ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: TGFβR1

TGFβR1 Kinase Assay

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Scientific Background:

TGFβR1 or transforming growth factor, beta-receptor 1 is a member of the TGFβ receptor subfamily and is a ser/thr protein kinase that forms a heteromeric complex with type II TGF-beta receptors when bound to TGF-beta, transducing the TGF-beta signal from the cell surface to the cytoplasm. Mutations in TGFβR1gene have been associated with Marfan syndrome, Loeys-Deitz Aortic Aneurysm Syndrome, and the development of various types of tumors (1). TGFβR1-dependent signaling is required for angiogenesis but not for the development of hematopoietic progenitor cells and functional hematopoiesis (2).

1. Singh, K. et.al: TGFBR1 and TGFBR2 mutations in patients with features of Marfan syndrome and Loeys- Dietz syndrome. Hum. Mutat. 27: 770-777, 2006.

2. Larsson, J. et.al: Abnormal angiogenesis but intact hematopoietic potential in TGF-beta type I receptor-deficient mice. EMBO J. 20: 1663-1673, 2001.

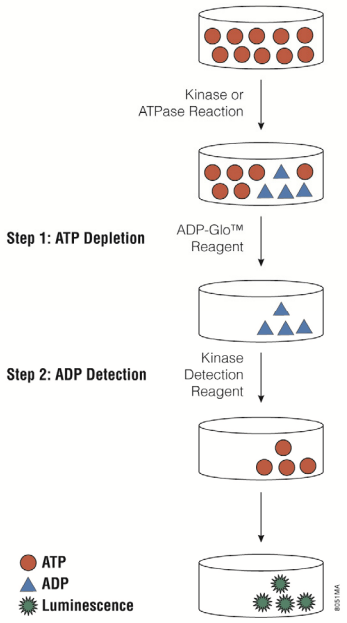

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

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.

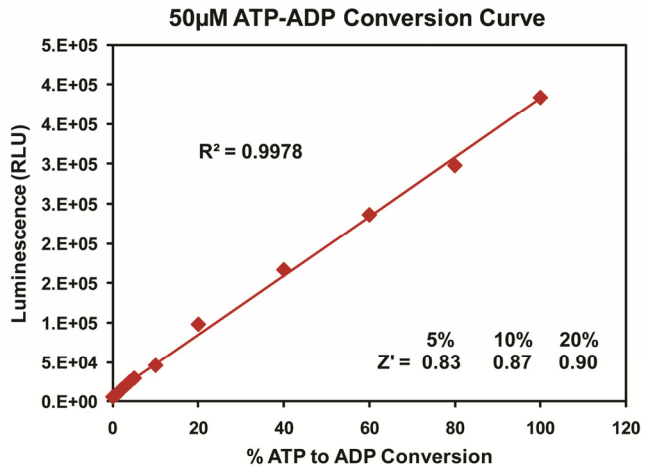

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 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 120 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. TGFβR1 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.

TGFβR1, ng	200	100	50	25	13	6.3	3.1	0
RLU	283729	143397	42180	17269	7668	3067	2687	625
S/B	454	230	68	28	12	5	4	1
% Conversion	100	50	14	5	2	0.24	0.10	0

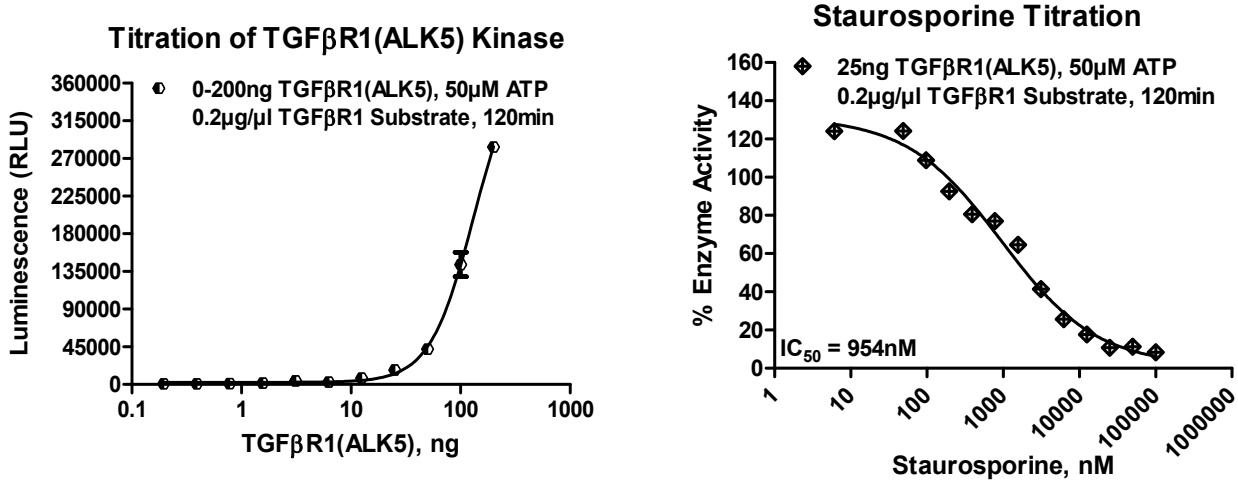

Figure 3. TGFβR1 Kinase Assay Development. (A) TGFβR1 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 25ng of TGFβR1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
TGFβR1 Kinase Enzyme System	Promega	V4092
ADP-Glo™ + TGFβR1 Kinase Enzyme System	Promega	V4093

TGFβR1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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