ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: ROCK2

ROCK2 Kinase Assay

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

Scientific Background:

ROCK2 is a ubiquitously expressed serine/threonine kinase localized in the nucleus that regulates cytokinesis, smooth muscle contraction, the formation of actin stress fibers and focal adhesions, and the activation of the c-fos serum response element (1). ROCK2 is an immediate downstream target of the small GTPase RhoA. ROCK2 may play a pivotal role in cardiovascular diseases such as vasospastic angina, ischemic stroke, and heart failure. Inhibition of ROCKs by statins or other selective inhibitors leads to the upregulation and activation of endothelial nitric oxide synthase (eNOS) and reduction of vascular inflammation and atherosclerosis (2).

1. Zhao, Z. et al: Rho-associated kinases play a role in endocardial cell differentiation and migration. Dev Biol. 2004 Nov 1;275(1):183-91.

2. Noma, K. et al: Physiological role of ROCKs in the cardiovascular system. Am J Physiol Cell Physiol. 2006 Mar;290(3):C661-8.

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.

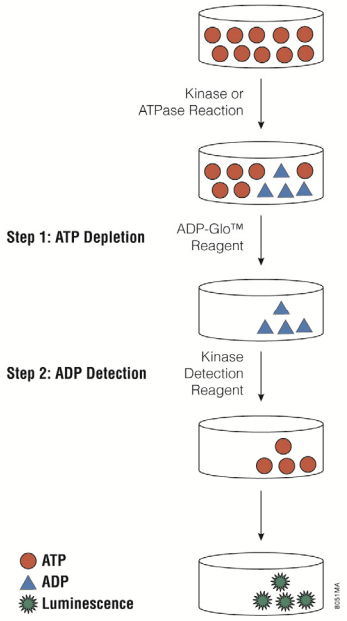

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

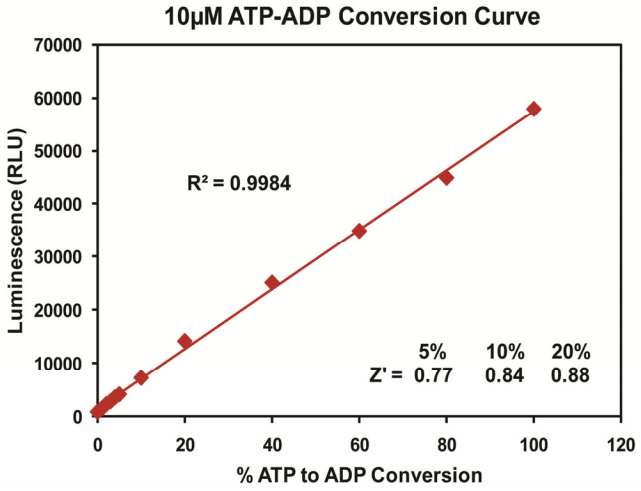

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 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. ROCK2 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.

ROCK2, ng	38	19	9.4	4.7	2.3	1.2	0.6	0.3	0.15	0
RLU	74273	73119	68026	52882	30117	12879	7323	4177	1934	324
S/B	229	226	210	163	93	40	23	13	6	1
% Conversion	100	100	93	72	40	17	9	5	2	0

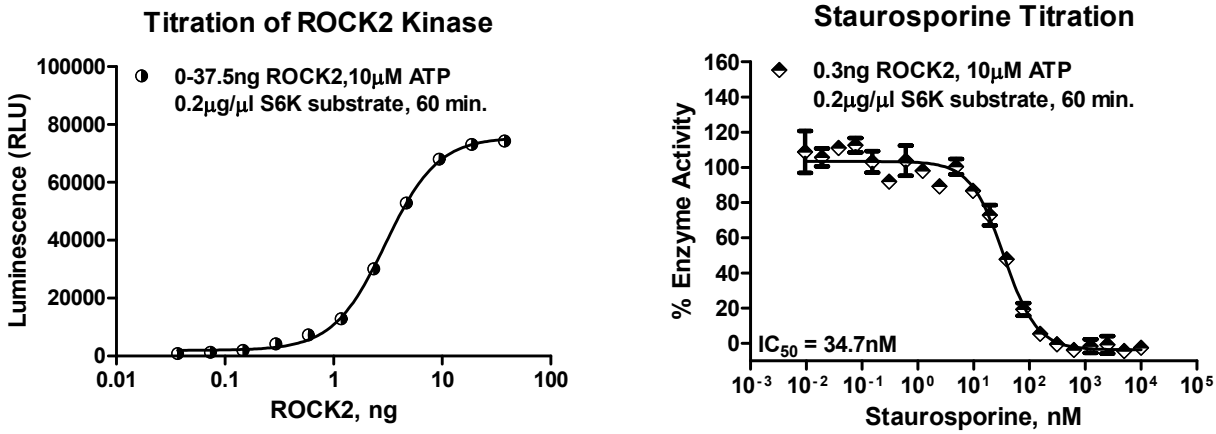

Figure 3. ROCK2 Kinase Assay Development. (A) ROCK2 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 0.3ng of ROCK2 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
ROCK2 Kinase Enzyme System	Promega	V4044
ADP-Glo™ + ROCK2 Kinase Enzyme System	Promega	V4045

ROCK2 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.