ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: ALK6

ALK6 Kinase Assay

By Juliano Alves, Ph.D., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

ALK6 (also known as BMPR1B) is a member of the transmembrane serine/threonine kinase that is the member of the bone morphogenetic protein (BMP) receptor, which is closely related to the activin receptors, ACVR1 and ACVR2. ALK6 is mainly involved in the endochondral bone formation and embryogenesis. ALK6 expressed in normal and cancerous prostate tissues and used in the endocrine therapy that given to the prostate cancer patients (1). ALK6 receptor trafficking also play a significant role in FOP pathogenesis and used in human T-cell differentiation (2).

1. Ide, H. et.al: Cloning of human bone morphogenetic protein type 1B receptor (BMPR-1B) and its expression in prostate cancer in comparison with other BMPRs. Oncogene 14: 1377-1382, 1997.

2. Cejalvo, T. et.al: Bone morphogenetic protein-2/4 signalling pathway components are expressed in the human thymus and inhibit early T-cell development. Immunology 121: 94-104, 2007.

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.

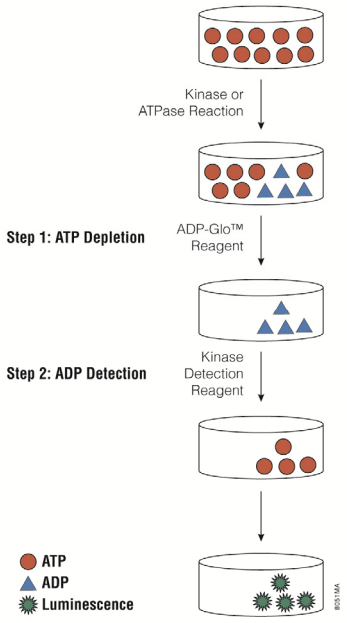


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

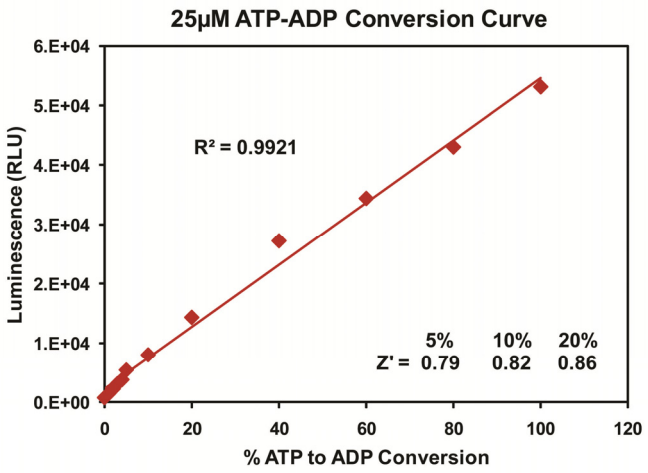


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

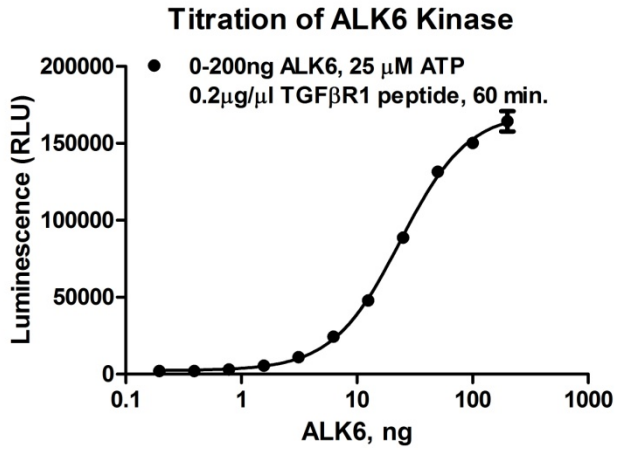
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

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 60 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. ALK6 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.

ALK6, ng	200	100	50	25	12.5	6.3	3.1	1.6	0.8	0
RLU	164341	150323	131568	88748	47890	24390	11108	5534	3036	1359
S/B	121	111	97	65	35	18	8	4.1	2.2	1
% Conversion	91	83	73	49	26	13	5	2.0	0.6	0



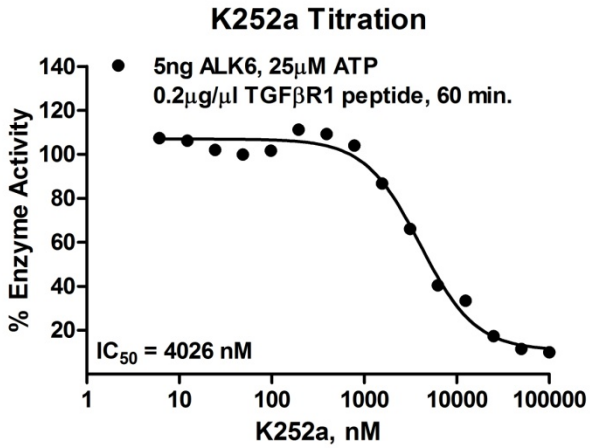


Figure 3. ALK6 Kinase Assay Development. **(A) ALK6 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 5ng of ALK6 to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
ALK6 Kinase Enzyme System	Promega	V4052
ADP-Glo™ + ALK6 Kinase Enzyme System	Promega	V4053

ALK6 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.