ADP-Glo™ Kinase Assay Application Note

Lipid Kinase Series

DGKZ Kinase Assay

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Scientific Background:

Diacylglycerol kinase zeta (DAG kinase zeta) is a member of class IV diacylglycerol kinase family, which contains two zinc fingers, an ATP binding site, and four ankyrin repeats with the MARCKS phosphorylation site, and exhibits the preference for 1,2-diacylglycerols but not for long chain diacylglycerols (1). DGK zeta regulates Ras activation via terminating the diacylglycerol signaling induced RasGRP (2). DGK zeta deficient mice were hyper-responsive to T cell receptor stimulation. Also, increased expression levels of DGK zeta promotes Rho GTPase activity and cancer cell invasion.

1. Bunting, M. et al. Molecular Cloning and Characterization of a Novel Human Diacylglycerol Kinase zeta. The Journal of Biological Chemistry. (271) 10230–10236, 1996.

2. Topham, M. et al. Diacylglycerol Kinase zeta Regulates Ras Activation by a Novel Mechanism. The Journal of Cell Biology. (152) 1135–1143, 2001.

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.

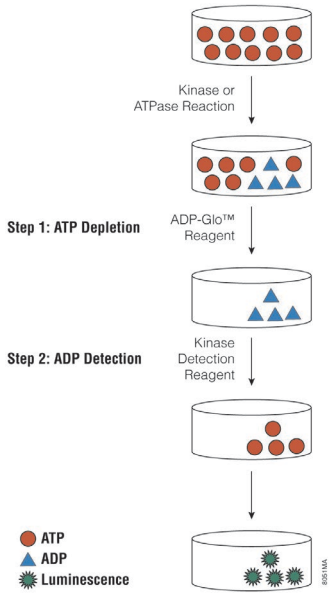

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

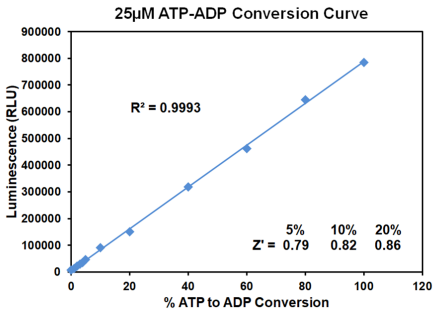

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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/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	1.56	0
Luminescence	233,476	105,422	63,222	40,796	30,864	26,850	19,317	14,166	3,468
S/B	67	30	18	12	9	8	6	4	1
% Conversion	24	10	6	3	2	2	1	0	0

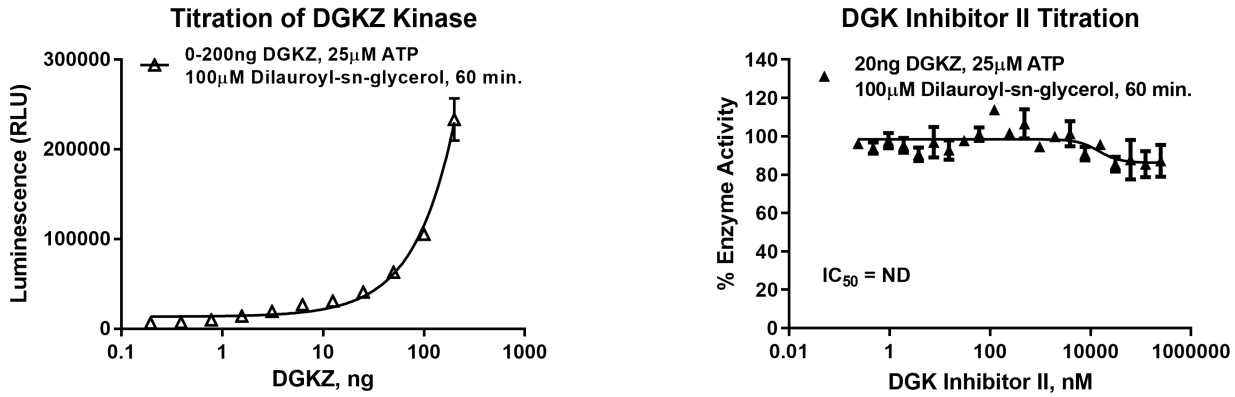

Figure 3. DGKZ Kinase Assay Development. (A) DGKZ enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 20ng of DGKZ to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
DGKZ Kinase Enzyme System	10µg	VA7618
	1mg	VA7619
ADP-Glo™ + DGKZ Kinase Enzyme System	1 Each	VA7620