ADP-Glo™ Kinase Assay Application Note
Tyrosine Kinase Series

EPHA2 Kinase Assay

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Scientific Background:

EPHA2 is a member of the ephrin receptor subfamily of protein-tyrosine kinases that bind the ephrin-A ligand and have diverse cellular function. EPHA2 has been shown to be an oncoprotein of importance in a range of cancers. EPHA2 is overexpressed in several human cancer types and promotes malignancy through a mechanism involving RhoA-dependent destabilization of adherens junctions (1). EPHA2 overexpression induces a FAK-dependent increase in MMP-2 expression and invasiveness and this process that can be reversed by ligation of EPHA2 (2).

1. Fang, W B. et al: Overexpression of EPHA2 receptor destabilizes adherens junctions via a RhoA-dependent mechanism. J Cell Sci. 2008 Feb 1;121(Pt 3):358-68.

2. Duxbury, M S. et al: Ligation of EphA2 by Ephrin A1-Fc inhibits pancreatic adenocarcinoma cellular invasiveness. Biochem Biophys Res Commun. 2004 Aug 6;320(4):1096-102.

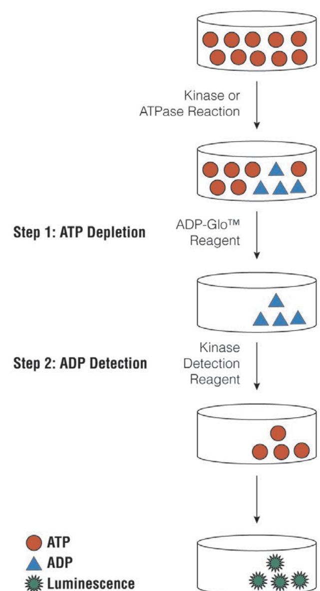

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

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.

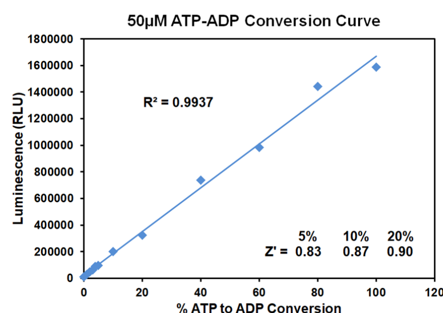

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	240	120	60	30	15	7.50	3.75	1.88	0
Luminescence	632,867	390,839	239,348	162,003	82,479	44,639	18,962	13,013	3,702
S/B	171	106	65	44	22	12	5	4	1
% Conversion	54	33	20	13	6	3	1	0	0

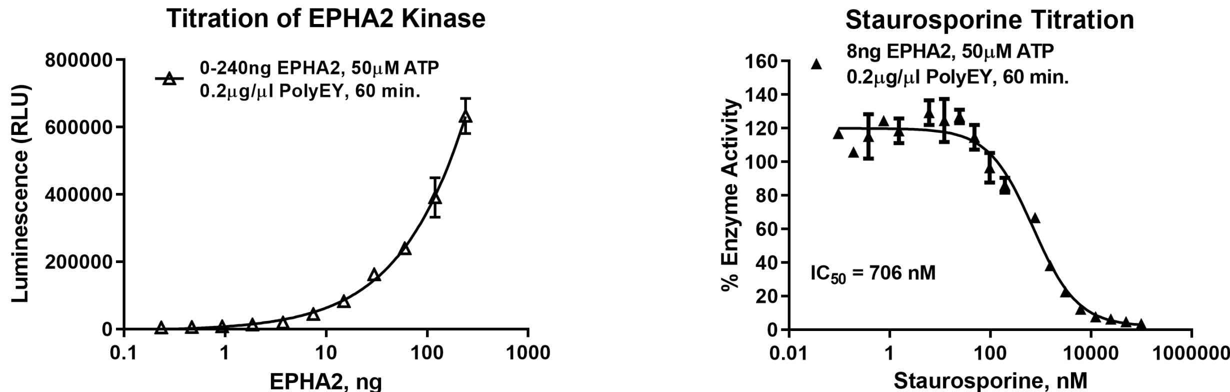

Figure 3. EPHA2 Kinase Assay Development. (A) EPHA2 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 8ng of EPHA2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
EPHA2 Kinase Enzyme System	10µg	VA7441
	1mg	VA7442
ADP-Glo™ + EPHA2 Kinase Enzyme System	1 Each	VA7443