ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

RSK4 Kinase Assay

By Juliano Alves, Laurie Engel, Said A. Goueli, and Hicham Zegzouti, Promega Corporation

Scientific Background:

RSK4 is a member of the 90-kDa ribosomal S6 kinase family which are important components of growth factor mediated stimulation of cellular proliferation, survival and differentiation. RSK4 is activated via coordinated phosphorylation by ERK and 3-phosphoinositide-dependent protein kinase-1 (PDK1) (1). RSK4 is often associated with contiguous gene syndromes consisting of X-linked deafness type 3 (DFN3), mental retardation (MRX) and choroideremia (CHM). RSK4 is most abundantly expressed in the brain and kidney (2).

1. Yntema, H.G. et al: A novel ribosomal S6-kinase (RSK4; RPS6KA6) is commonly deleted in patients with complex X-linked mental retardation. Genomics. 1999 ;15;62(3):332-43

2. Bettina, A. et al: Functional Characterization of Human RSK4, a New 90-kDa Ribosomal S6 Kinase, Reveals Constitutive Activation in Most Cell Types. J. Biol. Chem., 2005; 280, Issue 14,13304-13314

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.

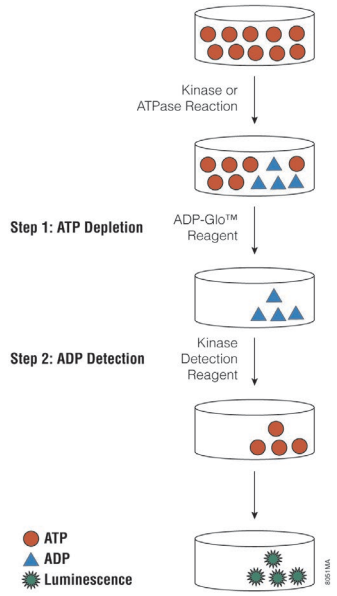

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

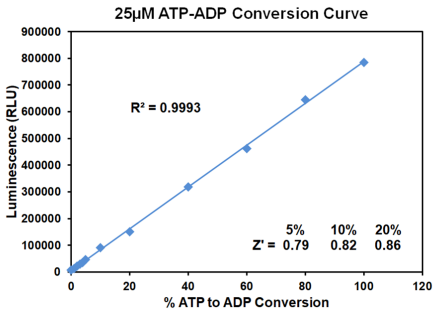

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	100	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0
Luminescence	561,899	489,820	516,976	434,481	378,232	210,752	143,465	80,490	39,936	20,597	15,997	3,254
S/B	173	151	159	134	116	65	44	25	12	6	5	1
% Conversion	66	57	61	51	44	24	16	8	3	1	0	0

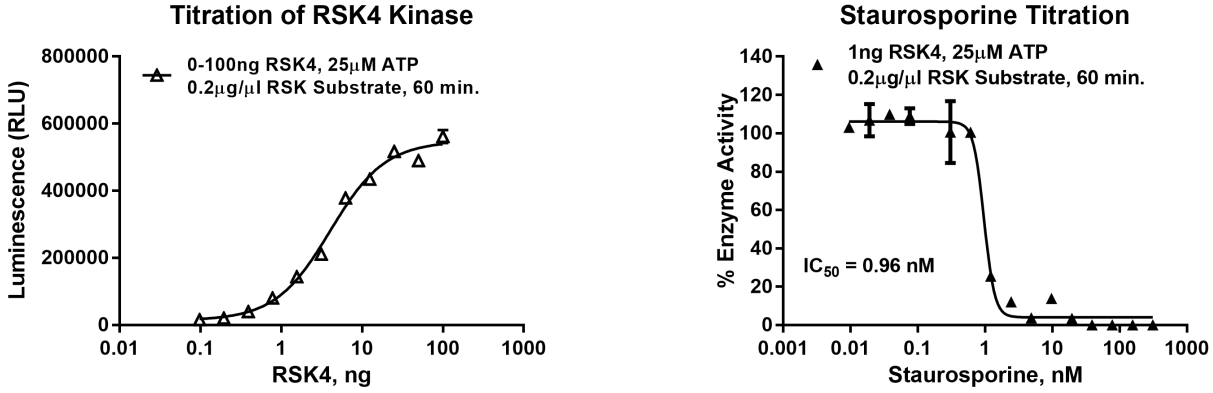

Figure 3. RSK4 Kinase Assay Development. (A) RSK4 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1ng of RSK4 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
RSK4 Kinase Enzyme System	10µg	VA7552
	1mg	VA7553
ADP-Glo™ + RSK4 Kinase Enzyme System	1 Each	VA7554