ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: p70S6Kb

p70S6Kb Kinase Assay

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Scientific Background:

p70S6Kb is a member of the RSK (ribosomal S6 kinase) family of serine/threonine kinases and is activated by mitogenic stimuli, including growth factors, cytokines, and phorbol esters. p70S6Kb contains 2 nonidentical kinase catalytic domains and phosphorylates the S6 ribosomal protein and eucaryotic translation initiation factor 4B. Phosphorylation of S6 leads to an increase in protein synthesis and cell proliferation. PI3 kinase pathway and mTOR are involved in the activation of p70S6Kb but other pathways can also activate this target protein.

1. Gout, I. et al: Molecular cloning and characterization of a novel p70 S6 kinase, p70 S6 kinase beta containing a proline-rich region. J. Biol. Chem. 273: 30061-30064, 1998.

2. Saitoh, M. et al: Cloning and characterization of p70(S6K-beta) defines a novel family of p70 S6 kinases. Biochem. Biophys. Res. Commun. 253: 470-476, 1998.

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.

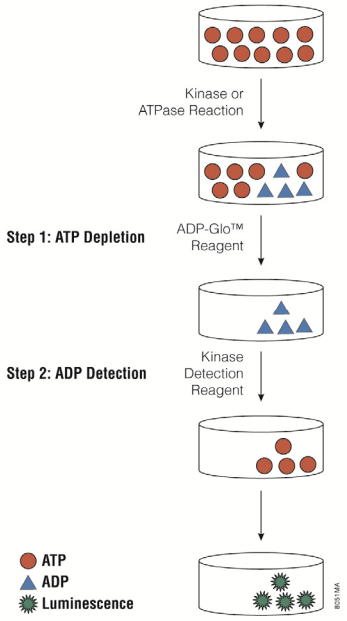

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

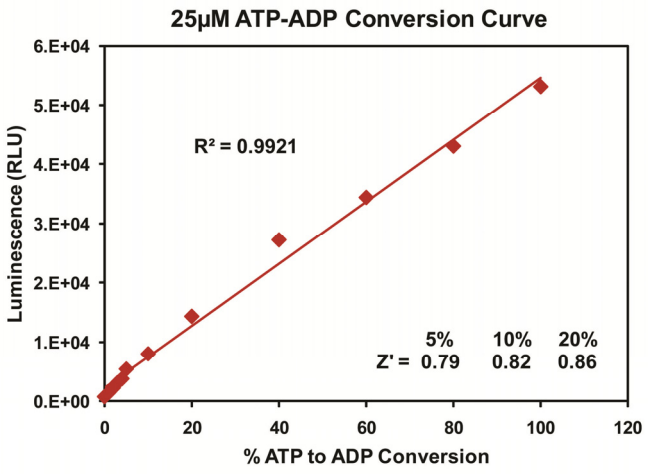

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. p70S6Kb 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.

P70S6Kb, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0
RLU	77234	70683	38224	16259	9421	4578	2946	1620	1095	849
S/B	91	83	45	19	11	5	3.5	1.9	1.3	1
% Conversion	70	64	34	14	8	3	2.0	0.8	0.3	0

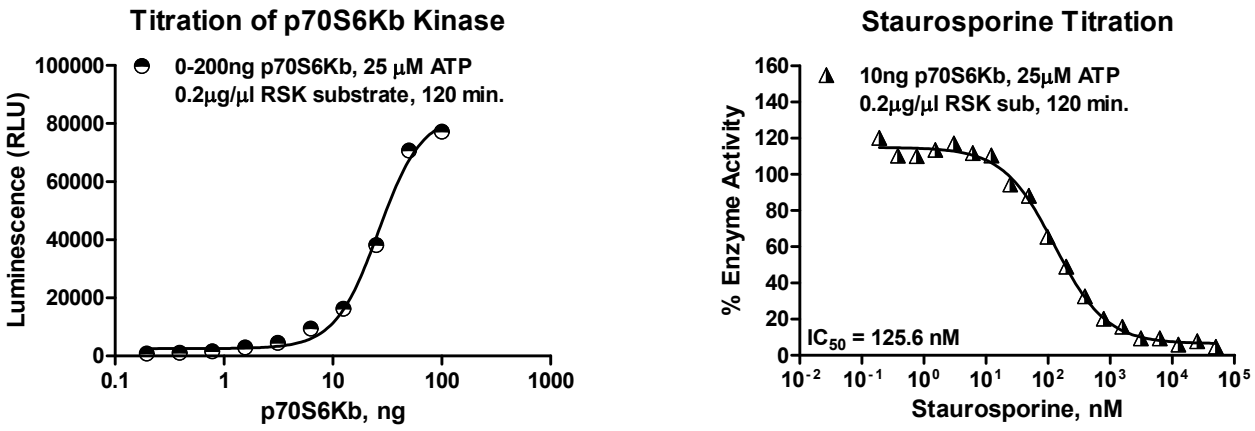

Figure 3. p70S6Kb Kinase Assay Development. **(A) P70S6Kb enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of P70S6Kb to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
p70S6Kb Kinase Enzyme System	Promega	V4030
ADP-Glo™ + p70S6Kb Kinase Enzyme System	Promega	V4031

p70S6Kb Kinase Buffer: 5 mM MOPS, pH 7.2, 2.5mM β-glycerophosphate, 5mM $MgCl_2$, 1mM EGTA, 0.4mM EDTA; 50µM DTT

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