ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

EPHB1 Kinase Assay

By Juliano Alves, Laurie Engel, Said A. Goueli, and Hicham Zegzouti, Promega Corporation

Scientific Background:

EPHB1 is a member of the Eph family of receptor tyrosine kinases. Ligand-activated EPHB1 forms a signaling complex with Nck, paxillin, and focal adhesion kinase and induces tyrosine phosphorylation of paxillin in a c-Src-dependent manner to promote cell migration (1). In addition, activated EPHB1 recruits the adaptor proteins Grb2 and p52Shc and promotes p52Shc and c-Src tyrosine phosphorylation as well as MAPK/extracellular signal-regulated kinase (ERK) activation. Expression of dominant-negative c-Src significantly reduced EPHB1-dependent ERK1/2 activation and chemotaxis (2).

1. Vindis, C. et al: EphB1-mediated cell migration requires the phosphorylation of paxillin at Tyr-31/Tyr-118. J Biol Chem. 2004 Jul 2;279(27):27965-70.

2. Vindis, C. et al: EphB1 recruits c-Src and p52Shc to activate MAPK/ERK and promote chemotaxis. J Cell Biol. 2003 Aug 18;162(4):661-71.

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.

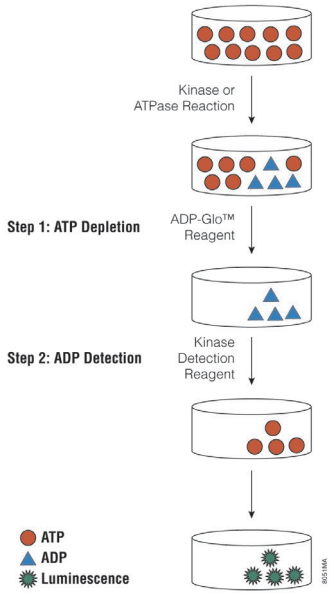

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

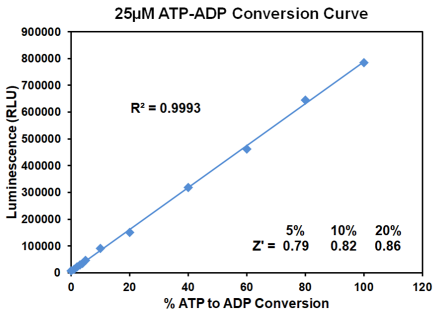

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

ADP-Glo™ Kinase Assay Application Note
Tyrosine Kinase Series

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	100	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0
Luminescence	725,986	552,312	469,773	361,643	274,778	184,056	112,898	56,159	31,853	19,629	12,357	4,349
S/B	167	127	108	83	63	42	26	13	7	5	3	1
% Conversion	90	69	58	45	34	23	14	7	4	2	1	0

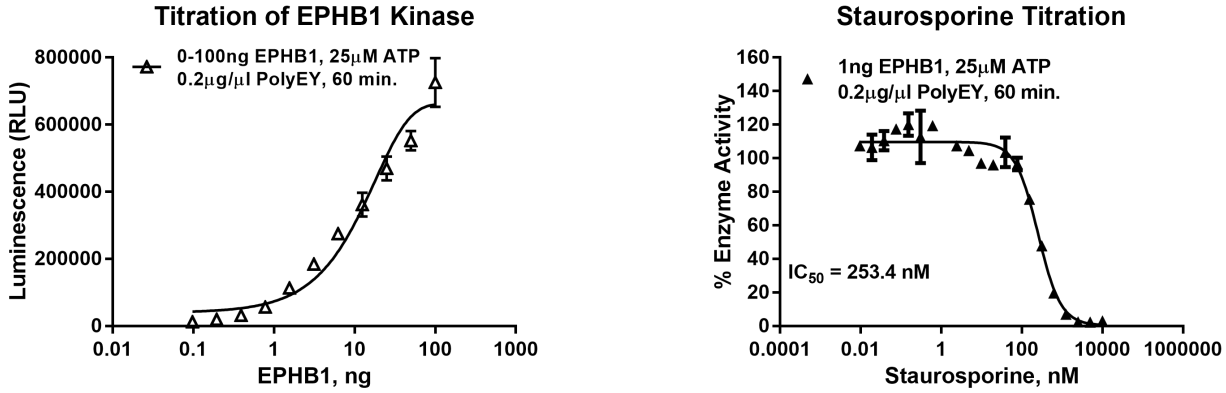

Figure 3. EPHB1 Kinase Assay Development. (A) EPHB1 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1ng of EPHB1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
EPHB1 Kinase Enzyme System	10µg	VA7141
	1mg	VA7142
ADP-Glo™ + EPHB1 Kinase Enzyme System	1 Each	VA7143