ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

GCK Kinase Assay

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Scientific Background:

GCK is a serine/threonine protein kinase that is activated by TNFα and interacts with the TNF receptor-associated factor 2 (TRAF2). GCK is upstream of MAP kinases and particularly activates the SAPK pathway (1). Although GCK is found in many tissues, its expression in lymphoid follicles is restricted to the cells of germinal centre, where it may participate in B-cell differentiation. A mouse protein Rab8ip that has high homology to GCK can interact with the vesicular transport protein Rab8. Based on its interaction with Rab8, it is postulated that Rab8ip/GCK may modulate secretion in response to stress stimuli (2).

1. Pombo C M, et al: Activation of the SAPK pathway by the human STE20 homologue germinal centre kinase. Nature 377: 750-754, 1995.

2. Ren M, et al: In its active form, the GTP-binding protein rab8 interacts with a stress-activated protein kinase. Proc. Nat. Acad. Sci. 93: 5151-5155, 1996.

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.

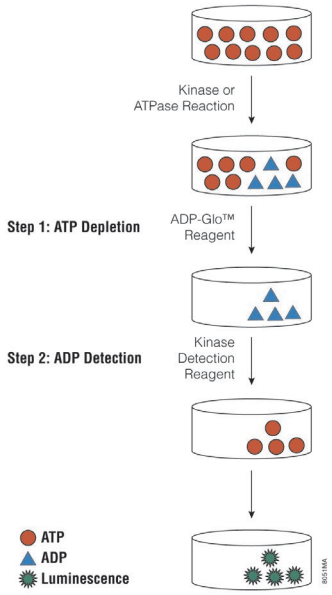

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

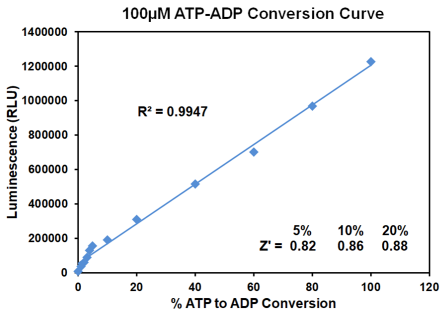

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	200	100	50	25	12.50	6.25	3.13	1.56	0.78	0
Luminescence	1,630,365	881,282	421,636	206,113	117,051	74,984	46,514	28,975	19,261	11,570
S/B	141	76	36	18	10	6	4	2	2	1
% Conversion	94	49	21	8	3	1	0	0	0	0

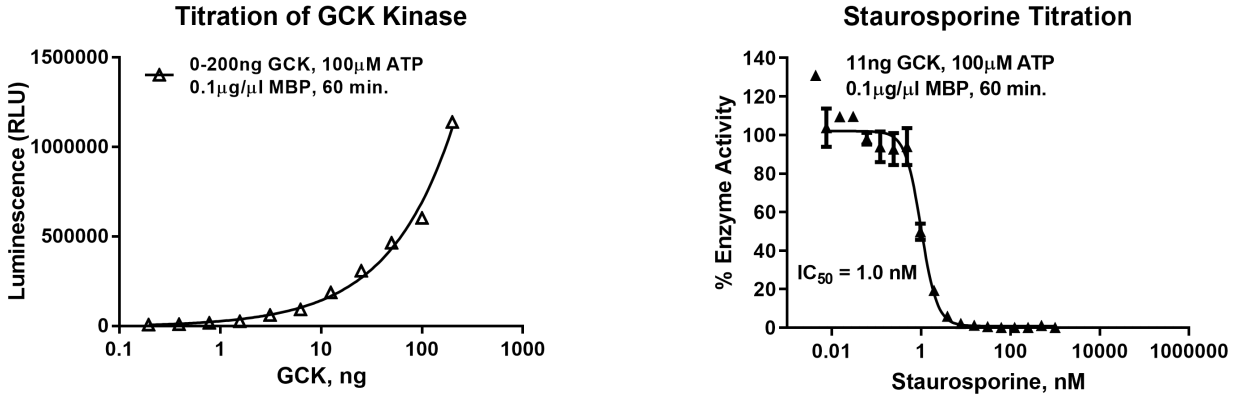

Figure 3. GCK Kinase Assay Development. (A) GCK enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 11ng of GCK to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
GCK Kinase Enzyme System	10µg	VA7174
	1mg	VA7175
ADP-Glo™ + GCK Kinase Enzyme System	1 Each	VA7176