ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

GLK Kinase Assay

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

Scientific Background:

GLK is a member of the STE20 family of serine/threonine protein kinases and contains N-terminal catalytic domain and C-terminal regulatory domain. GLK is upstream of the MEKK1 target and is stimulated by UV radiation and TNF-α and can lead to the activation of the JNK signaling pathway. GLK can directly activate PKC-θ during TCR signaling and people with systemic lupus erythematosus show considerable enhanced GLK expression and activation of PKC-θ and the kinase IKK in T cells (1). GLK can act as a pro-apoptotic kinase which orchestrates activation of BAX via the concerted posttranscriptional modulation of PUMA, BAD, and BIM (2).

1. Chuang, H C. et al: The kinase GLK controls autoimmunity and NF-κB signaling by activating the kinase PKC-θ in T cells. Nat Immunol. 2011 Oct 9;12(11):1113-8.

2. Lam, D. et al: MAP4K3 modulates cell death via the post-transcriptional regulation of BH3-only proteins. Proc Natl Acad Sci U S A. 2009 Jul 21;106(29):11978-83.

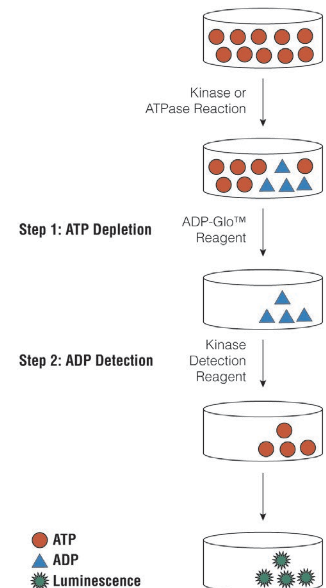

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

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.

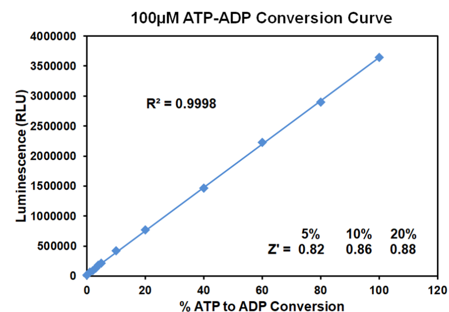

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

ADP-Glo™ Kinase Assay Application Note
Ser/Thr Kinase Series

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	140	70	35	17.50	8.75	4.38	2.19	0
Luminescence	2,227,510	1,335,510	789,749	369,825	171,424	78,767	45,098	12,242
S/B	182	109	65	30	14	6	4	1
% Conversion	61	36	21	9	4	1	0	0

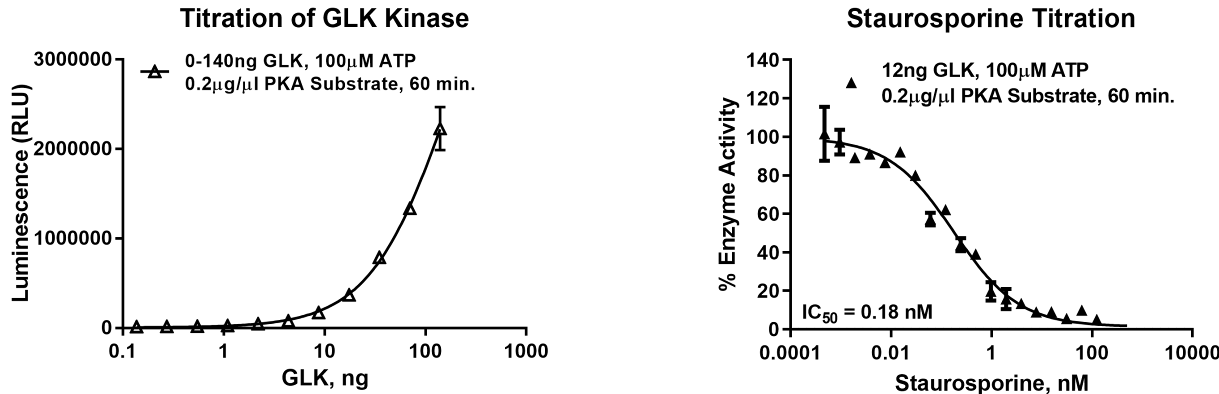

Figure 3. GLK Kinase Assay Development. (A) GLK enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 12ng of GLK to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
GLK Kinase Enzyme System	10µg	VA7177
	1mg	VA7178
ADP-Glo™ + GLK Kinase Enzyme System	1 Each	VA7179