ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

ROS1 Kinase Assay

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Scientific Background:

ROS1 is a proto-oncogene and member of the sevenless subfamily of tyrosine kinase insulin receptor genes. ROS1 is highly expressed in a variety of tumor cell lines and functions as a growth or differentiation factor receptor. The FIG gene can fuse with the ROS1 gene in glioblastoma cell lines (1). The resulting ROS1/FIG fusion protein is a constitutively activated tyrosine kinase. Direct interaction of ROS1 and the phosphatase SHP-1 can lead to efficient downregulation of ROS1-mediated signaling (2). Binding sites in the ROS1 cytoplasmic domain display high affinity binding to the SHP-1 N-terminal SH2 domain.

1. Charest, A. et al: Fusion of FIG to the receptor tyrosine kinase ROS in a glioblastoma with an interstitial del(6)(q21q21). Genes Chromosomes Cancer 37: 58-71, 2003.

2. Biscup, C. et al: Visualization of SHP-1-target interaction. J Cell Sci. 2004 Oct 1;117(Pt 21):5165-78.

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.

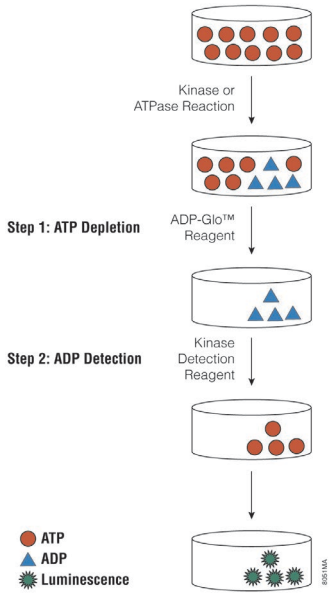

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

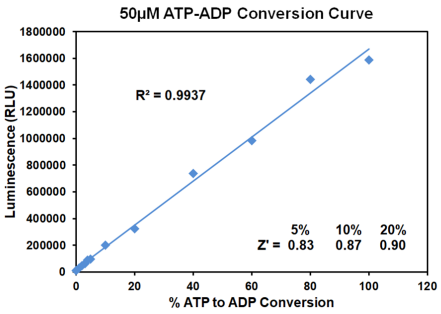

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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 (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	160	80	40	20	10	5	2.50	1.25	0.63	0.31	0.16	0
Luminescence	1,380,190	1,090,635	928,039	756,884	576,062	373,388	248,206	133,178	70,933	35,118	19,502	4,344
S/B	318	251	214	174	133	86	57	31	16	8	4	1
% Conversion	68	54	46	37	28	18	12	6	3	1	0	0

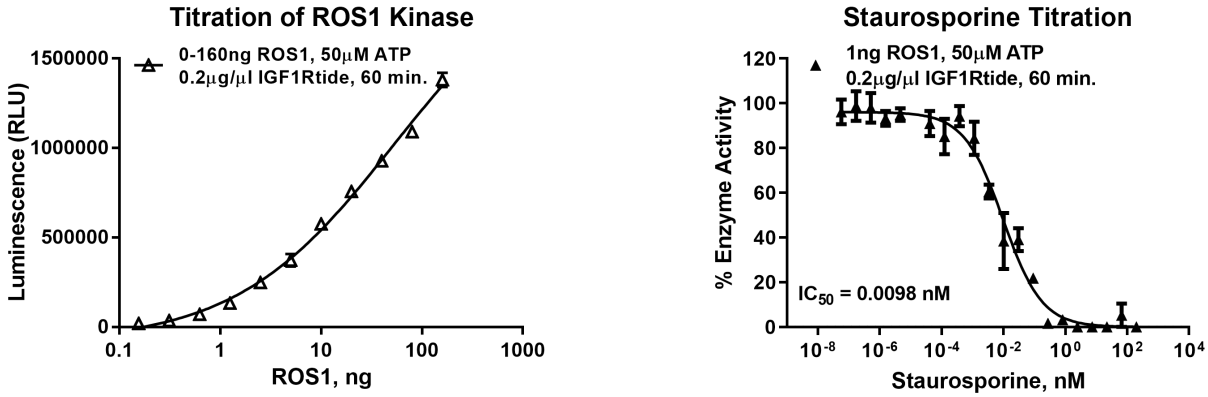

Figure 3. ROS1 Kinase Assay Development. (A) ROS1 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1ng of ROS1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
ROS1 Kinase Enzyme System	10µg	VA7546
	1mg	VA7547
ADP-Glo™ + ROS1 Kinase Enzyme System	1 Each	VA7548