ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

EPHB4 Kinase Assay

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Scientific Background:

EPHB4 is a member of the Eph family of receptor tyrosine kinases which play a role in regulating cell adhesion and cell movement during embryonic development (1). Furthermore, because tissue disorganization and abnormal cell adhesion, movement, and survival characterize the more advanced stages of cancer, the inappropriate functioning of Eph receptor could play a causal role in malignancy (2). EPHB4 is uniquely expressed in the vascular endothelial and endocardial cells and animal knockout studies with EPHB4 reveal a phenotype that is similar to Ephrin B2 gene knockdown. Furthermore, EPHB4 has been shown to be the major essential interaction partner of Ephrin B2 in angiogenesis.

1. Andres, A.C. et al: Expression of two novel eph-related receptor protein tyrosine kinases in mammary gland development and carcinogenesis. Oncogene, 1994;9(8):2431.

2. Kumar, S.R. et al: The receptor tyrosine kinase EphB4 is overexpressed in ovarian cancer, provides survival signals and predicts poor outcome. Br. J. Cancer. 2007, 96(7):1083-91.

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.

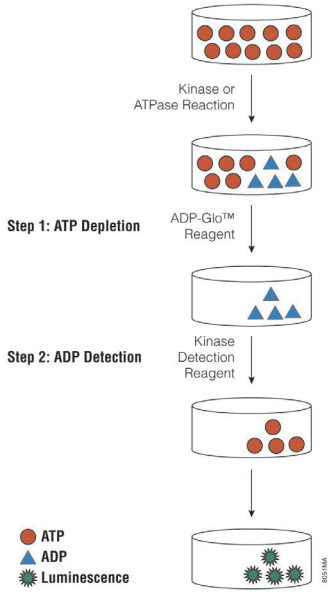

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

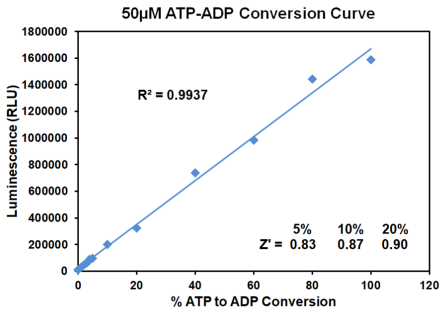

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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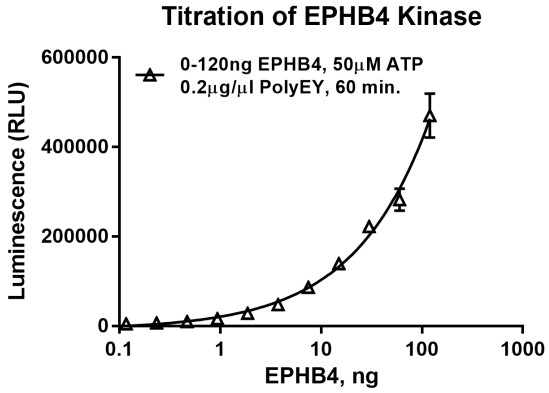
The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

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	120	60	30	15	7.50	3.75	1.88	0.94	0.23	0
Luminescence	470,201	282,033	222,383	139,152	86,375	47,701	28,451	16,019	6,585	3,836
S/B	123	74	58	36	23	12	7	4	2	1
% Conversion	40	23	18	11	6	3	1	0	0	0



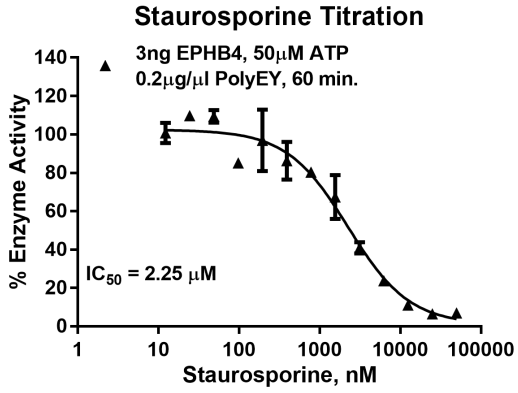


Figure 3. EPHB4 Kinase Assay Development. (A) EPHB4 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 3ng of EPHB4 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
EPHB4 Kinase Enzyme System	10µg	VA7450
	1mg	VA7451
ADP-Glo™ + EPHB4 Kinase Enzyme System	1 Each	VA7452