MLCK Kinase Assay

By Juliano Alves, Ph.D., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

MLCK or myosin light chain kinase is a muscle member of the immunoglobulin gene superfamily and is a calcium/calmodulin dependent protein kinase. MLCK is a regulatory protein for smooth muscle contraction, which acts by phosphorylating 20-kDa myosin light chain (MLC20) to activate the myosin ATPase activity. The kinase activity for the phosphorylation is localized at the central part of MLCK, which is also furnished with actin-binding activity at its N terminal and myosin-binding activity at its C terminal (1). Phosphorylation of regulatory light chain of myosin plays an important role in controlling the morphological changes seen during cell division (2).

1. Gao, Y. et al: Myosin light chain kinase as a multifunctional regulatory protein of smooth muscle contraction. IUBMB Life. 2001 Jun;51(6):337-44.

2. Matsumura, F. et al: Role of myosin light chain phosphorylation in the regulation of cytokinesis. Cell Struct Funct. 2001 Dec;26(6):639-44.

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.

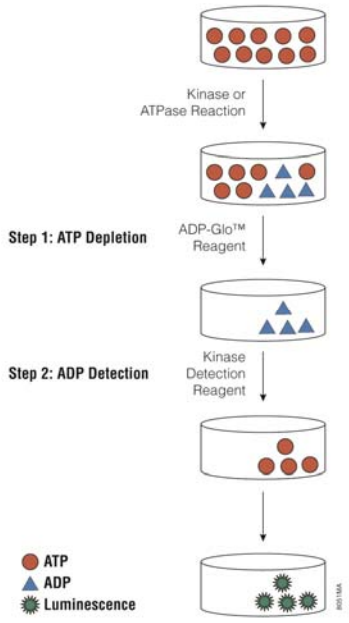


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

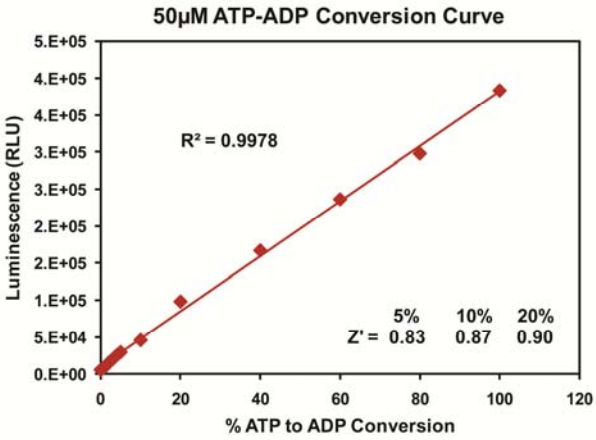


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

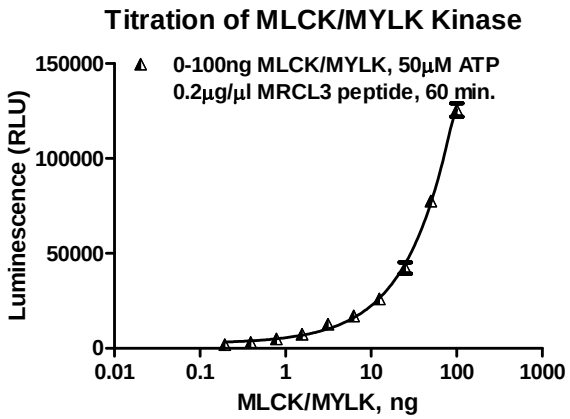
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, and the KES Protocol available at: http://www.promega.com/tbs/tm313/tm313.html, and http://www.promega.com/KESProtocol, respectively.

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 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 0.5-1sec).

Table 1. MLCK 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.

MLCK, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0
RLU	125510	77558	42341	26114	17075	12658	7397	4941	3043	1298
S/B	97	60	33	20	13	10	6	4	2	1
% Conversion	29	18	9	6	4	3	1.4	0.9	0.4	0



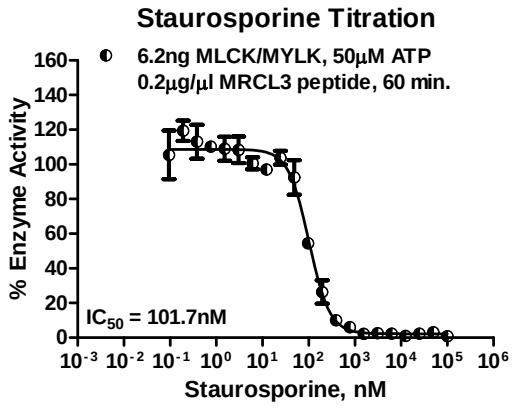


Figure 3. MLCK Kinase Assay Development. **(A) MLCK enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 6.2ng of MLCK to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
MLCK Kinase Enzyme System	Promega	V4496
ADP-Glo™ + MLCK Kinase Enzyme System	Promega	V4497

MLCK Kinase Buffer: 40mM Tris, pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT; Ca^{2+}/Calmodulin Solution II (0.05µg/µl Calmodulin, 10mM Tris, pH 7.2, 4mM $CaCl_2$)