ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

KRS1 (L639F) Kinase Assay

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Scientific Background:

KSR1 or Kinase Suppressor of Ras 1 interacts with various kinases of the Raf/MEK/extracellular signal-regulated kinase pathway to enhance its activation. KSR1 is regulated in response to a specific mode of dimerization of its kinase domain, which is termed the side-to-side dimer, whereas KSR1 also participates in forming side-to-side heterodimers with RAF and this can trigger RAF activation. KSR1 has an essential protective role in the intestinal epithelial cell during inflammation through activation of cell survival pathways (1). KSR1 functions as a scaffold that enhances iNOS activity and is crucial for the pulmonary response to P. aeruginosa infection (2).

1. Yan. et.al: Kinase suppressor of Ras-1 protects intestinal epithelium from cytokine-mediated apoptosis during inflammation. J. Clin. Invest. 114: 1272-1280, 2004.

2. Zhang. et.al: Kinase suppressor of Ras-1 protects against pulmonary Pseudomonas aeruginosa infections. Nature Med. 17: 341-346, 2011.

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.

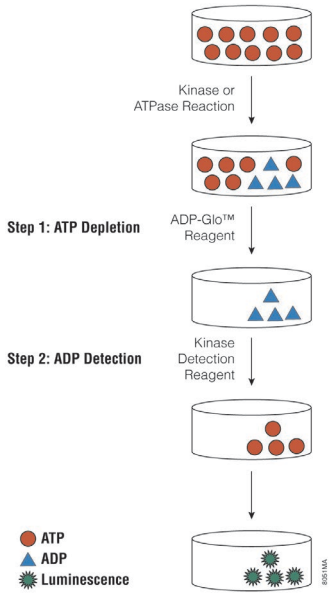

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

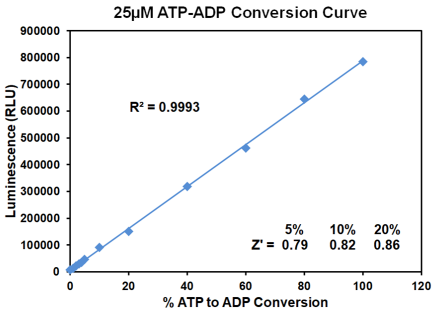

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	150	75	37.50	18.75	9.38	4.69	0
Luminescence	854,049	534,883	224,898	76,556	41,462	20,191	7,473
S/B	114	72	30	10	6	3	1
% Conversion	85	53	21	6	3	1	0

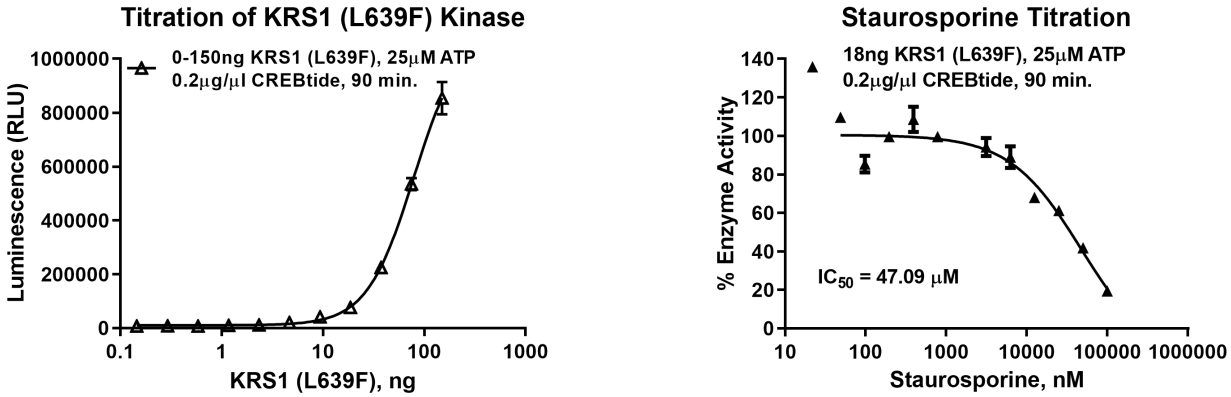

Figure 3. KRS1 (L639F) Kinase Assay Development. (A) KRS1 (L639F) enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 18ng of KRS1 (L639F) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
KRS1 (L639F) Kinase Enzyme System	10µg	VA7594
	1mg	VA7595
ADP-Glo™ + KRS1 (L639F) Kinase Enzyme System	1 Each	VA7596