ADP-Glo™ Kinase Assay Application Note
Tyrosine Kinase Series

# EPHA6 Kinase Assay

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## Scientific Background:

EPHA6 is a member of the ephrin receptor subfamily of protein-tyrosine kinases which have been implicated in axon guidance, neuron-target interactions, regional compartmentalization, and synaptic functions in nervous systems. EPHA6 is highly expressed in the brain and testis. Reduction in EPHA6 has been detected in Hypospadias, a common defect affecting the growth and closure of the external genitalia (1). Genetic inhibition of EPHA6 in mice produces behavioral deficits specifically in tests of learning and memory. Mice deficient in EPHA6 show reduced memory of the consequences of the training context (2).

1. Shaut C A, et al: HOXA13 directly regulates EphA6 and EphA7 expression in the genital tubercle vascular endothelia. Dev Dyn. 2007 Apr;236(4):951-60.

2. Savelieva K V, et al: Learning and memory impairment in Eph receptor A6 knockout mice. Neurosci Lett. 2008 Jun 20;438(2):205-9.

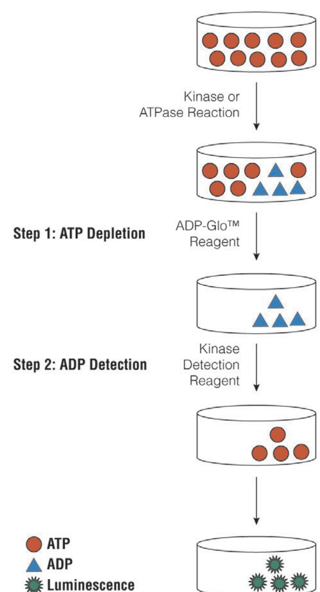


**Figure 1. Principle of the ADP-Glo™ Kinase Assay.** The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

## 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.

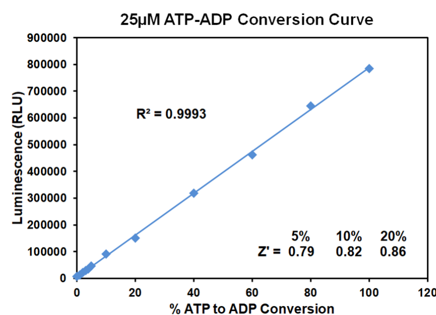


**Figure 2. Linearity of the ADP-Glo Kinase Assay.** ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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 | 2.34 | 1.17 | 0.59 | 0 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Luminescence | 811,163 | 588,470 | 406,926 | 209,178 | 111,230 | 53,769 | 30,411 | 15,993 | 9,999 | 6,458 | 3,149 |
| S/B | 258 | 187 | 129 | 66 | 35 | 17 | 10 | 5 | 3 | 2 | 1 |
| % Conversion | 95 | 69 | 48 | 24 | 13 | 6 | 3 | 2 | 1 | 0 | 0 |

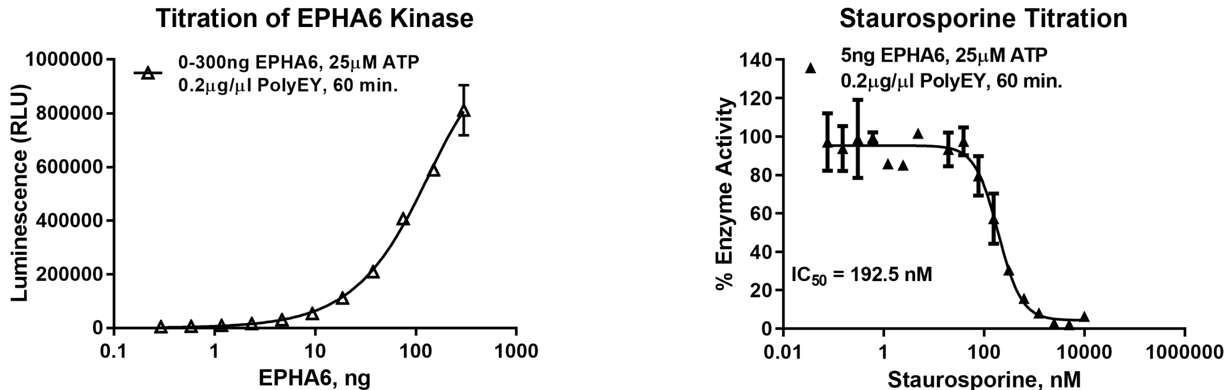


**Figure 3. EPHA6 Kinase Assay Development.** (A) EPHA6 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 5ng of EPHA6 to determine the potency of the inhibitor ($IC_{50}$).

## Ordering Information:

| Products | Size | Cat. # |
|---|---|---|
| EPHA6 Kinase Enzyme System | 10µg | VA7138 |
| | 1mg | VA7139 |
| ADP-Glo™ + EPHA6 Kinase Enzyme System | 1 Each | VA7140 |