FLT1 Kinase Assay

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Scientific Background:

FLT1, also known as fms-like tyrosine kinase 1, is a member of the VEGFR family and is related to the oncogene ROS (1). The tyrosine kinase activity of FLT1 has been shown to play a key role in control of cell proliferation and differentiation. FLT1 is a receptor for vascular endothelial growth factor (VEGF) and it has been observed that the ratio of FLT1 mRNA to VEGF mRNA correlates with tumor angiogenesis and prognosis in non-small cell lung cancers (2). In addition to the full-length receptor, FLT1 gene also encodes a soluble form of receptor due to alternative splicing. Both the full length and soluble form of FLT1 show strong binding affinity for VEGF.

1. Autiero, M. et al: Role of PlGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1. Nature, 2003; 9: 936-943.

2. Fong, G.-H. et al: Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. Nature,1995; 376: 65-69.

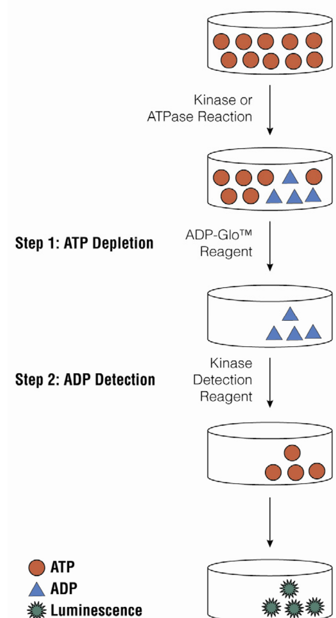


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

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.

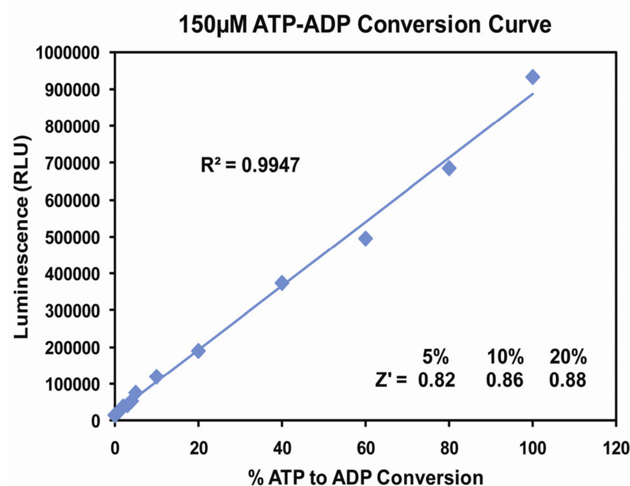


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 150µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.

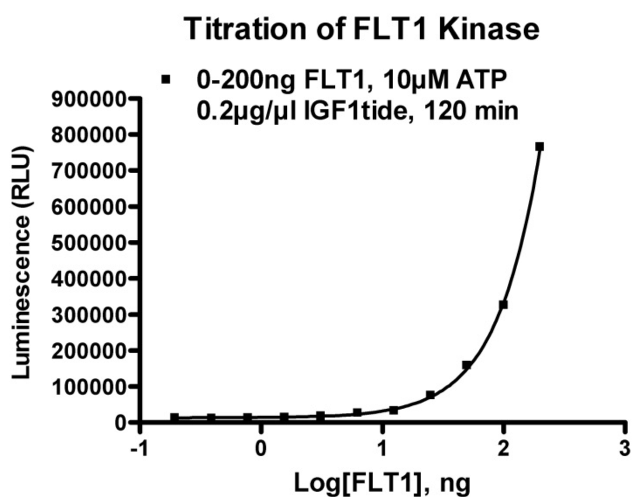
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 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. FLT1 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.

FLT1, ng	200	100	50	25	12.5	6.25	0
Luminescence	765363	326118	158451	75353	32780	26934	13527
S/B	56.6	24.1	11.7	5.6	2.4	2.0	1.0
% Conversion	86	35	16	6	2	1	0



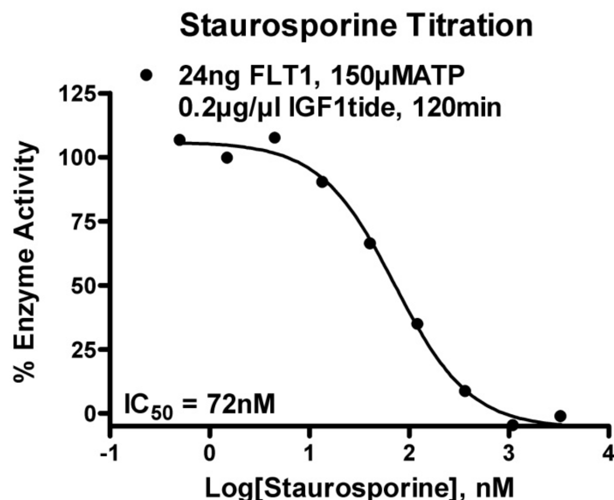


Figure 3. FLT1 Kinase Assay Development: (A) FLT1 enzyme was titrated using 150μM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 24ng of FLT1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
ABL1 Kinase Enzyme System	Promega	V3001
ADP-Glo + ABL1 Kinase Enzyme System	Promega	V9331

FLT1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 2mM $MnCl_2$; 50μM DTT.

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