ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

FGFR1 (V561M) Kinase Assay

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Scientific Background:

FGFR1 (also known as FLT2) is a member of the Fibroblast Growth Factor Receptor family that constitute a family of four membrane-spanning tyrosine kinases (FGFR1-4) which serve as high-affinity receptors for 17 growth factors (FGF1-17). The FGF Receptor family plays an important role in multiple biological processes, including mesoderm induction and patterning, cell growth and migration, organ formation and bone growth (1). FGFR1 is alternatively spliced generating multiple splice variants that are differentially expressed during embryo development and in the adult body (2).

1. Xu, X. et al: Fibroblast growth factor receptors (FGFRs) and their roles in limb development. Cell Tissue Res. 1999 Apr;296(1):33-43.

2. Groth, C. et al: The structure and function of vertebrate fibroblast growth factor receptor 1. Int J Dev Biol. 2002;46(4):393-400.

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.

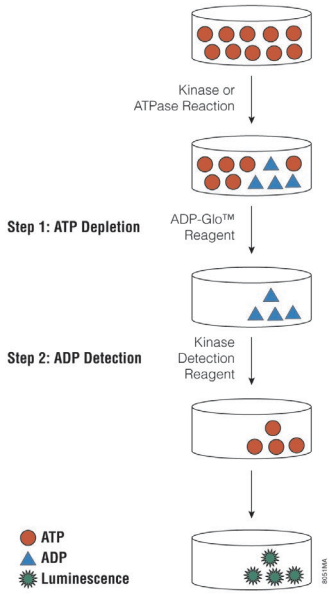

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

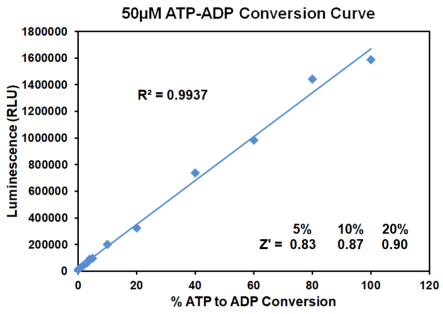

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	300	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0.59	0
Luminescence	1,067,535	781,842	556,432	338,300	212,738	94,265	52,115	22,839	12,224	7,214	3,939
S/B	271	198	141	86	54	24	13	6	3	2	1
% Conversion	82	60	42	25	15	6	2	0	0	0	0

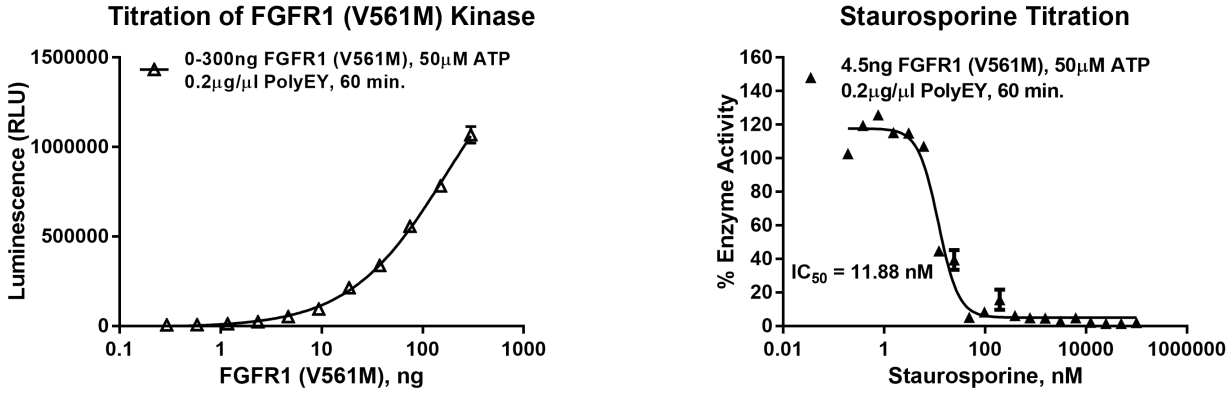

Figure 3. FGFR1 (V561M) Kinase Assay Development. (A) FGFR1 (V561M) enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 4.5ng of FGFR1 (V561M) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
FGFR1 (V561M) Kinase Enzyme System	10µg	VA7456
	1mg	VA7457
ADP-Glo™ + FGFR1 (V561M) Kinase Enzyme System	1 Each	VA7458