ADP-Glo™ Kinase Assay Application Note

Lipid Kinase Series

DGKB Kinase Assay

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Scientific Background:

Diacylglycerol kinase beta (DAG kinase beta) is a member of class I diacylglycerol kinase family and is regulated by Ca+ (1). Via converting diacylglycerol into phosphatidic acid, diacylglycerol kinases decrease the diacylglycerol levels to regulate the activity of PKC, Rho and Ras families (2). DGK beta is localized in brain and is involved in emotion and long-term memory linked to cognitive function. Impaired levels of diacylglycerol by DGK beta limits the NADPH oxidase activation in phagosomes.

1. Merida, I. et al. Diacylglycerol kinases: at the hub of cell signaling. Biochem. J. (409), 1–18, 2008.

2. Kano, T. et al. Both the C1 domain and a basic amino acid cluster at the C-terminus are important for the neurite and branch induction ability of DGKb. Biochemical and Biophysical Research Communications. (447) 89–94, 2014.

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.

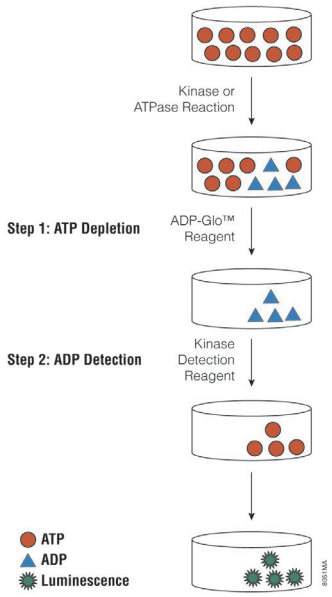

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

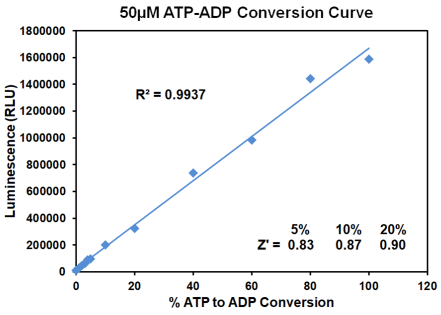

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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/1mM $CaCl_2$/0.05% Triton X-100 (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	0
Luminescence	569,897	241,912	117,551	62,504	38,223	23,325	17,291	8,084
S/B	70	30	15	8	5	3	2	1
% Conversion	32	13	6	3	1	1	0	0

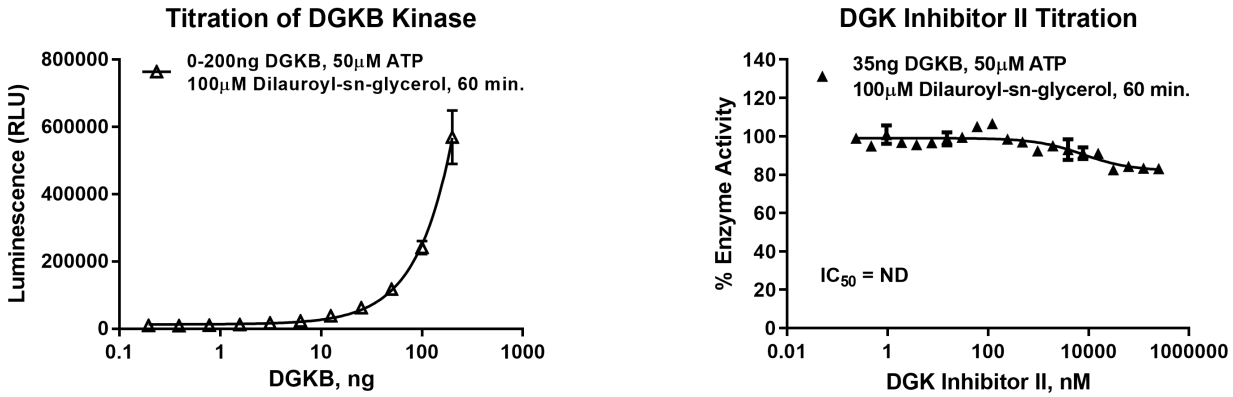

Figure 3. DGKB Kinase Assay Development. (A) DGKB enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 35ng of DGKB to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
DGKB Kinase Enzyme System	10µg	VA7609
	1mg	VA7610
ADP-Glo™ + DGKB Kinase Enzyme System	1 Each	VA7611