ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: RSK1

RSK1 Kinase Assay

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Scientific Background:

RSK1 is a member of the RSK (ribosomal S6 kinase) family that are growth factor-regulated serine/threonine kinases. RSK1 contains 2 nonidentical kinase catalytic domains and phosphorylates various substrates, including members of the mitogen-activated kinase (MAPK) signalling pathway. RSK1 encodes a predicted 735-amino acid protein containing 2 distinct consensus ATP-binding site sequences. RSK1 transcript is present in lymphocytes, skeletal muscle, liver, and adipose tissue (1). RSKs are implicated in the activation of the mitogen-activated kinase (MAPK) cascade and the stimulation of cell proliferation and differentiation (2).

1. Moller, D E. et al: Human rsk isoforms: cloning and characterization of tissue-specific expression. Am. J. Physiol. 266: C351-C359, 1994.

2. Gross, S D. et al: Induction of metaphase arrest in cleaving Xenopus embryos by the protein kinase p90(Rsk). Science 286: 1365-1367, 1999.

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.

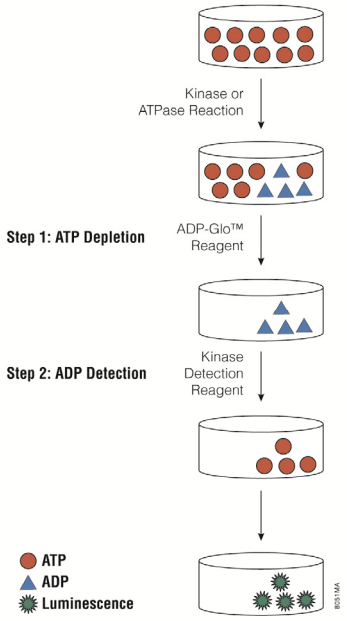

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

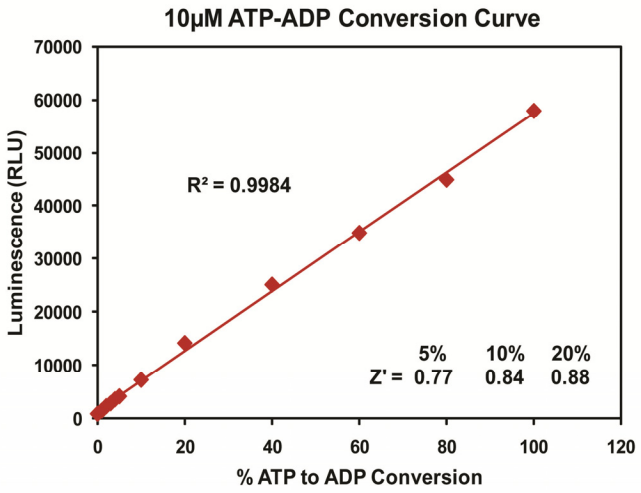

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. RSK1 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.

RSK1, ng	25	13	6.3	3.1	1.6	0.8	0.4	0.2	0
RLU	72341	55820	34619	17080	7407	3376	1543	908	316
S/B	229	177	110	54	23	11	5	3	1
% Conversion	84	65	40	19	8	3.1	1.2	0.6	0

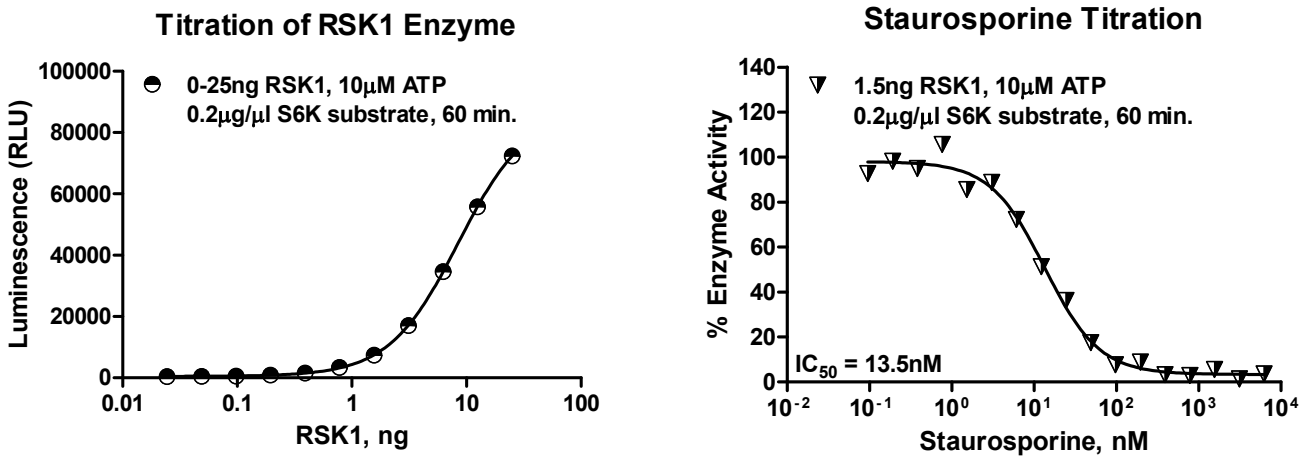

Figure 3. RSK1 Kinase Assay Development. (A) RSK1 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1.5ng of RSK1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
RSK1 Kinase Enzyme System	Promega	V4046
ADP-Glo™ + RSK1 Kinase Enzyme System	Promega	V4047

RSK1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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