ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

LIMK1 Kinase Assay

By Juliano Alves, Laurie Engel, Said A. Goueli, and Hicham Zegzouti, Promega Corporation

Scientific Background:

LIMK1 or LIM domain kinase 1 contains a unique combination of 2 N-terminal LIM domain and a C-terminal protein kinase domain. LIM domains are highly conserved cysteine-rich structures containing 2 zinc fingers that can bind to DNA/RNA as well as mediating protein-protein interactions (1). LIMK1 is thought to be a component of an intracellular signaling pathway that may be involved in brain development especially development of nerve cells. LIMK1 may play an important role in areas of the brain that are responsible for processing visual-spatial information (visuospatial constructive cognition). LIMK1 can regulate aspects of the cytoskeleton, the structural framework that helps to determine cell shape, size, and movement (2).

1. Davila, M. et al: LIM kinase 1 is essential for the invasive growth of prostate epithelial cells: implications in prostate cancer. J Biol Chem. 2003;19;278(38):36868-75.

2. Davila, M. et al: Expression of LIM kinase 1 is associated with reversible G1/S phase arrest, chromosomal instability and prostate cancer. Mol Cancer. 2007;8;6:40.

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.

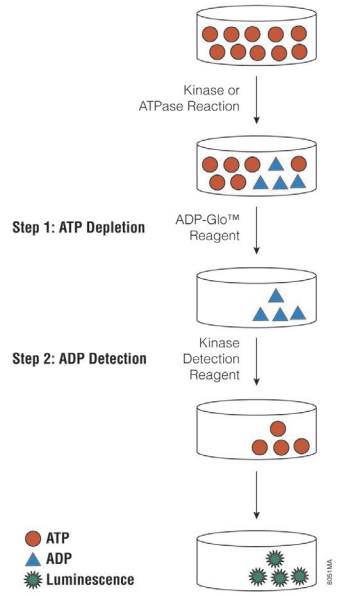

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

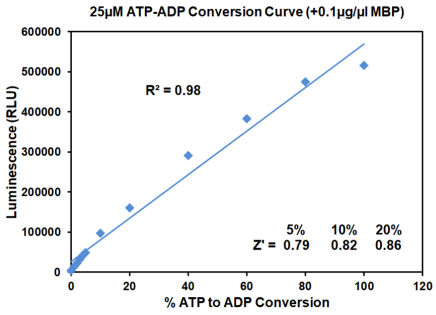

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

ADP-Glo™ Kinase Assay Application Note
Ser/Thr Kinase Series

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	300	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0
Luminescence	437,697	353,931	336,494	203,846	95,560	39,816	17,550	8,717	5,087	2,287
S/B	191	155	147	89	42	17	8	4	2	1
% Conversion	78	62	59	35	15	4	0	0	0	0

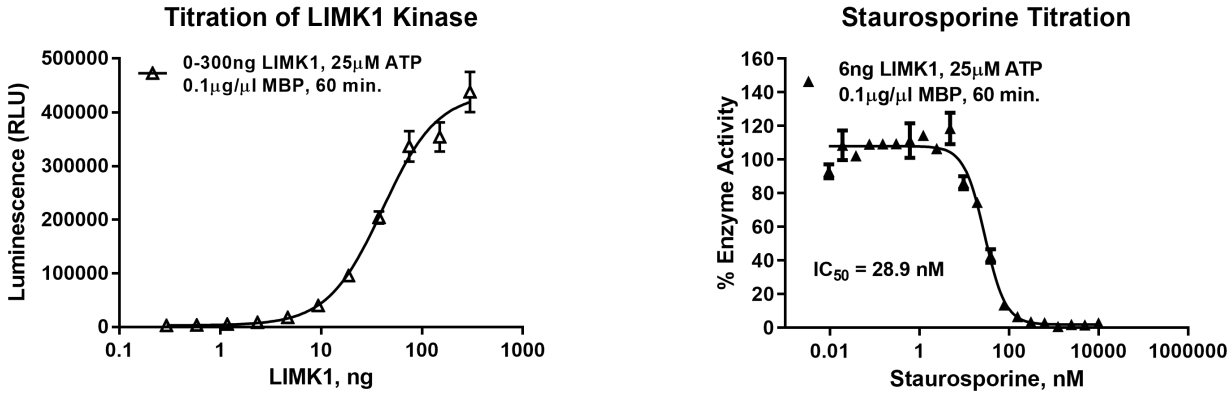

Figure 3. LIMK1 Kinase Assay Development. (A) LIMK1 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 6ng of LIMK1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
LIMK1 Kinase Enzyme System	10µg	VA7480
	1mg	VA7481
ADP-Glo™ + LIMK1 Kinase Enzyme System	1 Each	VA7482