

## c-KIT (T670E) Kinase Assay

By Juliano Alves, Laurie Engel, Said A. Goueli, and Hicham Zegzouti, Promega Corporation

### Scientific Background:

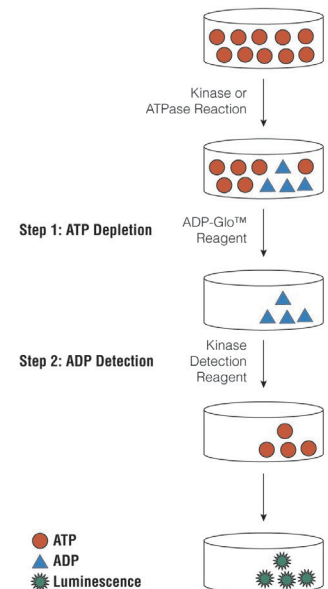
c-KIT (T670E) is the human homolog of the proto-oncogene c-kit which was first identified as the cellular homolog of the feline sarcoma viral oncogene v-kit. c-KIT (T670E) is a type 3 transmembrane receptor for MGF (mast cell growth factor, also known as stem cell factor). c-KIT (T670E) function in hematopoiesis, melanogenesis, and gametogenesis (1) and mutations in this gene are associated with gastrointestinal stromal tumors, mast cell disease, acute myelogenous leukemia, and piebaldism. c-KIT (T670E) signaling is involved in human skin pigmentation and that this signaling pathway is regulated by sKIT (2). It is also help in regulating primordial germ cell growth.

1. Rothschild, G. et.al: A role for Kit receptor signaling in Leydig cell steroidogenesis. *Biol. Reprod.* 69: 925-932, 2003.
2. Kasamatsu, S. et.al: Production of the soluble form of KIT, s-KIT, abolishes stem cell factor-induced melanogenesis in human melanocytes. *J. Invest. Derm.* 128: 1763-1772, 2008.

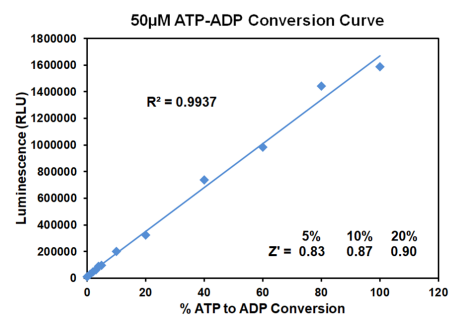
### ADP-Glo™ Kinase Assay

#### 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 50µM 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.



# ADP-Glo™ Kinase Assay Application Note Tyrosine Kinase Series

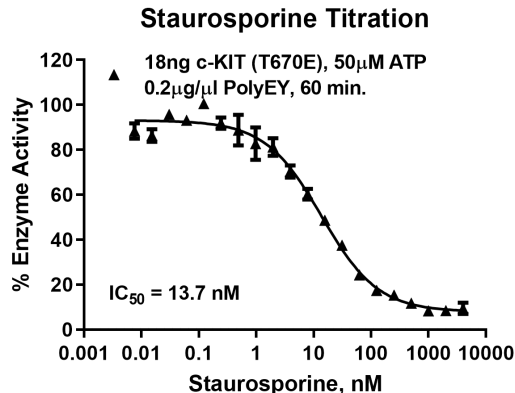
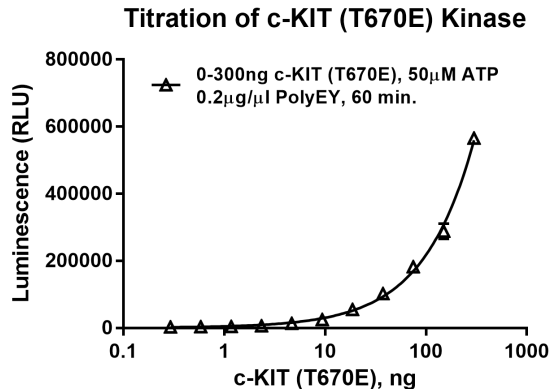
The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: <http://www.promega.com/KESProtocol>

## Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction buffer.
- Add to the wells of 384 low volume plate:
  - ✓ 1 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	300	150	75	37.50	18.75	9.38	2.34	1.17	0
Luminescence	565,796	288,237	182,023	102,272	55,297	24,736	6,558	4,465	2,674
S/B	212	108	68	38	21	9	2	2	1
% Conversion	43	21	13	7	3	1	0	0	0



**Figure 3. c-KIT (T670E) Kinase Assay Development.** (A) c-KIT (T670E) enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 18ng of c-KIT (T670E) to determine the potency of the inhibitor (IC<sub>50</sub>).



## Ordering Information:

Products	Size	Cat. #
c-KIT (T670E) Kinase Enzyme System	10µg	VA7411
	1mg	VA7412
ADP-Glo™ + c-KIT (T670E) Kinase Enzyme System	1 Each	VA7413