ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

FGFR3 (K650Q) Kinase Assay

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Scientific Background:

Fibroblast growth factor receptor 3 (FGFR3) is part of a family of fibroblast growth factor receptors that share similar structure and function. FGFR3 plays a role in several important cellular processes, including regulation of cell growth and division, determination of cell fate, formation of blood vessels, wound healing and embryo development (1). FGFR3 is involved in the development and maintenance of bone and brain tissue. Mutations in FGFR3 have been implicated in causing bladder cancer, cancer of white blood cells (multiple myeloma) and cervical cancer (2).

1. Chen, L. and Deng, C.X. Roles of FGF signaling in skeletal development and human genetic diseases. Front Biosci. 2005; 1(10):1961-1976.

2. Mhawech-Fauceglia, P. et al. 2006. FGFR3 and p53 protein expressions in patients with pTa and pT1 urothelial bladder cancer. Eur. J. Surg. Oncol. 2006; 32(2):231-237.

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.

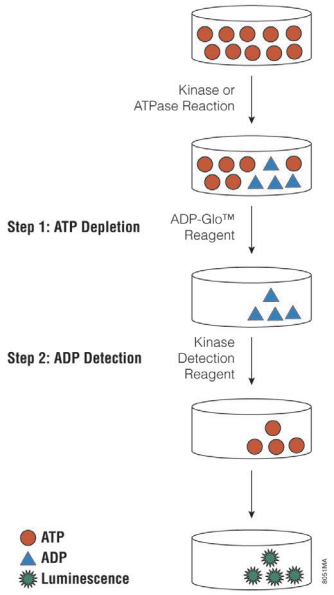

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

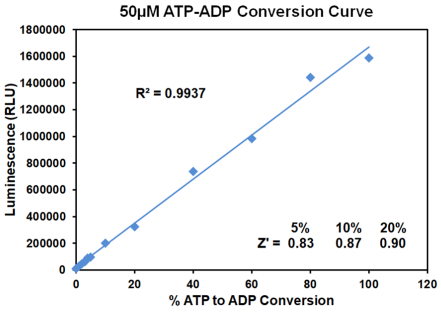

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	240	120	60	30	15	7.50	3.75	1.88	0.94	0.23	0
Luminescence	1,458,365	1,063,430	1,026,042	767,916	522,616	249,083	144,364	60,919	30,836	9,153	4,951
S/B	295	215	207	155	106	50	29	12	6	2	1
% Conversion	97	70	68	50	34	16	8	3	1	0	0

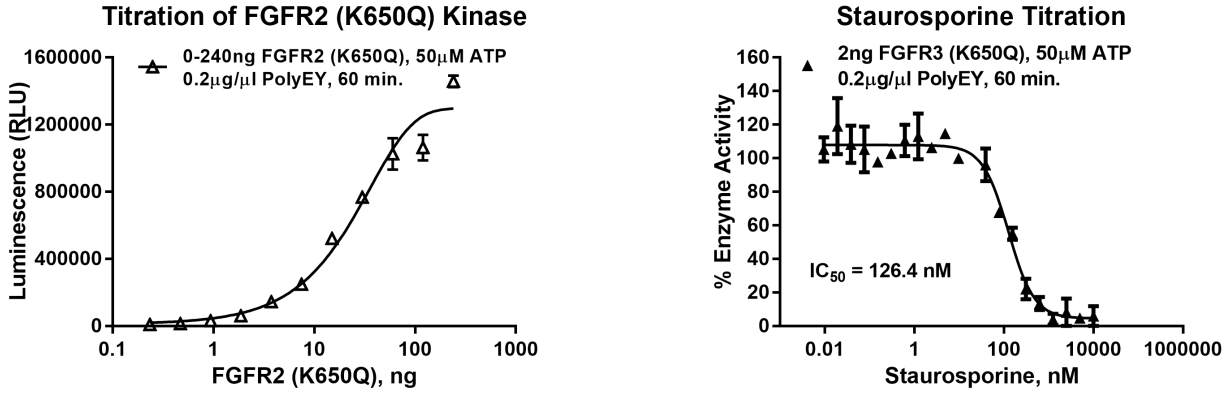

Figure 3. FGFR3 (K650Q) Kinase Assay Development. (A) FGFR3 (K650Q) enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2ng of FGFR3 (K650Q) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
FGFR3 (K650Q) Kinase Enzyme System	10µg	VA7162
	1mg	VA7163
ADP-Glo™ + FGFR3 (K650Q) Kinase Enzyme System	1 Each	VA7164