SPHK2 Kinase Assay

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Scientific Background:

SPHK2 is a member of the SPHK family and catalyzes the phosphorylation of the lipid sphingosine, creating the bioactive lipid sphingosine-1-phosphate (S1P). Sphingolipid metabolism regulates proper uterine decidualization and blood vessel stability (1). SPHK2 is involved in the regulation of chemosensitivity by controlling ceramide formation and the downstream Akt pathway in human colon cancer cells. SPHK2 plays an important role in migration of MDA-MB-453 cells toward the ligand EGF. The N-terminal-extended form of SPHK2 has a role in serum-dependent regulation of cell proliferation and apoptosis (2).

1. Mizugishi, K. et al: Maternal disturbance in activated sphingolipid metabolism causes pregnancy loss in mice. J. Clin. Invest. 1172993-3006, 2007.

2. Hait, N. C.et al: Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. Science 3251254-1257, 2009. NoteErratumScience 326366 only, 2009.

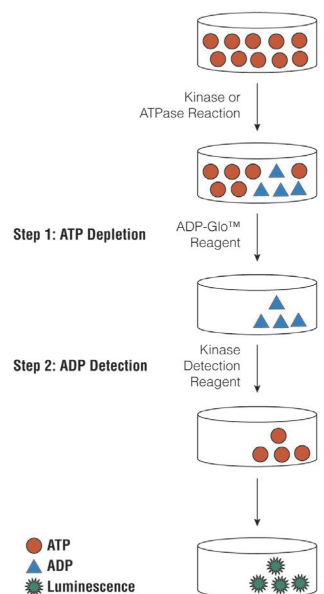

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

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.

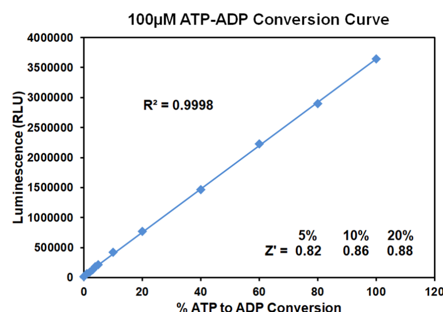

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

ADP-Glo™ Kinase Assay Application Note
Lipid Kinase Series

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 (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	240	120	60	30	15	7.50	3.75	0
Luminescence	1,451,270	1,533,750	1,210,145	469,073	155,588	60,759	27,537	12,054
S/B	120	127	100	39	13	5	2	1
% Conversion	44	47	37	13	3	0	0	0

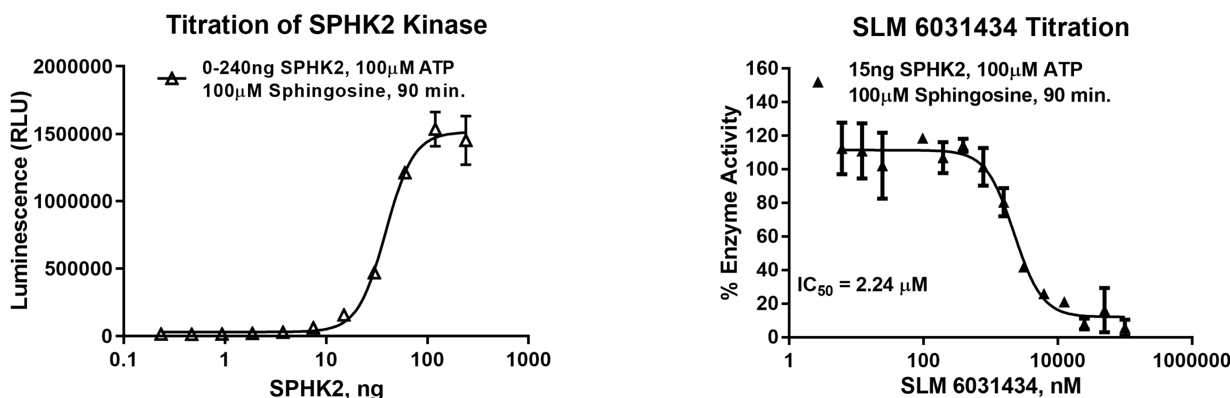

Figure 3. SPHK2 Kinase Assay Development. (A) SPHK2 enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 15ng of SPHK2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
SPHK2 Kinase Enzyme System	10µg	VA7600
	1mg	VA7601
ADP-Glo™ + SPHK2 Kinase Enzyme System	1 Each	VA7602