ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

EPHA3 Kinase Assay

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Scientific Background:

EPHA3 is a member of the ephrin receptor subfamily of protein-tyrosine kinases that bind the ephrin-A ligand and have diverse cellular function. Analysis of human colorectal, breast, lung and pancreatic cancer samples shows somatic mutations in the EPHA3 gene (1). EPHA3 gene expression can be regulated by CD28 and IGF-1 in Jurkat cells and expression of EPHA3 is associated with adherence and motility of malignant T cells (2).

1. Wood, L D. et al: Somatic mutations of GUCY2F, EPHA3, and NTRK3 in human cancers. Hum Mutat. 2006 Oct;27(10):1060-1.

2. Smith, L M. et al: EphA3 is induced by CD28 and IGF-1 and regulates cell adhesion. Exp Cell Res. 2004 Jan 15;292(2):295-303.

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.

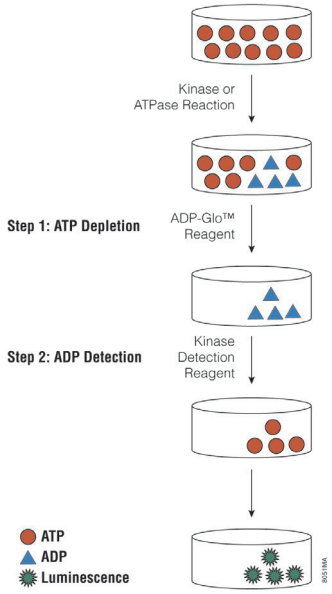

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

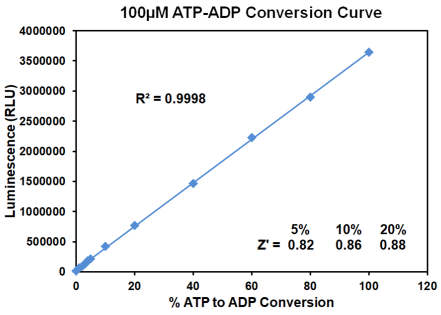

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	200	100	50	25	12.50	6.25	3.13	0.78	0
Luminescence	1,359,950	809,202	527,841	321,224	188,290	99,906	51,671	16,923	9,332
S/B	146	87	57	34	20	11	6	2	1
% Conversion	38	22	14	8	4	2	0	0	0

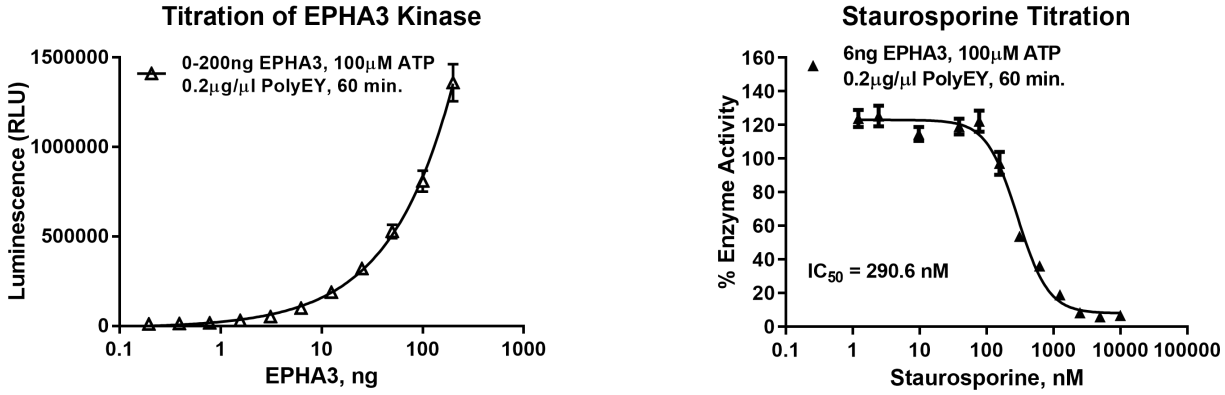

Figure 3. EPHA3 Kinase Assay Development. (A) EPHA3 enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 6ng of EPHA3 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
EPHA3 Kinase Enzyme System	10µg	VA7135
	1mg	VA7136
ADP-Glo™ + EPHA3 Kinase Enzyme System	1 Each	VA7137