ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: ALK2

ALK2 Kinase Assay

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Scientific Background:

ALK 2 is a receptor serine/threonine kinase that is member of the ALK family and is upstream of signaling pathway involving the SMAD proteins especially SMAD1/5/8. Knockdown of ALK2, but not TGFβRI (ALK5), abrogates endoglin-mediated decrease in cell motility of prostate cancer cells and constitutively active ALK2 is sufficient to restore a low-motility phenotype in endoglin deficient cells (1). Therefore, endoglin decreases prostate cancer cell motility through activation of the ALK2-Smad1 pathway. ALK2 is the key gene involved in Fibrodysplasia ossificans progressiva (FOP), a rare autosomal dominant congenital disorder characterized by progressive heterotopic bone formation in muscle tissues (2).

1. Craft, C.S. et al: Endoglin inhibits prostate cancer motility via activation of the ALK2-Smad1 pathway. Oncogene. 2007 Nov 8;26(51):7240-50.

2. Shore, E. M. et al: A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. Nature Genet. 38: 525-527, 2006.

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.

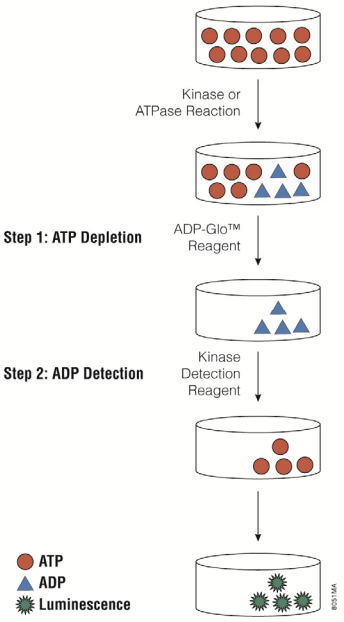

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

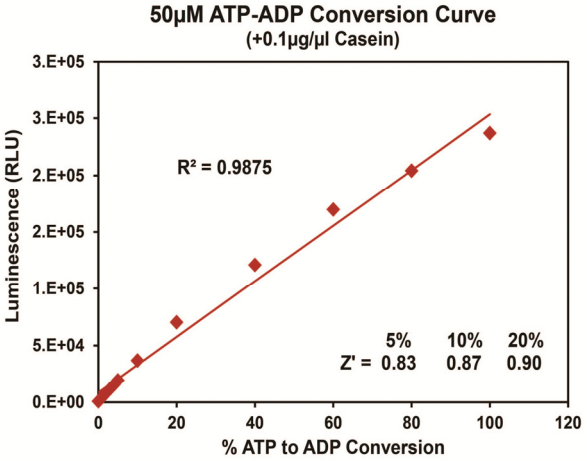

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. ALK2 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.

ALK2, ng	100	50	25	13	6.3	3.1	0
RLU	51691	27725	14975	8248	3806	1228	1018
S/B	51	27	15	8	4	1.2	1
% Conversion	14	8	4	2	0.6	0.2	0

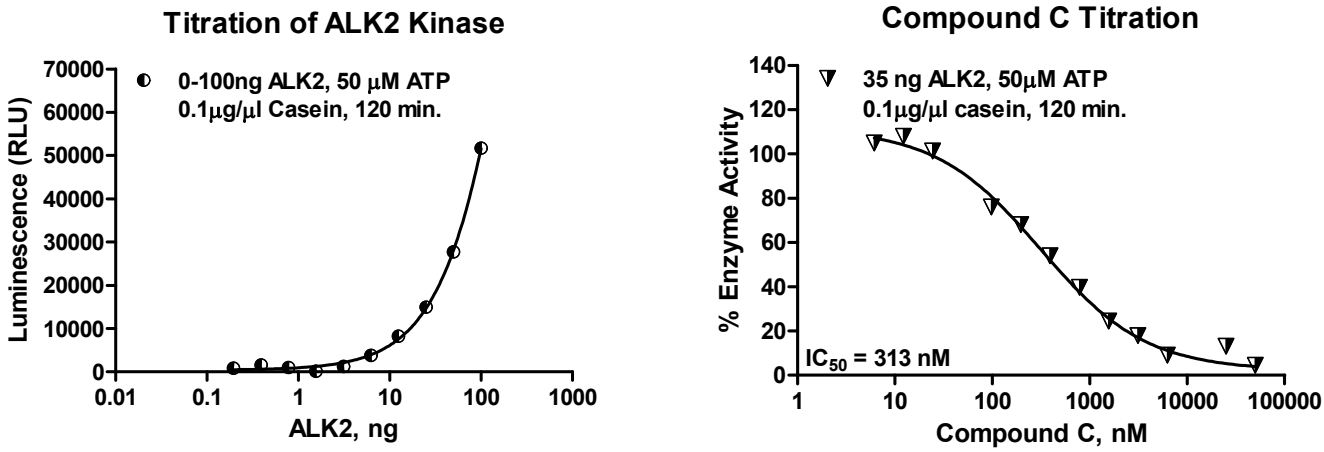

Figure 3. ALK2 Kinase Assay Development. (A) ALK2 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Compound C dose response was created using 35ng of ALK2 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
ALK2 Kinase Enzyme System	Promega	V4492
ADP-Glo™ + ALK2 Kinase Enzyme System	Promega	V4493

ALK2 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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