ADP-Glo™ Kinase Assay Application Notes

TYROSINE KINASE SERIES: LCK

LCK Kinase Assay

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Scientific Background:

LCK (p56lck) is a member of the src family of non-receptor tyrosine kinases. It was identified as a gene rearranged and overexpressed in the murine lymphoma LSTRA, most likely as a result of the insertion of Moloney murine leukemia virus DNA immediately adjacent to the gene (1). Lck behaves as a proto-oncogene and can lead to cell transformation upon activation. A number of human cancer cell lines show overexpression of LCK, pointing to a possible role for this kinase in the initiation and maintenance of the transformed state in human cancers (2).

1. Fischer, S. et al: The amino terminal region of the p56 lck from LSTRA exerts negative modulation on the tyrosine kinase activity. Biochem Biophys Res Commun. 1987 Mar 30;143(3):819-26.

2. Veillette, A. et al: Expression of the lck tyrosine kinase gene in human colon carcinoma and other non-lymphoid human tumor cell lines. Oncogene Res. 1987 Sep-Oct;1(4):357-74.

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.

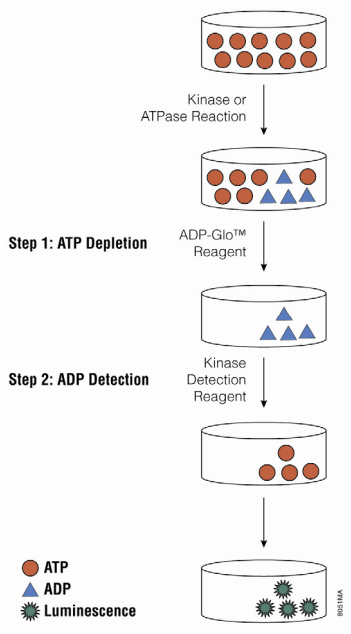

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

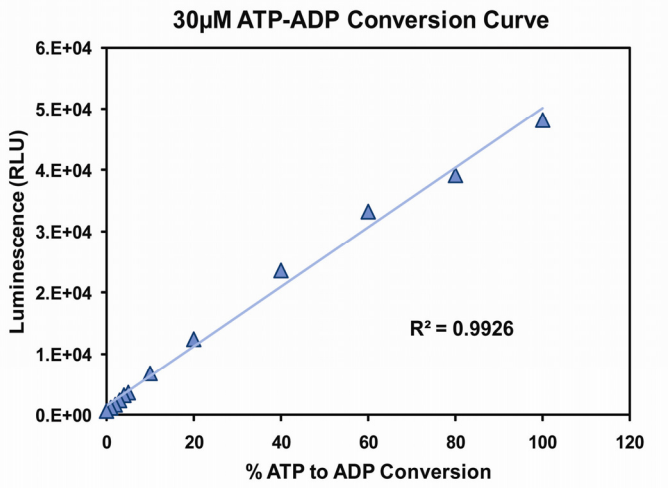

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 30µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction.

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 Tyrosine 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. LCK 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.

LCK, ng	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0
Luminescence	40003	34773	27937	18370	11628	6819	4143	2591	678
S/B	59.0	51.3	41.2	27.1	17.2	10.1	6.1	3.8	1
% Conversion	79.2	68.4	54.3	34.6	20.7	10.8	5.3	2.1	0

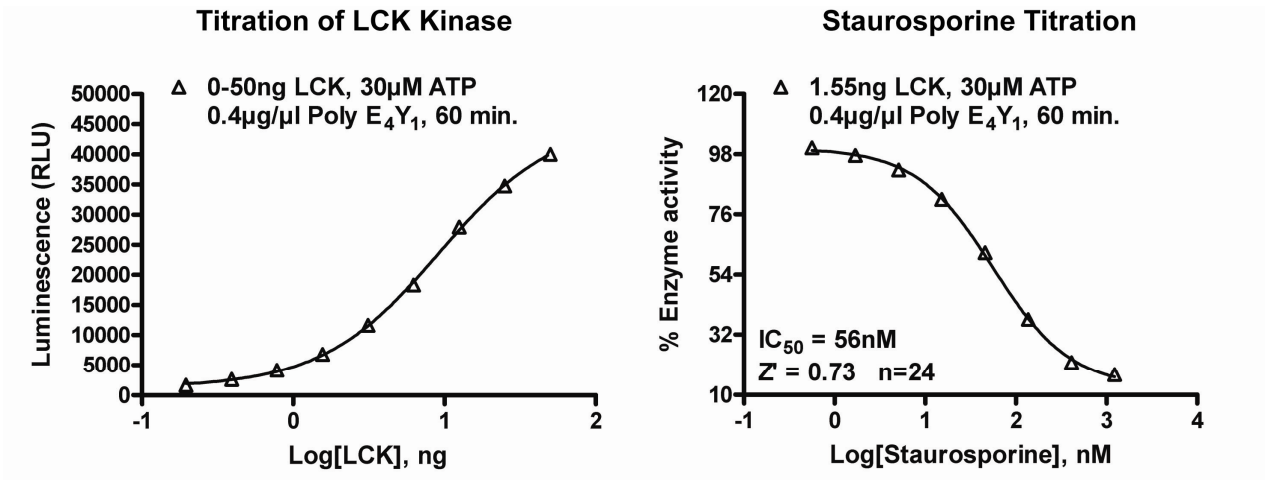

Figure 3. LCK Kinase Assay Development. (A) LCK enzyme was titrated using 30µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1.55ng of LCK to determine the potency of the inhibitor (IC_{50}). Z' factor was determined using 24 replicates of each of the minimum and maximum response (10 and 0µM staurosporine, respectively).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
LCK Kinase Enzyme System	Promega	V2691
ADP-Glo + LCK Kinase Enzyme System	Promega	V9491

LCK Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 2mM $MnCl_2$; 50µM DTT.