ADP-Glo™ Kinase Assay Application Note

Lipid Kinase Series

DGKE Kinase Assay

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Scientific Background:

Diacylglycerol kinase epsilon (DAG kinase epsilon) is the only member of class III diacylglycerol kinase family, which lacks the E-F hand motifs for Ca+ binding but possess a clear selectivity for arachidonoyl-containing species of diacylglycerol (1). DGK epsilon might decrease signals conducted via arachidonoyl-diacylglycerol or promotes the synthesis of phospholipids with certain fatty acids (1). DGK epsilon contributes to Huntington disease pathogenesis and its inhibition might be effect in the therapeutics (2). Also, recessive mutations in DGKE results in unrestricted complement activation in atypical hemolytic-uremic syndrome.

1. Tang, W. et al. Molecular Cloning of a Novel Human Diacylglycerol Kinase Highly Selective for Arachidonate-containing Substrates. The Journal of Biological Chemistry. (271) 10237–10241, 1996.

2. Zhang, N. et al. Inhibition of Lipid Signaling Enzyme Diacylglycerol Kinase Epsilon Attenuates Mutant Huntingtin Toxicity. The Journal of Biological Chemistry. (287) 21204–21213, 2012.

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.

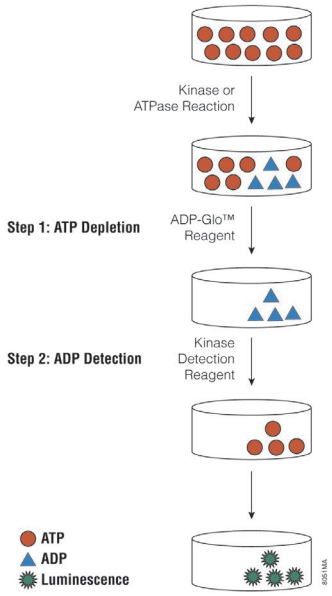

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

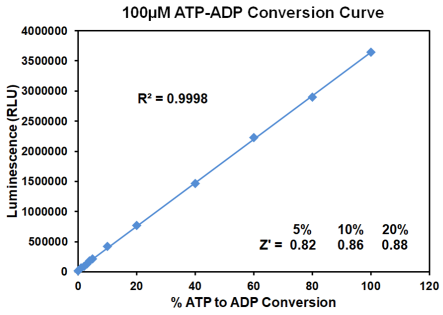

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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/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	100	50	25	12.50	6.25	1.56	0
Luminescence	623,090	309,918	161,133	105,944	46,755	20,744	12,182
S/B	51	25	13	9	4	2	1
% Conversion	18	8	4	2	0	0	0

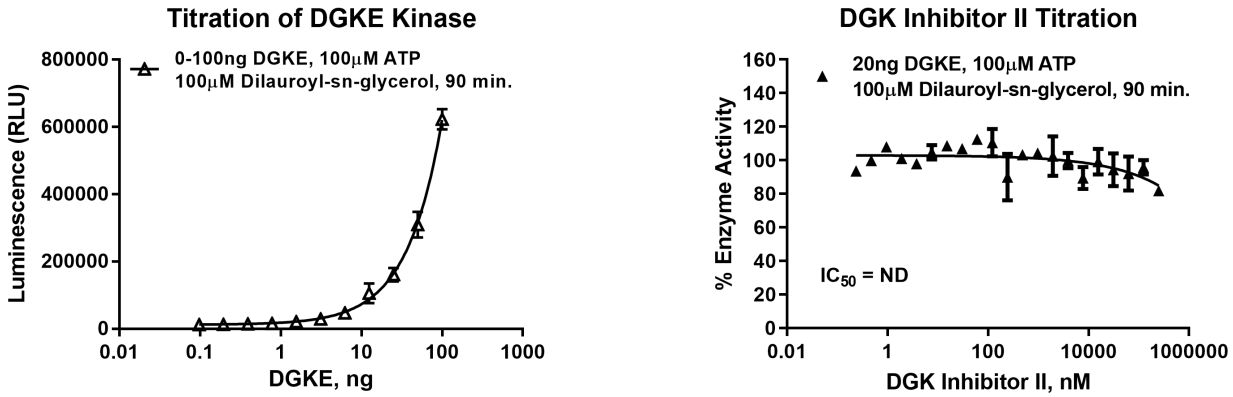

Figure 3. DGKE Kinase Assay Development. (A) DGKE enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 20ng of DGKE to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
DGKE Kinase Enzyme System	10µg	VA7612
	1mg	VA7613
ADP-Glo™ + DGKE Kinase Enzyme System	1 Each	VA7614