ADP-Glo™ Kinase Assay Application Note

Lipid Kinase Series

DGKQ Kinase Assay

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Scientific Background:

Diacylglycerol kinase theta (DAG kinase theta) is the only member of class V diacylglycerol kinase, which contains three cysteine-rich domains, a proline-rich region, and a Ras-associating domain and mediates the regeneration of phosphatidylinositol from diacylglycerol in cell signal transduction (1). DGKQ has been associated with Parkinson's disease in a genome-wide meta-analysis study (2). Stimulated DGK theta activity promotes the SF1-dependent transcription of CYP17 in human adrenocortical cells.

1. Houssa, B. et al. Cloning of a Novel Human Diacylglycerol Kinase (DGK theta) Containing Three Cysteine-rich Domains, a Proline-rich Region, and a Pleckstrin Homology Domain with an Overlapping Ras-associating Domain. The Journal of Biological Chemistry. (272) 10422–10428, 1997.

2. Nalls, M. et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. Nature Genetics. (46) 989-993, 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.

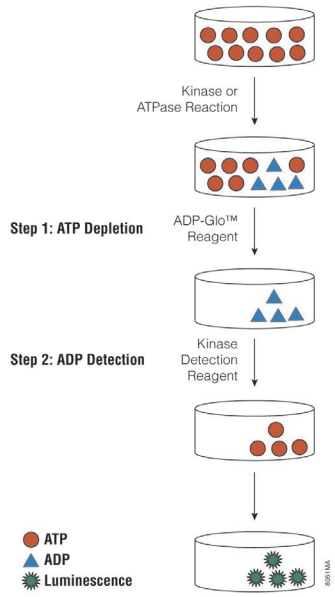

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

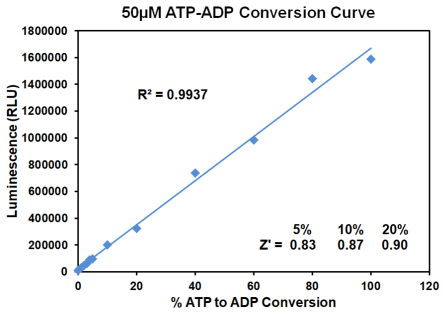

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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/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	0.78	0
Luminescence	743,691	601,313	325,830	255,139	126,213	68,543	38,141	24,674	17,549	7,816
S/B	95	77	42	33	16	9	5	3	2	1
% Conversion	36	29	16	12	6	3	1	1	0	0

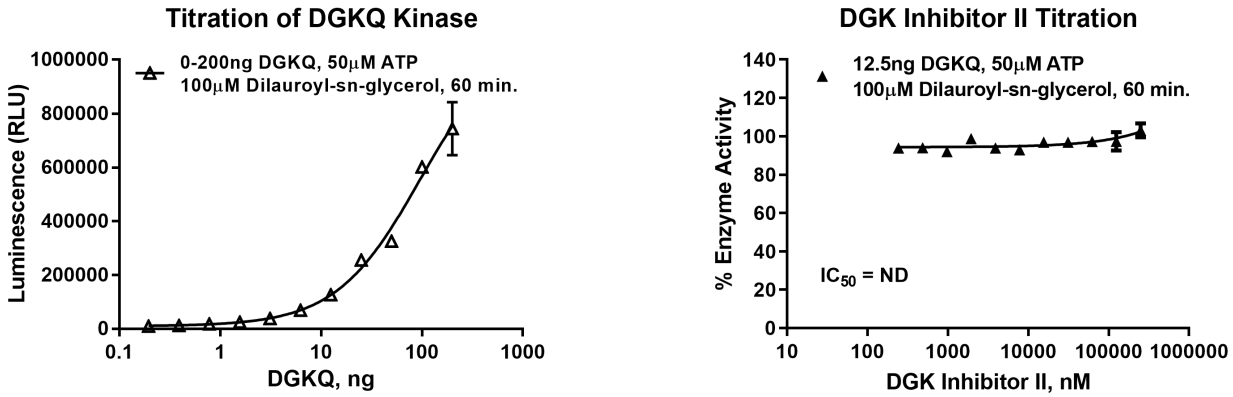

Figure 3. DGKQ Kinase Assay Development. (A) DGKQ enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 12.5ng of DGKQ to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
DGKQ Kinase Enzyme System	10µg	VA7615
	1mg	VA7616
ADP-Glo™ + DGKQ Kinase Enzyme System	1 Each	VA7617