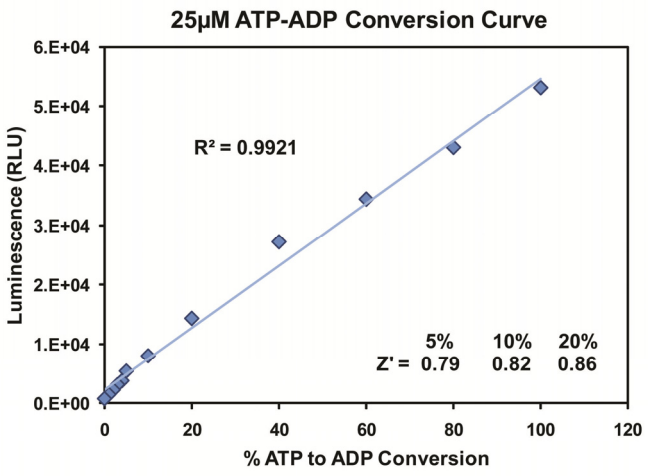


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

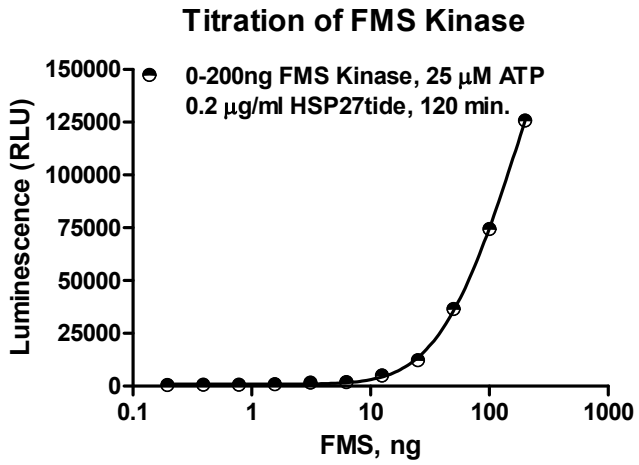
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

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. FMS 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.

FMS, ng	200	100	50	25	12.5	6.3	3.1	0
Luminescence	125778	74381	36569	12324	5019	1938	1641	592
S/B	212	126	62	21	8	3	2.8	1
% Conversion	69	41	20	6	2	1	0.5	0



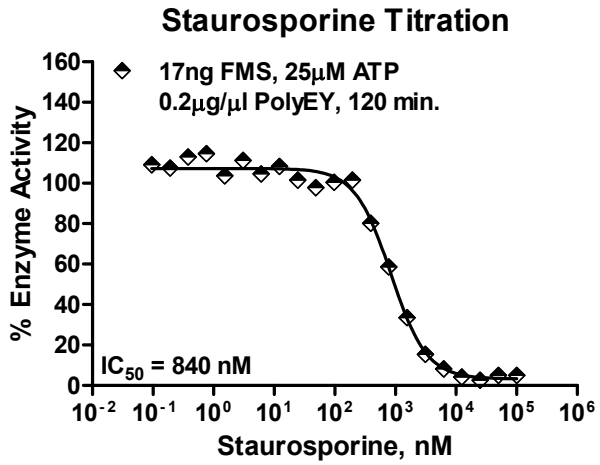


Figure 3. FMS Kinase Assay Development. **(A) FMS enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 17ng of FMS to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
FMS Kinase Enzyme System	Promega	V4022
ADP-Glo™ + FMS Kinase Enzyme System	Promega	V4023

FMS Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 2.5mM $MnCl_2$, 50µM DTT.

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