

## p70S6K Kinase Assay

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### Scientific Background:

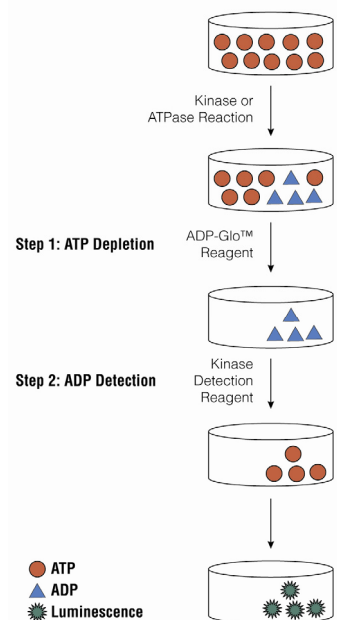
p70S6K is responsible for the phosphorylation of 40S ribosomal protein S6 and is ubiquitously expressed in human adult tissues (1). p70S6K is activated by serum stimulation and this activation is inhibited by wortmannin and rapamycin. p70S6K activity undergoes changes in the cell cycle and increases 20-fold in G1 cells released from G0 (2). p70S6K activation requires sequential phosphorylations at proline-directed residues in the putative autoinhibitory pseudosubstrate domain, as well as threonine 389 a site phosphorylated by phosphoinositide-dependent kinase 1 (PDK-1).

1. Ferrari, S. et al: S6 phosphorylation and the p70s6k/p85s6k. *Crit Rev Biochem Mol Biol.* 1994;29(6):385-413. Review.
2. Edelman, HM. Et al: Cell cycle regulation of p70 S6 kinase and p42/p44 mitogen-activated protein kinases in Swiss mouse 3T3 fibroblasts. *J Biol Chem.* 1996 Jan 12;271(2):963-71.

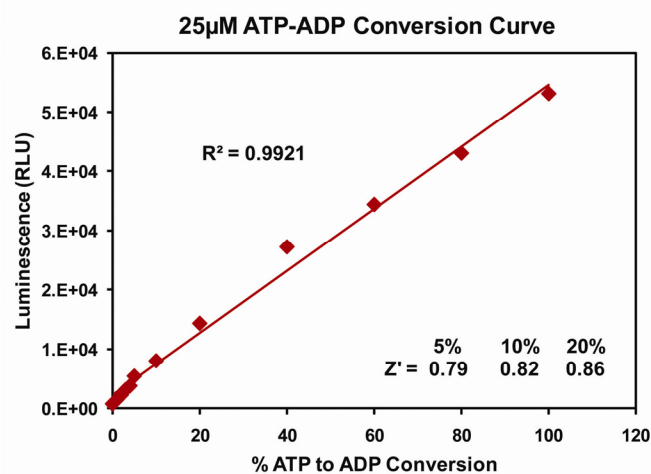
### ADP-Glo™ Kinase Assay

#### 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.



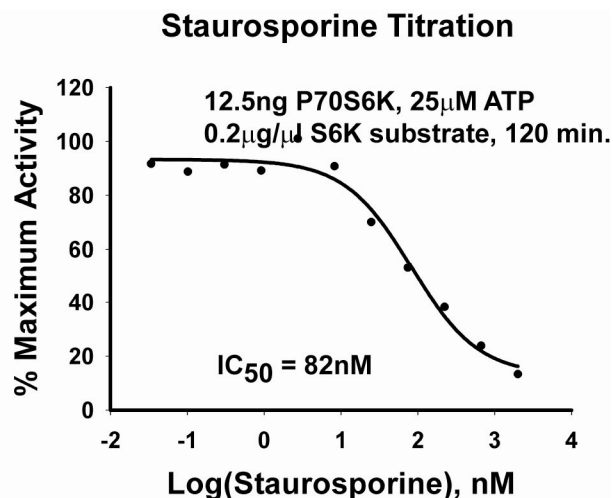
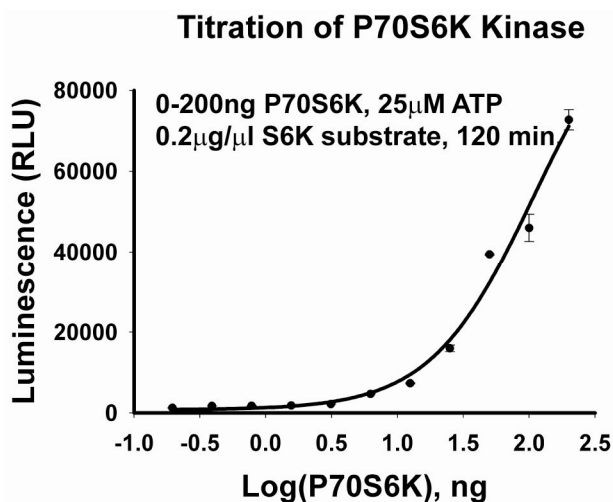
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay Technical Manual #TM313*, available at [www.promega.com/tbs/tm313/tm313.html](http://www.promega.com/tbs/tm313/tm313.html)

## Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase Buffer.
- Add to the wells of 384 low volume plate:
  - 1  $\mu$ l of inhibitor or (5% DMSO)
  - 2  $\mu$ l of enzyme (defined from table 1)
  - 2  $\mu$ l of substrate/ATP mix
- Incubate at room temperature for 60 minutes.
- Add 5  $\mu$ l of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10  $\mu$ l of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1second).

**Table 1. p70S6K Enzyme Titration.** Data are shown as relative light units (RLU) that directly correlate to the amount of ADP produced. The correlation between the % ATP converted to ADP and corresponding signal to background ratio is indicated for each kinase amount.

p70S6K, ng	200	100	50	25	12.5	6.25	3.13	1.56	0
RLU	72711	45986	39386	15961	7315	4687	2194	1827	1221
S/B	59.5	37.7	32.3	13.1	6.0	3.8	1.8	1.5	1.0
% Conversion	60.26	37.12	31.41	11.13	3.64	1.37	0.90	0.70	0.00



**Figure3. p70S6K Kinase Assay Development.** (A) p70S6K enzyme was titrated using 25 $\mu$ M ATP and the luminescence signal generated from each of the amounts is shown. (B) Staurosporine dose response was created using 12.5ng of p70S6K to determine the potency of the inhibitor (IC<sub>50</sub>).

### Assay Components and Ordering Information:



#### Products

ADP-Glo™ Kinase Assay  
p70S6K Kinase Enzyme System  
ADP-Glo + p70S6K Kinase Enzyme System

#### Company

Promega  
Promega  
Promega

#### Cat.#

V9101  
V2741  
V9611

P70S6K Kinase Buffer: 40mM Tris, 7.5; 20mM MgCl<sub>2</sub>; 0.1mg/ml BSA; 50 $\mu$ M DTT.