EPHA1 Kinase Assay

By Hicham Zegzouti, Ph.D., Jolanta Vidugiriene, Ph.D., Gediminas Vidugiris, Ph.D., and Said A. Goueli, Ph.D., Promega Corporation

Scientific Background:

EPHA1 is a member of the Eph family of receptor tyrosine kinases that have been implicated in mediating developmental events, particularly in the nervous system (1). Receptors in the Eph subfamily typically have a single kinase domain and an extracellular region containing a Cys-rich domain and 2 fibronectin type III repeats. EPHA1 seems to be a marker of the differentiated normal epidermis and its downregulation in nonmelanoma skin cancer may contribute to carcinogenesis of these very frequent human tumors. EPHA1 represents a new potential prognostic marker and therapeutic target in nonmelanoma skin cancer (2).

1. Kullander, K. et al: Mechanisms and functions of Eph and ephrin signalling. Nat Rev Mol Cell Biol. 2002 Jul;3(7):475-86.

2. Hafner, C. et al: Expression profile of Eph receptors and ephrin ligands in human skin and downregulation of EphA1 in nonmelanoma skin cancer. Mod Pathol. 2006 Oct;19(10):1369-77

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.

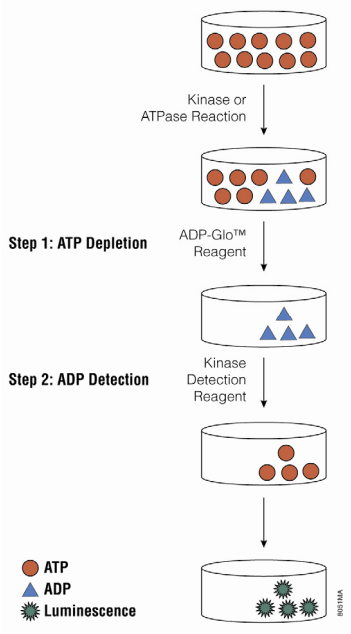

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

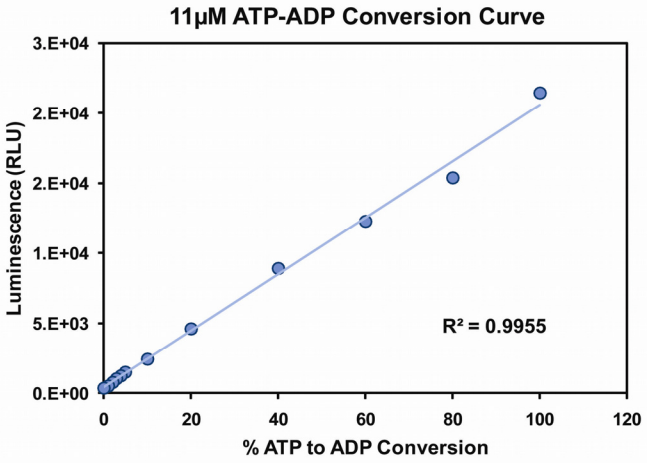

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 11µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction.

For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Tyrosine Kinase 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 60 minutes.
- 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 0.5-1second).

Table 1. EPHA1 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.

EPHA1, ng	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.20	0
Luminescence	12993	10509	9608	7385	3886	2368	1580	1209	698	305
S/B	42.6	34.5	31.5	24.2	12.7	7.8	5.2	4.0	2.3	1
% Conversion	62.4	50.1	45.6	34.6	17.3	9.7	5.8	4.0	1.5	0

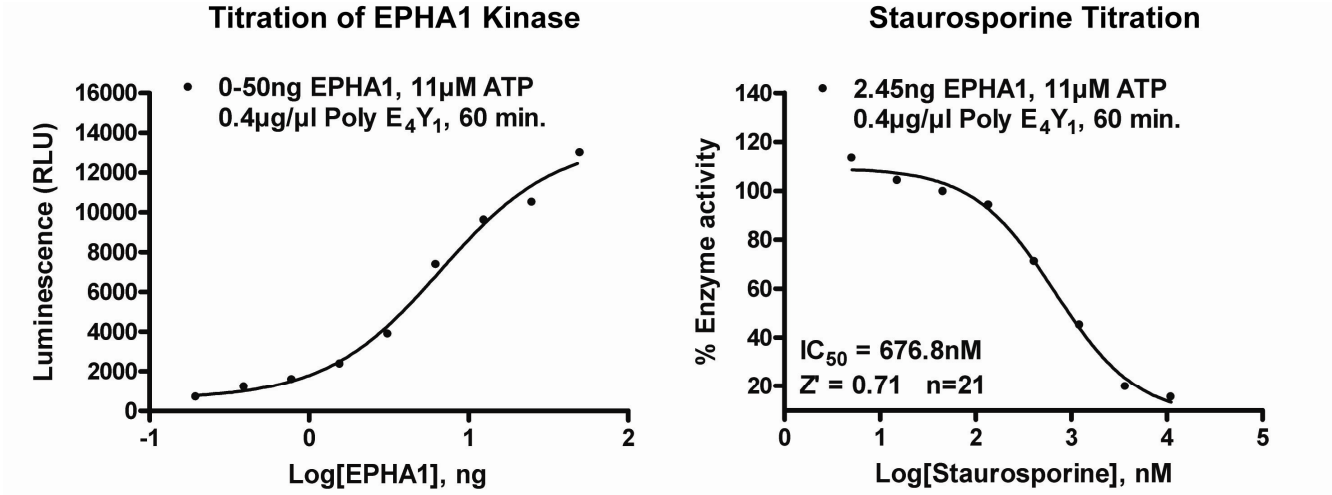

Figure 3. EPHA1 Kinase Assay Development. (A) EPHA1 enzyme was titrated using 11µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 2.45ng of EPHA1 to determine the potency of the inhibitor (IC_{50}). Z' factor was determined using 21 replicates of each of the minimum and maximum response (10 and 0µM, respectively).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
EPHA1 Kinase Enzyme System	Promega	V3561
ADP-Glo + EPHA1 Kinase Enzyme System	Promega	V9271

EPHA1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 2mM $MnCl_2$; 50µM DTT.

Promega Corporation • 2800 Woods Hollow Road • Madison, WI 53711-5399 USA • Telephone 608-274-4330 • Fax 608-277-2601
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