ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

TYRO3 Kinase Assay

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Scientific Background:

TYRO3 receptor protein tyrosine kinase together with AXL and MER form the AXL/TYRO3 family of receptor tyrosine kinases (1). Members of this family play an essential role in spermatogenesis, immunoregulation, and phagocytosis. In addition to signal transduction, members of this family of receptor tyrosine kinases participate in cell adhesion (2). Gas6, a product of growth arrest-specific gene, activates the kinase activity of all three receptors. TYRO3 is expressed in the brain (where it is involved in the protection of neurons from apoptosis), expressed in lymphoid, vascular and reproductive tissue and in primary and tumor cell lines.

1. Angelillo-Scherrer, A. et al: Role of Gas6 receptors in platelet signaling during thrombus stabilization and implications for antithrombotic therapy. J. Clin. Invest. 2005; 115: 237-246.

2. Liao, X. et al: Receptor tyrosine kinase gene Tyro3 maps to mouse chromosome 2, closely linked to Ltk. Mammalian Genome, 1996. 7: 395-396.

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.

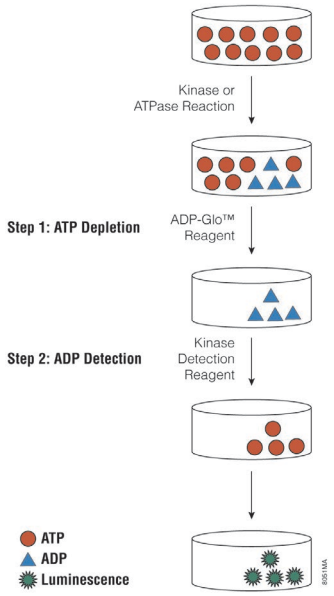

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

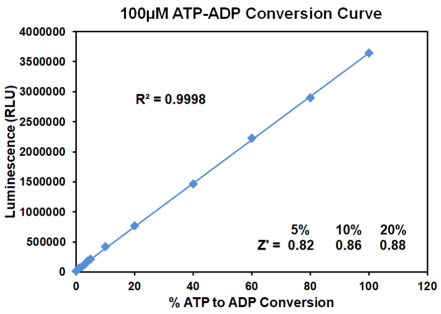

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	160	80	40	20	10	5	2.50	1.25	0.63	0
Luminescence	2,339,890	1,689,835	1,277,145	822,264	501,244	252,191	111,251	52,965	29,784	14,236
S/B	164	119	90	58	35	18	8	4	2	1
% Conversion	71	51	38	24	14	7	3	1	0	0

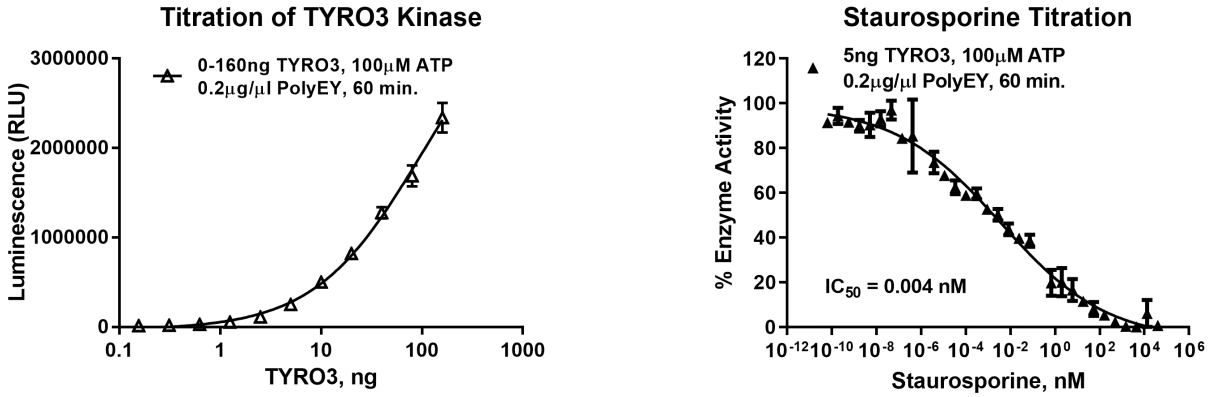

Figure 3. TYRO3 Kinase Assay Development. (A) TYRO3 enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 5ng of TYRO3 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
TYRO3 Kinase Enzyme System	10µg	VA7579
	1mg	VA7580
ADP-Glo™ + TYRO3 Kinase Enzyme System	1 Each	VA7581