ADP-Glo™ Kinase Assay Application Note

Lipid Kinase Series

DGKA Kinase Assay

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Scientific Background:

Diacylglycerol kinase alpha (DAG kinase alpha) is a member of the eukaryotic diacylglycerol kinase family. Upon cell stimulation converts the second messenger diacylglycerol into phosphatidate, initiating the re-synthesis of phosphatidylinositols and attenuating protein kinase C activity. Involved in hepatocellular carcinoma progression through regulation of the Ras/Raf/MEK/ERK pathway. The DGKα/atypical PKC/β1 integrin signaling pathway is required for matrix invasion of breast carcinoma cells.

1. Takeishi, K. et al. (2012). Diacylglycerol kinase alpha enhances hepatocellular carcinoma progression by activation of Ras-Raf-MEK-ERK pathway. J. Hepatol. 57, 77–83.

2. Rainero, E. et al. (2014). The diacylglycerol kinase alpha/atypical PKC/beta1 integrin pathway in SDF-1alpha mammary carcinoma invasiveness. PLoS ONE 9:e97144.

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.

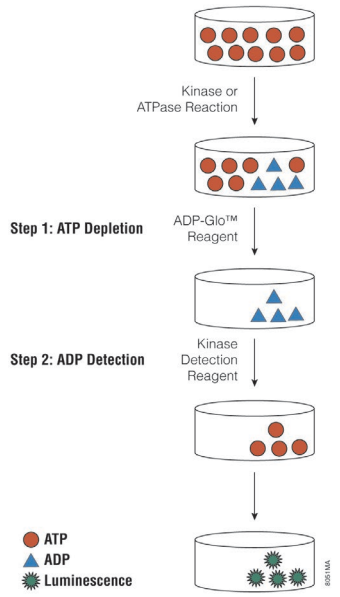

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

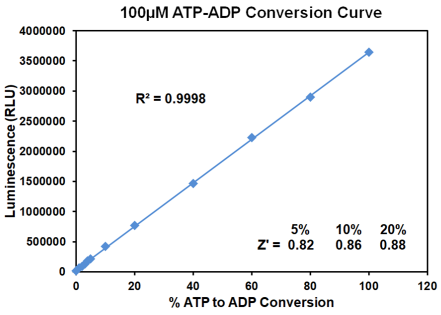

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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/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	1.56	0.78	0
Luminescence	2,182,830	2,046,520	2,056,390	1,923,990	1,560,185	1,043,656	629,847	332,805	185,608	12,015
S/B	182	170	171	160	130	87	52	28	15	1
% Conversion	66	62	62	58	47	31	18	9	5	0

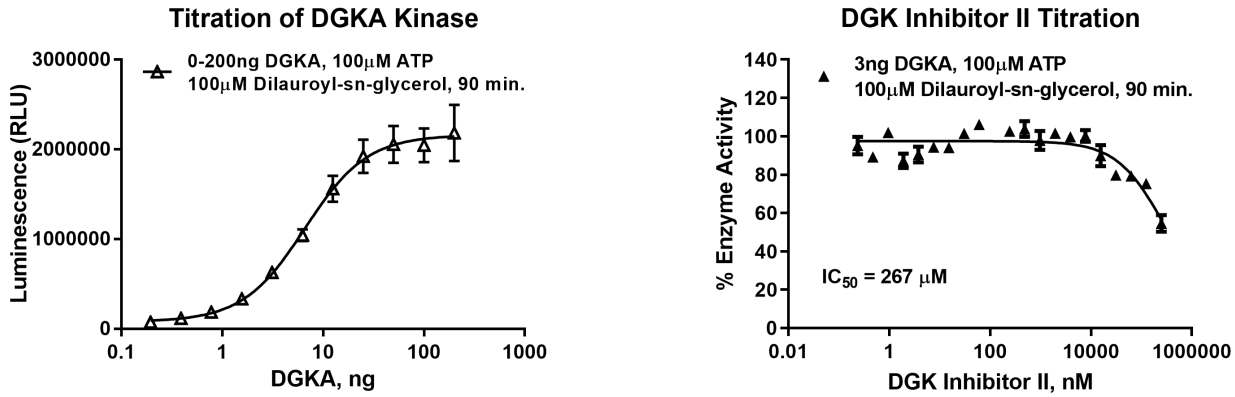

Figure 3. DGKA Kinase Assay Development. (A) DGKA enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 3ng of DGKA to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
DGKA Kinase Enzyme System	10µg	VA7606
	1mg	VA7607
ADP-Glo™ + DGKA Kinase Enzyme System	1 Each	VA7608