ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

FLT3 (ITD-NPOS) Kinase Assay

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Scientific Background:

Fms-related tyrosine kinase 3 (FLT3) is a gene that encodes for a tyrosine kinase that activates pathways in hematopoietic cells important in cellular proliferation. FLT3 is frequently mutated in acute myeloid leukemia (AML), myelodysplastic syndromes, other hematologic malignancies, and colorectal cancer. FLT3 mutations are observed in 24.3% of AML, of which internal tandem duplications (ITD) are the most frequent. FLT3 ITD occurs when sequences of less than ten to several hundred bases in length are repeated within the juxtamembrane domain are always “in-frame”.

1. http://www.mycancergenome.org/content/disease/acute-myeloid-leukemia/flt3/277/

2. Fathi AT, et al: Treatment of FLT3-ITD acute myeloid leukemia. Am J Blood Res. 1(2):175-89, 2011

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.

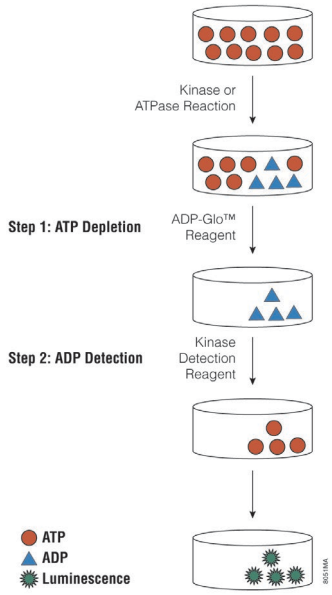

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

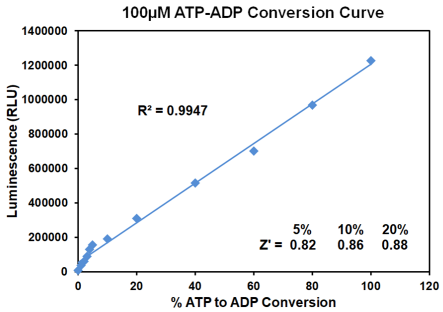

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z’ factors were determined using 200 replicates of each of the % conversions shown.

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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	150	75	37.50	18.75	9.38	4.69	2.34	0
Luminescence	744,772	287,663	151,468	94,198	42,784	20,633	10,797	5,660
S/B	132	51	27	17	8	4	2	1
% Conversion	60	20	9	4	0	0	0	0

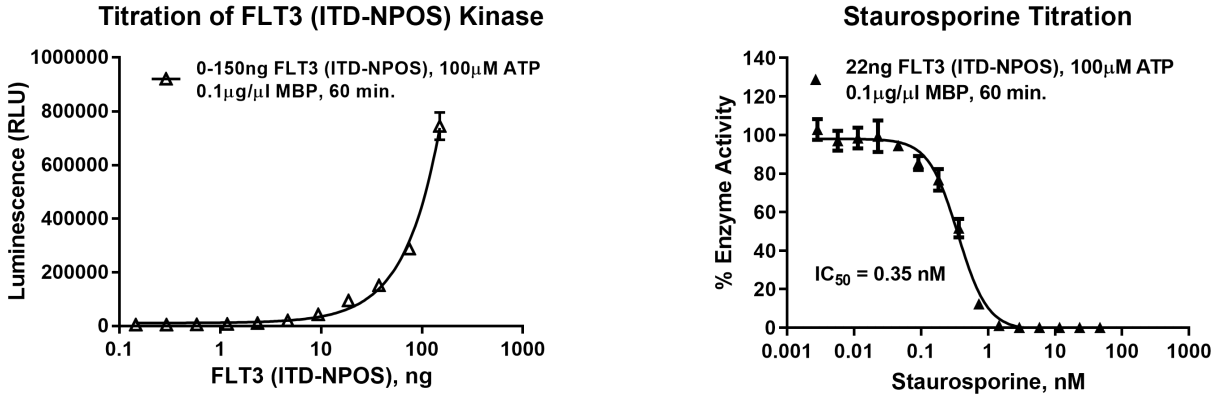

Figure 3. FLT3 (ITD-NPOS) Kinase Assay Development. (A) FLT3 (ITD-NPOS) enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 22ng of FLT3 (ITD-NPOS) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
FLT3 (ITD-NPOS) Kinase Enzyme System	10µg	VA7168
	1mg	VA7169
ADP-Glo™ + FLT3 (ITD-NPOS) Kinase Enzyme System	1 Each	VA7170