ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

c-KIT (N822K) Kinase Assay

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Scientific Background:

c-KIT is a proto-oncogene and a type 3 transmembrane receptor for MGF (mast cell growth factor, also known as stem cell factor). c-KIT was first identified as the cellular homolog of the feline sarcoma viral oncogene v-kit. c-KIT together with its ligand regulates growth and activation of a variety of hemopoietic and non-hemopoietic cells. Mutations in c-KIT are associated with gastrointestinal stromal tumors, mast cell disease, acute myelogenous leukemia, and piebaldism. Recently, deregulation of the KIT receptor TK by the prevalent activation loop mutation D816V has served as a focal point in therapeutic strategies aimed at curbing neoplastic mast cell growth (2).

1. Berger, S A.: Signaling pathways influencing SLF and c-kit-mediated survival and proliferation. Immunol Res. 2006;35(1-2):1-12.

2. Gotlib, J.: KIT mutations in mastocytosis and their potential as therapeutic targets. Immunol Allergy Clin North Am. 2006 Aug;26(3):575-92.

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.

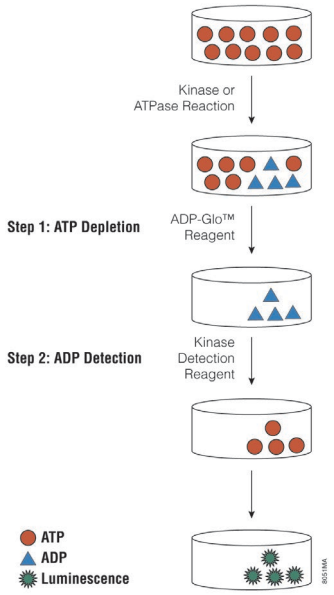

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

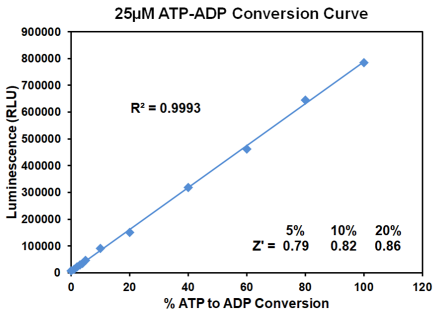

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	1.17	0.59	0.29	0
Luminescence	782,023	524,193	409,580	253,310	132,456	66,360	31,025	14,575	8,130	4,607	2,867
S/B	273	183	143	88	46	23	11	5	3	2	1
% Conversion	97	65	51	31	16	8	4	1	1	0	0

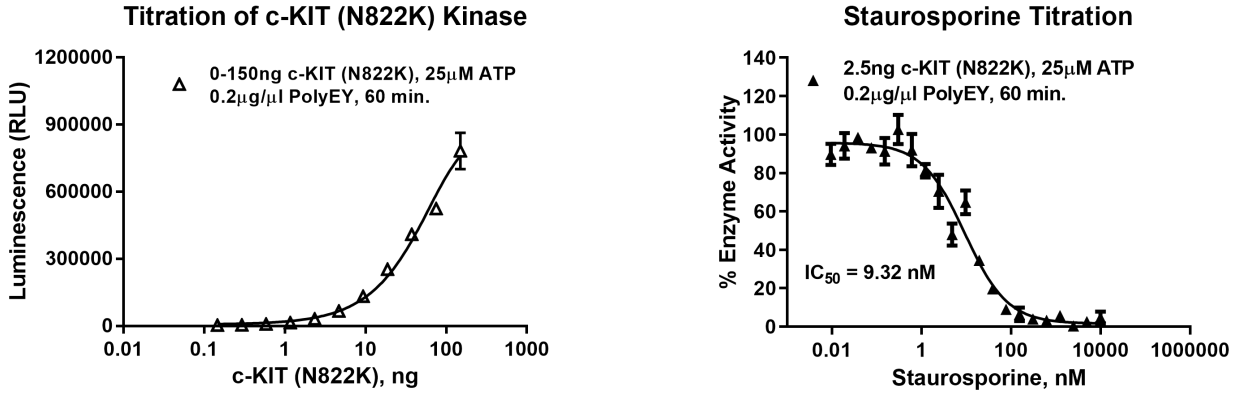

Figure 3. c-KIT (N822K) Kinase Assay Development. (A) c-KIT (N822K) enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2.5ng of c-KIT (N822K) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
c-KIT (N822K) Kinase Enzyme System	10µg	VA7054
	1mg	VA7055
ADP-Glo™ + c-KIT (N822K) Kinase Enzyme System	1 Each	VA7056