ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

FIP1L1-PDGFRα Kinase Assay

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Scientific Background:

FIP1L1 or factor interacting with PAPOLA and CPSF1 is a subunit of the CPSF (cleavage and polyadenylation specificity factor) complex that polyadenylates the 3' end of mRNA precursors. The FIP1L1-PDGFRα fusion gene encodes a constitutively activated tyrosine kinase that transforms hematopoietic cells and is inhibited by imatinib in which fusion of the PDGFRα and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome (1). FIP1L1-PDGFR-alpha requires disruption of the juxtamembrane protein of PDGFR-alpha and is FIP1L1-independent (2).

1. Cools, J. et.al: A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. New Eng. J. Med. 348: 1201-1214, 2003.

2. Stover, E. H. et.al: FIP1L1-PDGFR-alpha requires disruption of the juxtamembrane protein of PDGFR-alpha and is FIP1L1-independent. Proc. Nat. Acad. Sci. 103: 8078-8083, 2006.

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.

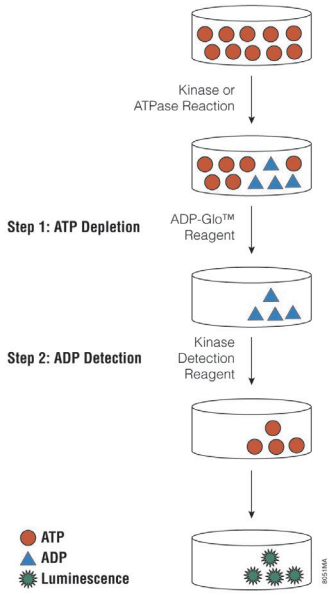

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

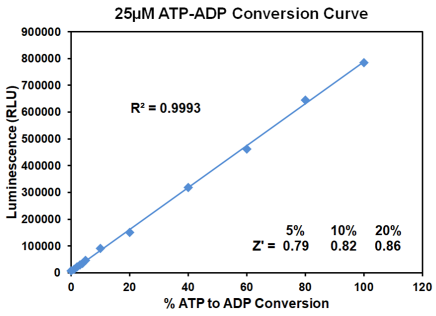

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	240	120	60	30	15	7.50	3.75	1.88	0.94	0
Luminescence	800,746	408,186	280,493	162,123	80,150	44,700	20,086	11,213	6,300	3,465
S/B	231	118	81	47	23	13	6	3	2	1
% Conversion	100	51	35	20	10	5	2	1	0	0

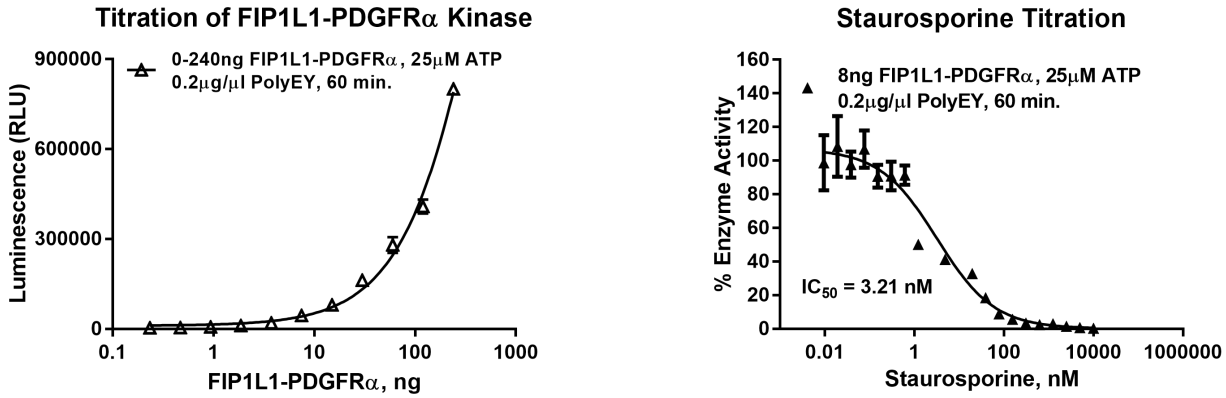

Figure 3. FIP1L1-PDGFRα Kinase Assay Development. (A) FIP1L1-PDGFRα enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 8ng of FIP1L1-PDGFRα to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
FIP1L1-PDGFRα Kinase Enzyme System	10µg	VA7165
	1mg	VA7166
ADP-Glo™ + FIP1L1-PDGFRα Kinase Enzyme System	1 Each	VA7167