ALK4 Kinase Assay

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Scientific Background:

ALK4 is a member of the subfamily of receptor ser/thr kinases that mediates signaling by the Activins. ALK4 is expressed in many human tissues, including kidney, pancreas, brain, lung, and liver. Truncated ALK4, predominantly expressed in human pituitary adenomas, function as dominant negative receptors to interfere with wild-type receptor function and blocks the antiproliferative effect of activin possibly contributing to development of human pituitary tumors (1). ALK4 is able to mediate Nodal signaling in the presence of Cripto during vertebrate development (2).

1. Zhou, Y. et al: Truncated activin type I receptor Alk4 isoforms are dominant negative receptors inhibiting activin signaling. Mol Endocrinol. 2000 Dec;14(12):2066-75.

2. Reissmann, E. et al: The orphan receptor ALK7 and the Activin receptor ALK4 mediate signaling by Nodal proteins during vertebrate development. Genes Dev. 2001 Aug 1;15(15):2010-22.

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.

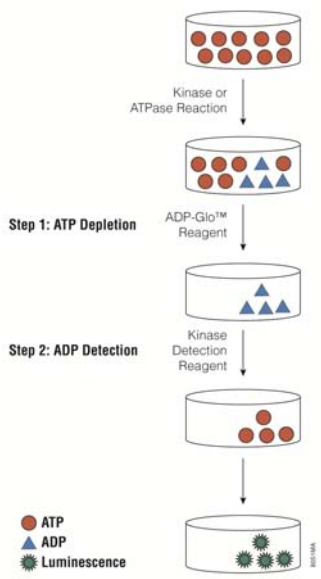

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

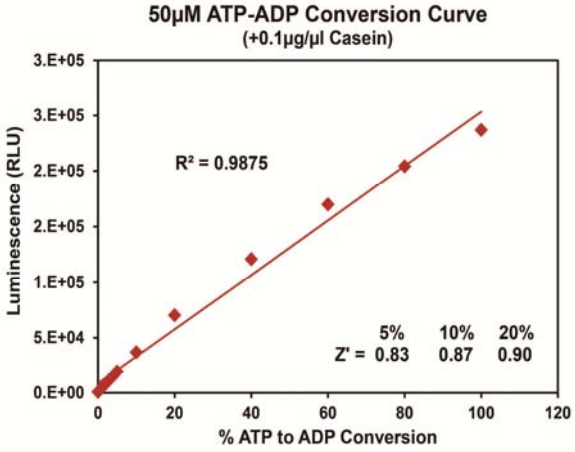

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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

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. ALK4 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.

ALK4, ng	200	100	50	25	12.5	6.3	3.1	1.6	0
RLU	309745	207272	82789	24554	11436	4303	3186	1812	958
S/B	323	216	86	26	12	4	3	2	1
% Conversion	77	51	20	6	3	1	0.5	0.1	0

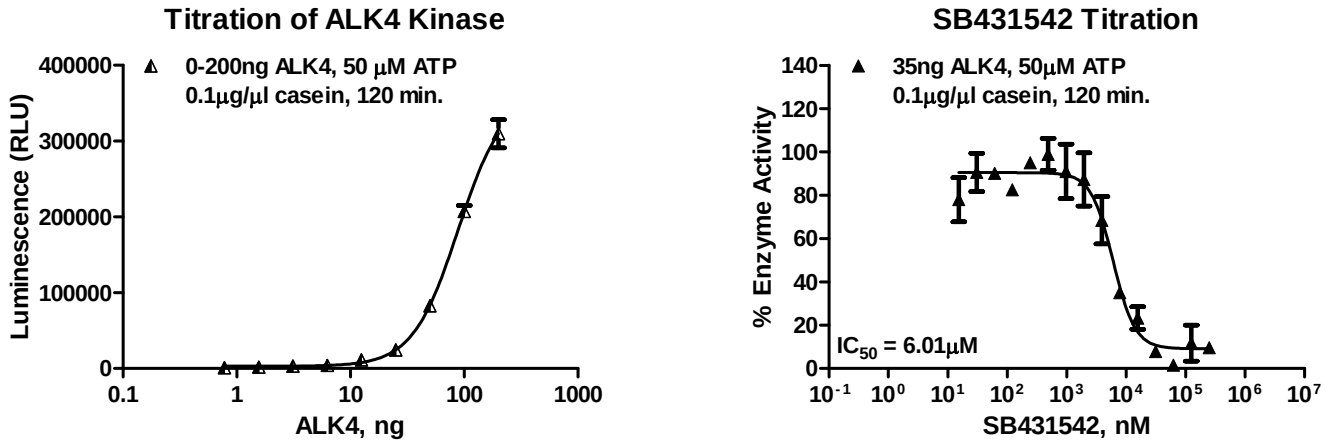

Figure 3. ALK4 Kinase Assay Development. (A) ALK4 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) SB431542 dose response was created using 35ng of ALK4 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
ALK4 Kinase Enzyme System	Promega	V4508
ADP-Glo™ + ALK4 Kinase Enzyme System	Promega	V4509

ALK4 Kinase Buffer: 40mM Tris, pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT