ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

EPHA7 Kinase Assay

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Scientific Background:

EPHA7 is a member of the ephrin receptor subfamily of protein-tyrosine kinases family that have been implicated in mediating developmental events, particularly in the nervous system. Receptors in the EPH subfamily typically have a single kinase domain and an extracellular region containing a Cys-rich domain and 2-fibronectin types III repeats. EPHA7 mediated signaling act as a physiologic trigger for apoptosis that can alter brain size and shape by regulating the number of neural progenitors (1). The high expression of EPHA7 protein plays an important role in the malignancy transformation, invasion progression and metastasis of primary hepatocellular carcinoma (2).

1. Depaepe, V. et.al: Ephrin signalling controls brain size by regulating apoptosis of neural progenitors. Nature 435: 1244-1250, 2005.

2. Zhang SJ. et.al: Expression of EphA7 protein in primary hepatocellular carcinoma and its clinical significance. Zhonghua Wai Ke Za Zhi. 2010 Jan 1;48(1):53-6.

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.

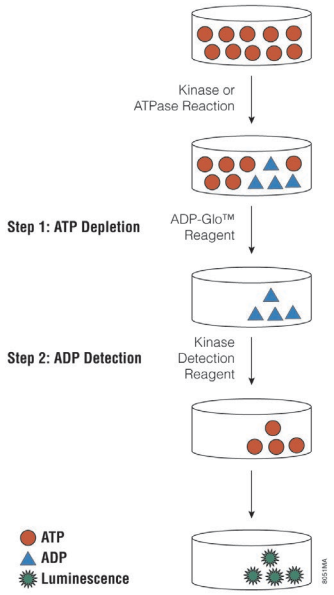

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

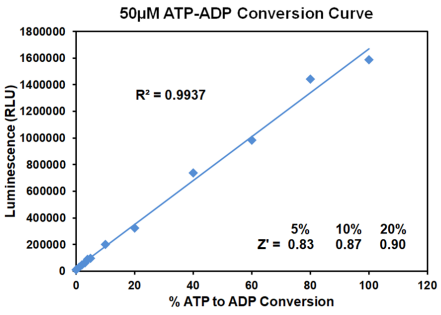

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	300	150	75	37.50	18.75	9.38	4.69	1.17	0
Luminescence	719,354	323,796	225,317	119,419	65,806	30,858	20,736	7,563	4,090
S/B	176	79	55	29	16	8	5	2	1
% Conversion	47	21	14	7	3	1	0	0	0

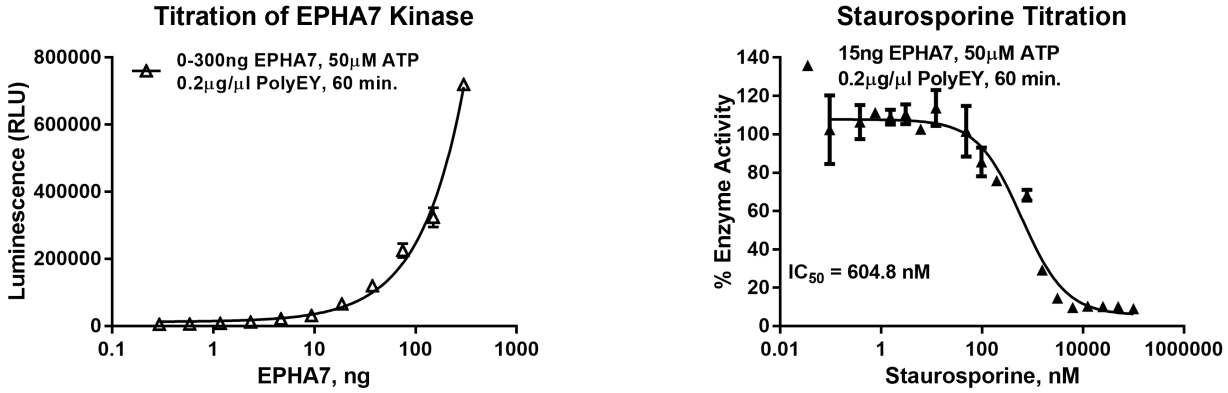

Figure 3. EPHA7 Kinase Assay Development. (A) EPHA7 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 15ng of EPHA7 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
EPHA7 Kinase Enzyme System	10µg	VA7447
	1mg	VA7448
ADP-Glo™ + EPHA7 Kinase Enzyme System	1 Each	VA7449