ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

GOPC-ROS1 Kinase Assay

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Scientific Background:

GOPC gene encodes a Golgi protein with a PDZ domain. Mice which are deficient in the GOPC protein have globozoospermia and are infertile. Multiple transcript variants encoding different isoforms of GOPC have been found for this gene and GOPC gene locus is involved in translocations with other loci including c-ros oncogene 1 (ROS1) which result in the formation of the GOPC-ROS1 fusion protein that act as an oncogene. The ros1 gene encodes a proto-oncogenic protein which has been implicated in the pathogenesis of several types of cancer, including lung cancer and gliomas (1). GOPC-ROS1 fusion gene product has been identified in lung cancer (2).

1. Maxwell, M. et al: Overexpression of the ros1 gene in primary human gliomas may contribute to malignant progression. Int J Oncol. 1996 Apr;8(4):713-8.

2. Suehara Y. et al: Identification of KIF5B-RET and GOPC-ROS1 fusions in lung adenocarcinomas through a comprehensive mRNA-based screen for tyrosine kinase fusions. Clin Cancer Res. 2012 Dec 15;18(24):6599-608.

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.

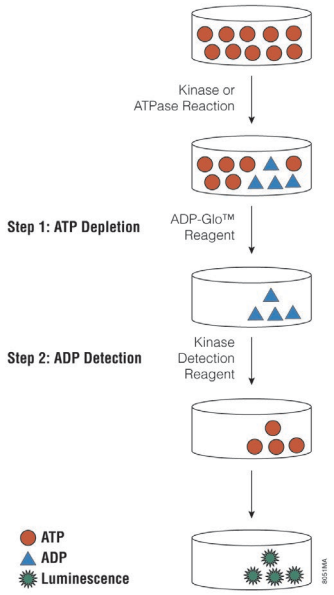

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

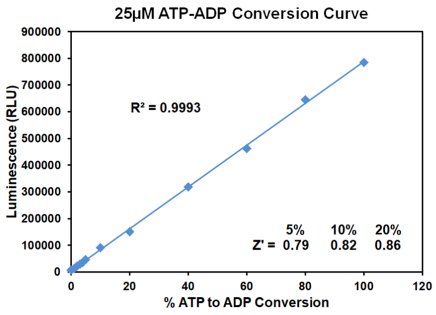

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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 (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	120	60	30	15	7.50	3.75	1.88	0.94	0.47	0.23	0.12	0
Luminescence	1,112,906	893,801	701,953	514,223	323,925	169,576	71,996	35,148	25,086	11,229	9,720	4,727
S/B	235	189	148	109	69	36	15	7	5	2	2	1
% Conversion	123	99	77	56	35	18	7	3	2	0	0	0

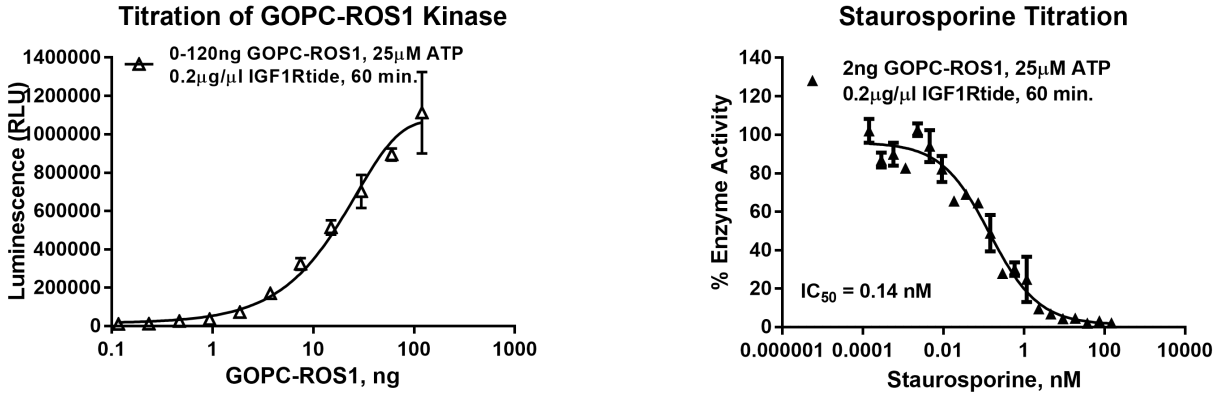

Figure 3. GOPC-ROS1 Kinase Assay Development. (A) GOPC-ROS1 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2ng of GOPC-ROS1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
GOPC-ROS1 Kinase Enzyme System	10µg	VA7180
	1mg	VA7181
ADP-Glo™ + GOPC-ROS1 Kinase Enzyme System	1 Each	VA7182