RSK2 Kinase Assay

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Scientific Background:

RSK2 is a member of the RSK (ribosomal S6 kinase) family that are growth factor-regulated serine/threonine kinases. RSK2 has been shown to mediate growth factor signaling via RAS and MAPK leading to the induction of CREB serine-133 phosphorylation and activation of gene expression (1). Mutations in RSK2 have been shown to be responsible for Coffin-Lowry syndrome (CLS) which is a X-linked disorder characterized by severe psychomotor retardation, facial and digital dysmorphisms, and progressive skeletal deformations (2).

1. Xing, J. et al: Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. Science. 1996 Aug 16;273(5277):959-63.
2. Jacquot, S. et al: Mutation analysis of the RSK2 gene in Coffin-Lowry patients: extensive allelic heterogeneity and a high rate of de novo mutations. Am J Hum Genet. 1998 Dec;63(6):1631-40

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.

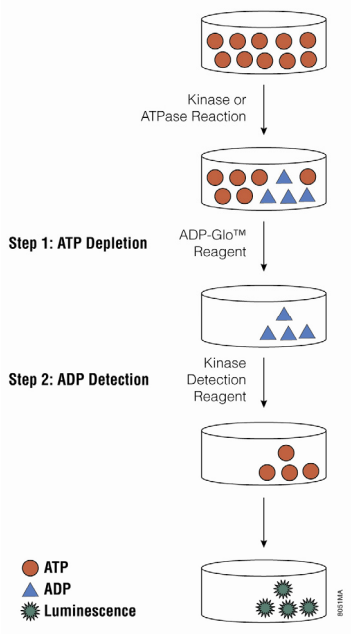

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

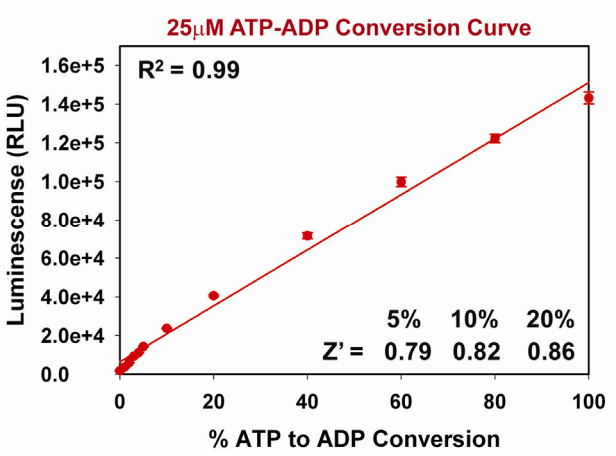

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. RSK2 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.

RSK2, ng	25	12.5	6.25	3.12	1.5	0.78	0.39	0.2	0
RLU	116612	100272	79850	52074	28476	15384	8807	5398	1781
S/B	65.476	56.301	44.834	29.238	15.988	8.6376	4.945	3.0309	1
% Conversion	58.282	49.902	39.429	25.185	13.083	6.3695	2.997	1.2487	0

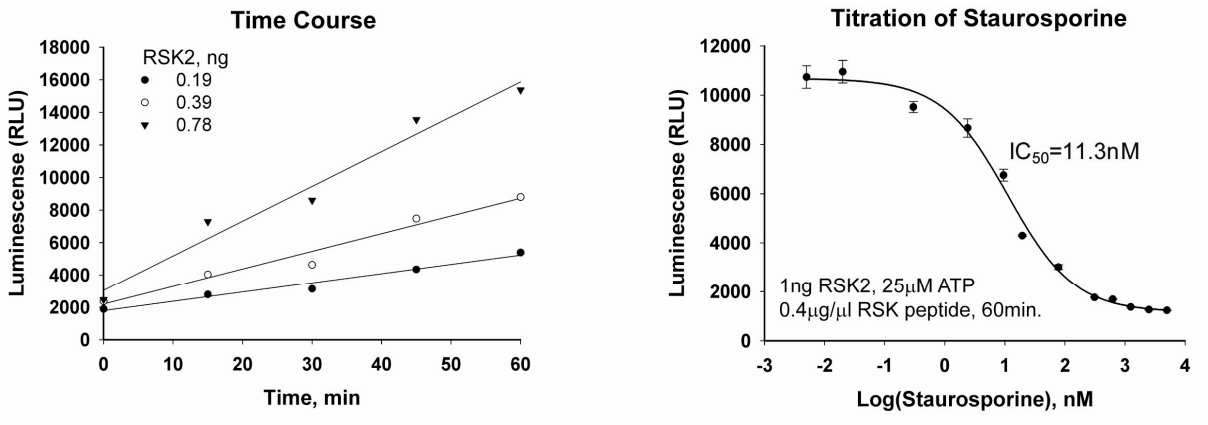

Figure 3. RSK2 Kinase Assay Development. RSK2 linear response curves were obtained at indicated amounts of enzyme using 0.4µg/µl of RSK peptide substrate and 25µM ATP. To determine the potency of the inhibitor (IC_{50}) staurosporine dose response was performed under conditions indicated in the figure.

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
RSK2 Kinase Enzyme System	Promega	V3501
ADP-Glo + RSK2 Kinase Enzyme System	Promega	V9651

RSK2 Kinase Buffer: 40mM Tris, 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT

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