ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

RSK2 (L608F) Kinase Assay

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Scientific Background:

RSK2 is a member of the RSK (ribosomal S6 kinase) family that consists of 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.

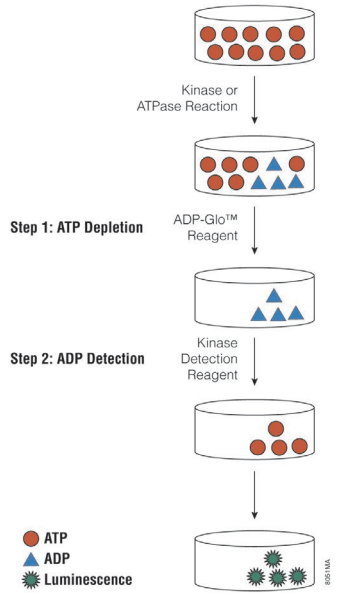

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

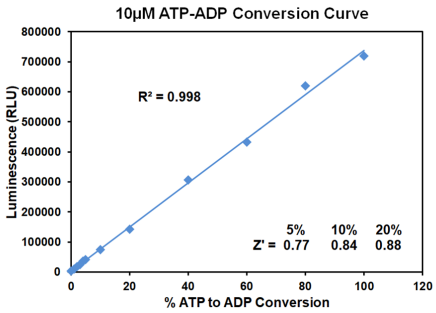

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction 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 indicated time (See Figure 3).
- 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 0.5-1 second).

Table 1. 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.

Enzyme, ng	125	62.50	31.25	15.63	7.81	3.91	1.95	0.98	0.49	0.24	0.12	0
Luminescence	405,546	400,922	364,489	285,710	189,438	115,783	59,317	31,821	17,426	9,437	6,106	2,023
S/B	200	198	180	141	94	57	29	16	9	5	3	1
% Conversion	86	85	77	60	40	24	12	6	3	1	1	0

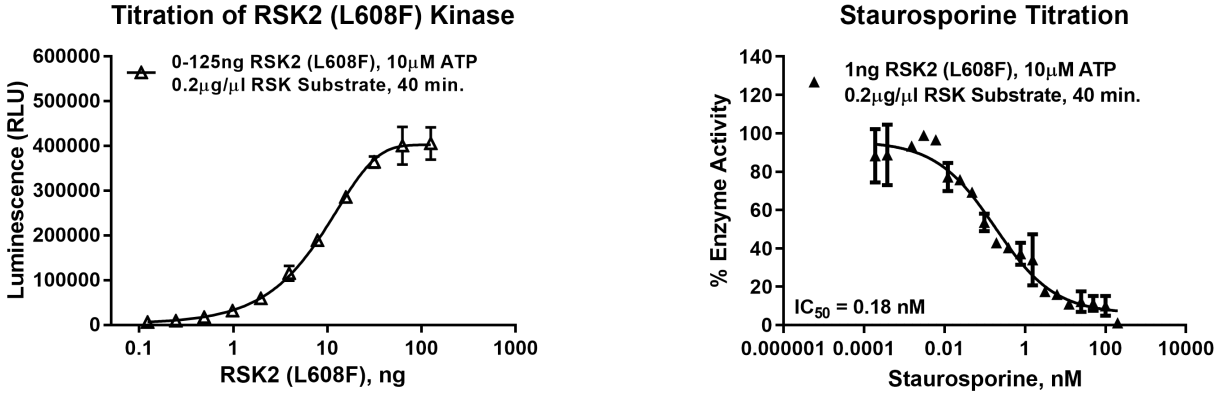

Figure 3. RSK2 (L608F) Kinase Assay Development. (A) RSK2 (L608F) enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1ng of RSK2 (L608F) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
RSK2 (L608F) Kinase Enzyme System	10µg	VA7297
	1mg	VA7298
ADP-Glo™ + RSK2 (L608F) Kinase Enzyme System	1 Each	VA7299