Promega

ADP-Glo™ Kinase Assay Application Note
Tyrosine Kinase Series

EPHA4 Kinase Assay

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

Scientific Background:

EPHA4 also known as EPH receptor A4, belongs to the ephrin receptor subfamily of protein-tyrosine kinases which have been implicated in mediating developmental events, particularly in the nervous system (1). The EPHA4 ligand ephrin-A3 is localized to the astrocytic processes that envelop the spine. Activation of EPHA4 by ephrin-A3 induces spinal retraction and reduces spine density and inhibits the interaction distorted spine shape and organization. EphA4-null mice possess defects in the corticospinal tract and anterior commissure indicating a model in which an ephrin ligand on the axons senses EPHA4 on spinal cord cells surrounding the corticospinal tract (2).

1. Flanagan, J.G. et al: The ephrins and Eph receptors in neural development.". Annu. Rev. Neurosci. 1998;21: 309–45.

2. Dottori, M. et al: EphA4 (Sek1) receptor tyrosine kinase is required for the development of the corticospinal tract. Proc. Nat. Acad. Sci. 95: 13248-13253, 1998.

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.

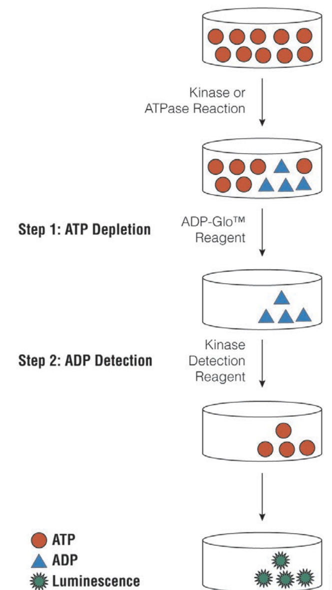

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

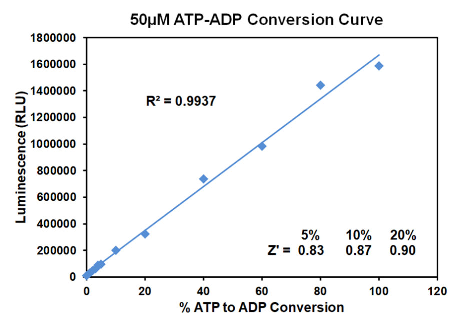

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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	240	120	60	30	15	7.50	3.75	1.88	0.94	0.47	0.23	0
Luminescence	844,010	478,983	427,812	318,154	234,902	140,927	92,784	53,523	33,038	18,466	13,030	3,933
S/B	215	122	109	81	60	36	24	14	8	5	3	1
% Conversion	72	41	36	27	19	11	7	4	2	0	0	0

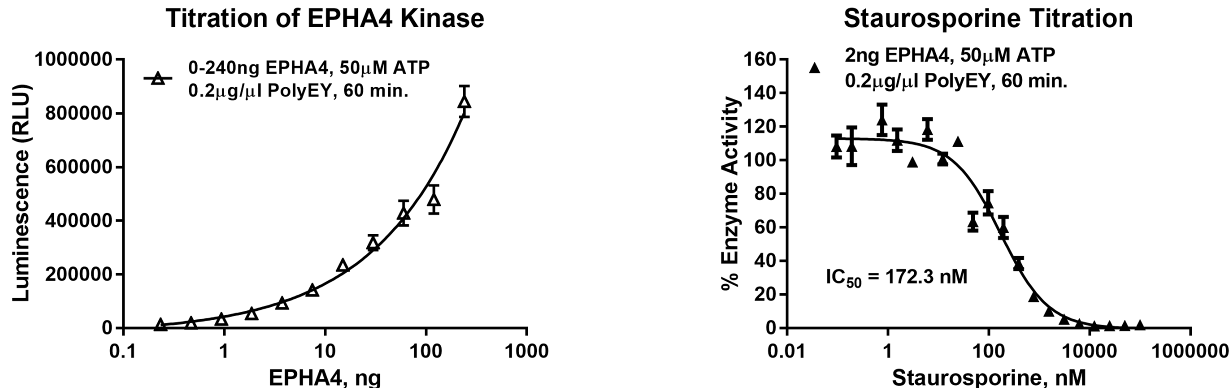

Figure 3. EPHA4 Kinase Assay Development. (A) EPHA4 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2ng of EPHA4 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
EPHA4 Kinase Enzyme System	10µg	VA7444
	1mg	VA7445
ADP-Glo™ + EPHA4 Kinase Enzyme System	1 Each	VA7446