ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: ROCK1

ROCK1 Kinase Assay

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Scientific Background:

ROCK1 is a ubiquitously expressed serine/threonine kinase that is a downstream target of the small GTPase RhoA. ROCK1 is involved in diverse cellular functions, including smooth muscle contraction, actin cytoskeleton organization, cell adhesion and motility, and gene expression (1). ROCK1 contributes to the development of cardiac fibrosis and induction of fibrogenic cytokines in cardiomyocytes in response to pathological stimuli. ROCK1 knockout mice exhibit reduced perivascular and interstitial fibrosis which is associated with reduced expression of a variety of extracellular matrix (ECM) proteins and fibrogenic cytokines (2).

1. Zhao, Y M. et al: Rho-associated kinases play a role in endocardial cell differentiation and migration. Dev Biol. 2004 Nov 1;275(1):183-91.
2. Zhang, C. et al: Targeted deletion of ROCK1 protects the heart against pressure overload by inhibiting reactive fibrosis. FASEB J. 2006 May;20(7):916-25.

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.

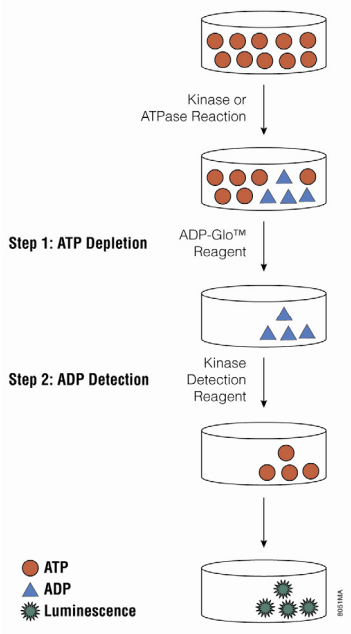

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

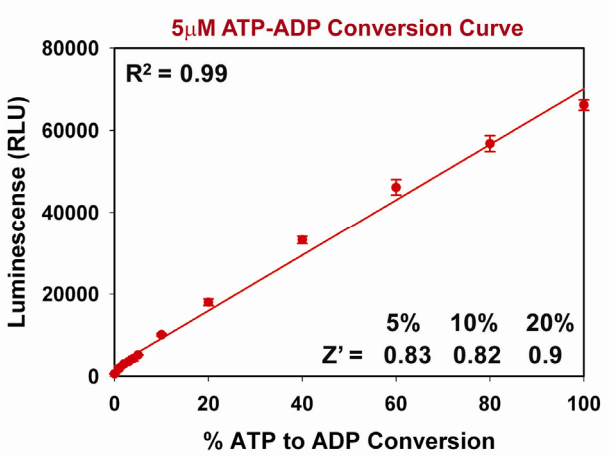

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 5μM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. ROCK1 Enzyme Titration. Reactions were carried out for 60 minutes and kinase activity was determined using ADP-Glo. 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.

ROCK1, ng	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0
RLU	19353	13545	7290	4838	2887	1766	1376	1006	536
S/B	36.1	25.2	13.6	9.1	5.3	3.3	2.5	1.8	1
% Conversion	21.2	14.6	7.5	4.7	2.5	1.3	0.8	0.4	0

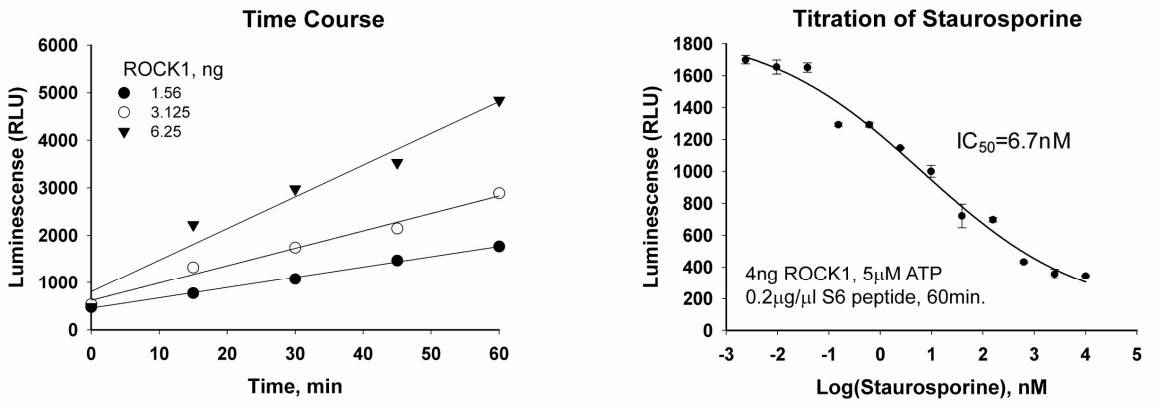

Figure 3. ROCK1 Kinase Assay Development. ROCK1 linear response curves were obtained at indicated amounts of enzyme using 0.2µg/µl of S6K peptide substrate and 5µM ATP. To determine the potency of the inhibitor (IC_{50}) staurosporine dose response was performed under conditions indicated in the figure.

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
ROCK1 Kinase Enzyme System	Promega	V3411
ADP-Glo + ROCK1 Kinase Enzyme System	Promega	V9581

ROCK1 Kinase Buffer: 40mM Tris, 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT

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