ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

CK1α1L Kinase Assay

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Scientific Background:

CK1α1L or casein kinase 1, alpha 1 like is a novel member of the CKI gene family which highlights a critical role for p53 in invasiveness control (1). CK1-alpha dynamically associates with the CBM complex on T cell receptor engagement to participate in cytokine production and lymphocyte proliferation which has a contrasting role by subsequently promoting the phosphorylation and inactivation of CARMA1 and it governs antigen-receptor-induced NF-kappa-B activation and human lymphoma cell survival (2).

1. Elyada, E.et.al: CKI-alpha ablation highlights a critical role for p53 in invasiveness control. Nature 470: 409-413, 2011.

2. Bidere, N.et.al: Casein kinase 1-alpha governs antigen-receptor-induced NF-kappa-B activation and human lymphoma cell survival. Nature 458: 92-96, 2009.

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.

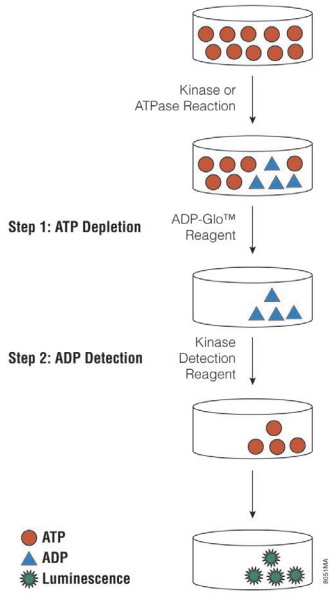

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

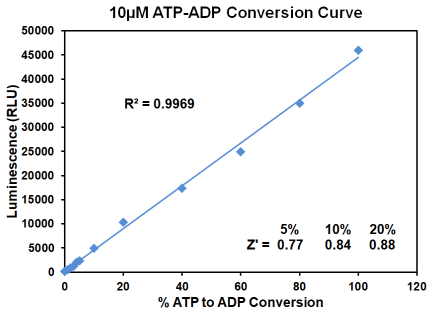

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	200	100	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0
Luminescence	58,521	42,974	32,084	21,968	14,029	7,731	4,853	2,867	354	226	81
S/B	722	531	396	271	173	95	60	35	4	3	1
% Conversion	132	97	72	49	31	17	11	6	1	0	0

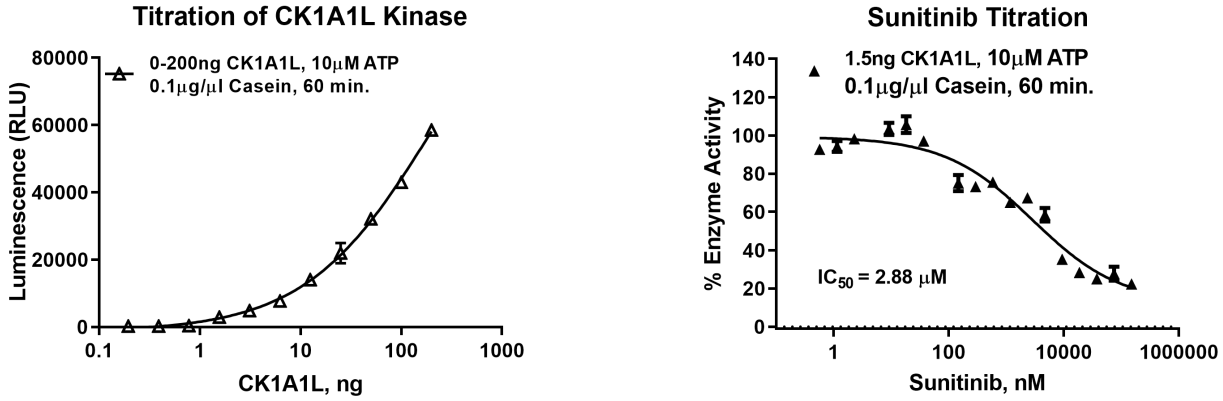

Figure 3. CK1α1L Kinase Assay Development. (A) CK1α1L enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1.5ng of CK1α1L to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
CK1α1L Kinase Enzyme System	10µg	VA7183
	1mg	VA7184
ADP-Glo™ + CK1α1L Kinase Enzyme System	1 Each	VA7185