ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

YES1 Kinase Assay

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Scientific Background:

YES1 is the cellular homolog of the Yamaguchi sarcoma virus oncogene that has tyrosine kinase activity and belongs to the SRC family. YES1 lies in close proximity to thymidylate synthase gene on chromosome 18 and chromosome 22 (1). The activation of YES1 may play a significant role in the malignant transformation of hepatocytes, and is important for maintaining embryonic stem cells in an undifferentiated state. YES1 is a useful marker to detect early-stage hepatocellular carcinoma, and it plays a key role in the tumorigenesis and metastasis of gastric cancer. YES1 induction results in increased cancer cell motility suggesting that YES1 may promote cancer spread and metastasis rather than tumor growth (2).

1. Silverman, G. et.al: Chromosomal reassignment: YACs containing both YES1 and thymidylate synthase map to the short arm of chromosome 18. Genomics 15: 442-445, 1993.

2. Barraclough, J. et al: Increases in c-Yes expression level and activity promote motility but not proliferation of human colorectal carcinoma cells. Neoplasia. 2007 Sep;9(9):745-54.

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.

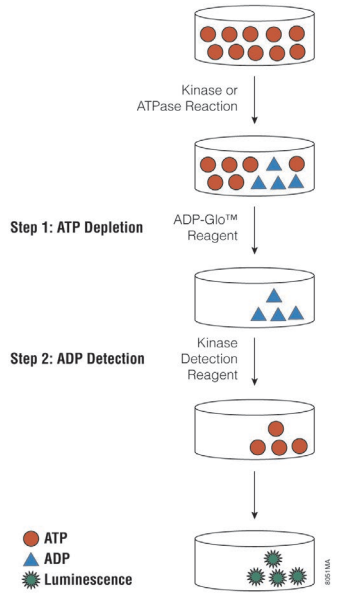

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

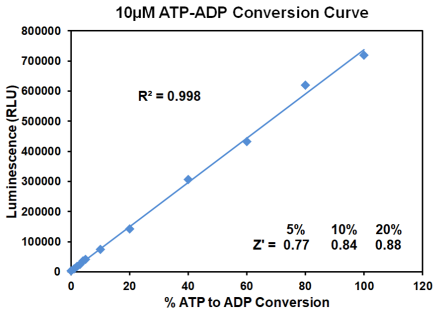

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	80	40	20	10	5	2.50	1.25	0.63	0.31	0.16	0.08	0
Luminescence	420,856	389,400	380,573	327,161	218,193	140,783	80,834	44,667	26,117	13,782	8,730	2,784
S/B	151	140	137	117	78	51	29	16	9	5	3	1
% Conversion	53	49	48	41	27	17	10	5	3	1	0	0

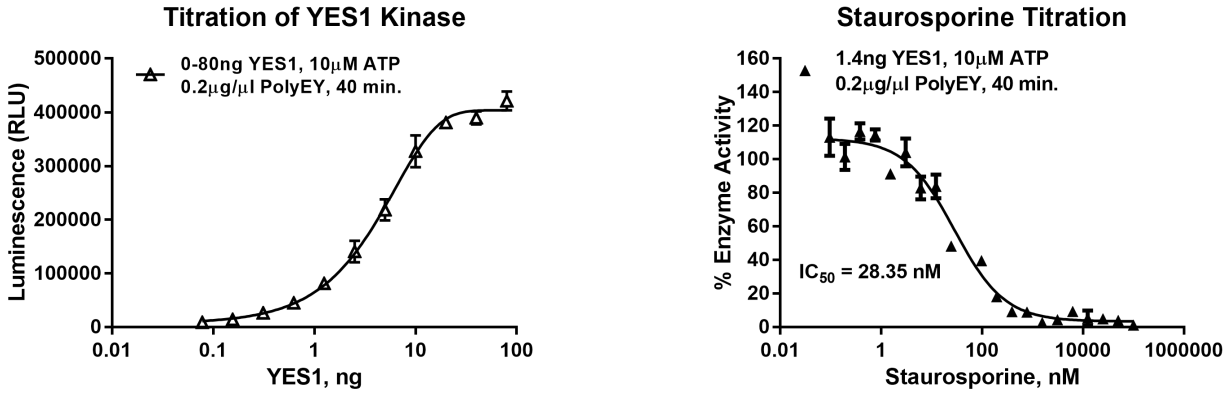

Figure 3. YES1 Kinase Assay Development. (A) YES1 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1.4ng of YES1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
YES1 Kinase Enzyme System	10µg	VA7351
	1mg	VA7352
ADP-Glo™ + YES1 Kinase Enzyme System	1 Each	VA7353