

FLT3 (N676K) Kinase Assay

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Scientific Background:

FLT3 is a receptor tyrosine kinase that has been shown to play a role in proliferation and survival of hematopoietic progenitor cells as well as differentiation of early B lymphoid progenitors (1). FLT3 consists of an extracellular domain composed of five immunoglobulin-like domains, one transmembrane region, and a cytoplasmic kinase domain split into two parts by a kinase-insert domain. FLT3 is the most frequently mutated gene in cases of acute myelogenous leukemia (AML). About 30% to 35% of patients have either internal tandem duplications (ITDs) in the juxtamembrane domain or mutations in the activating loop of FLT3 (2). The consequence of either FLT3-ITD or activating loop mutations is the constitutive activation of the tyrosine kinase activity.

1. Christensen, J L. et al : Flk-2 is a marker in hematopoietic stem cell differentiation: a simple method to isolate long-term stem cells. *Proc. Nat. Acad. Sci.* 98: 14541-14546, 2001.
2. Gilliland, D G. et al: Role of FLT3 in leukemia. *Curr Opin Hematol.* 2002 Jul;9(4):274-81.

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.

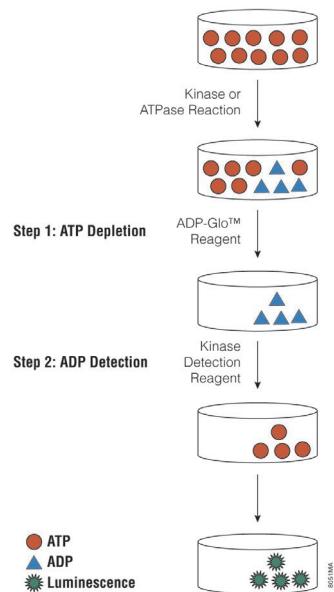


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

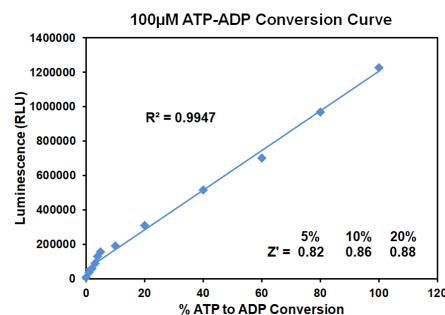


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

**Promega**

ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

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 μ l of inhibitor or (5% DMSO)
 - ✓ 2 μ l of enzyme (defined from table 1)
 - ✓ 2 μ l of substrate/ATP mix
- Incubate at room temperature for indicated time (See Figure 3).
- Add 5 μ l of ADP-Glo™ Reagent.
- Incubate at room temperature for 40 minutes.
- Add 10 μ l 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	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0
Luminescence	1,222,285	707,465	312,128	156,565	85,218	51,120	29,669	18,780	11,060
S/B	111	64	28	14	8	5	3	2	1
% Conversion	69	38	15	5	1	0	0	0	0

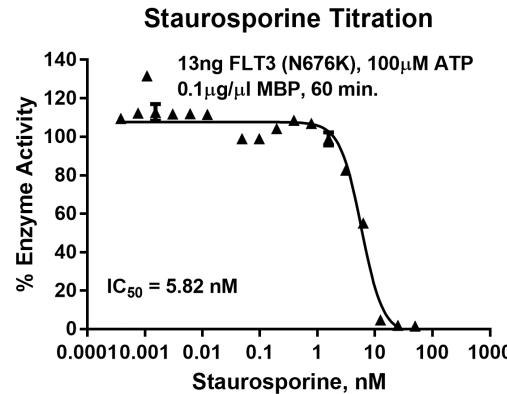
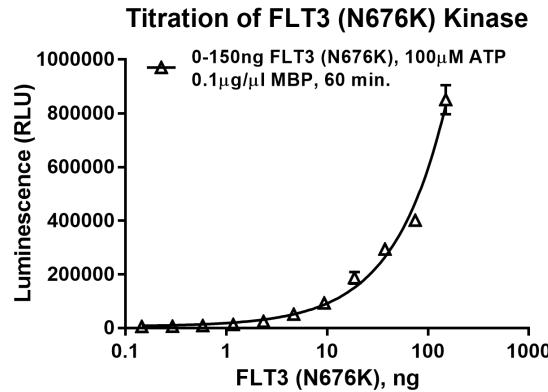


Figure 3. FLT3 (N676K) Kinase Assay Development. (A) FLT3 (N676K) enzyme was titrated using 100 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 13ng of FLT3 (N676K) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:



Products	Size	Cat. #
FLT3 (N676K) Kinase Enzyme System	10 μ g	VA7171
	1mg	VA7172
ADP-Glo™ + FLT3 (N676K) Kinase Enzyme System	1 Each	VA7173