ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: VRK2

VRK2 Kinase Assay

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Scientific Background:

VRK2 (also known as vaccinia related kinase 2) is a member of the vaccinia-related kinase (VRK) family of serine/threonine protein kinases. VRK2 is widely expressed in human tissues and highly expressed in actively dividing cells, such as those in testis, leukocytes, fetal liver and carcinomas (1). VRK2 can be used to phosphorylate casein and itself undergo autophosphorylation. VRK2 interacts specifically with Epstein-Barr virus BHRF1, a homologue of Bcl-2, and this interaction is involved in protecting cells from apoptosis (2).

1. Nezu, J. et.al: Identification of two novel human putative serine/threonine kinases, VRK1 and VRK2, with structural similarity to vaccinia virus B1R kinase. Genomics 45: 327-331, 1997.

2. Li, L.-Y. et.al: The cellular protein PRA1 modulates the anti-apoptotic activity of Epstein-Barr virus BHRF1, a homologue of Bcl-2, through direct interaction. J Biol Chem 276, 27354–27362, 2001.

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.

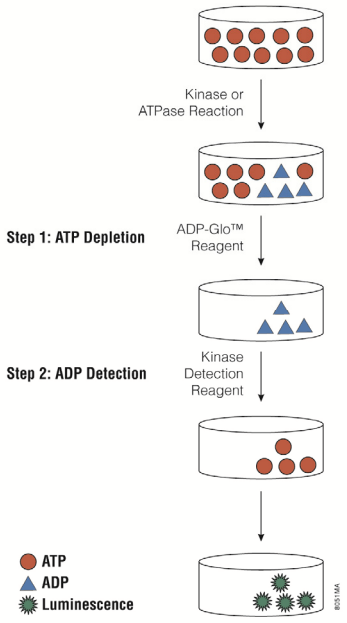

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

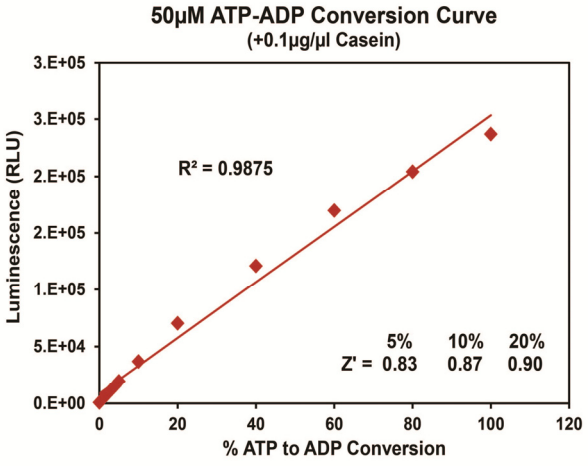

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase 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 120 minutes.
- 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 0.5-1second).

Table 1. VRK2 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.

VRK2, ng	100	50	25	13	6.3	3.1	1.6	0
RLU	139474	57602	24081	10641	5045	2890	2598	680
S/B	205	85	35	16	7	4.2	3.8	1
% Conversion	62	25	10	4	1.0	0.6	0.5	0

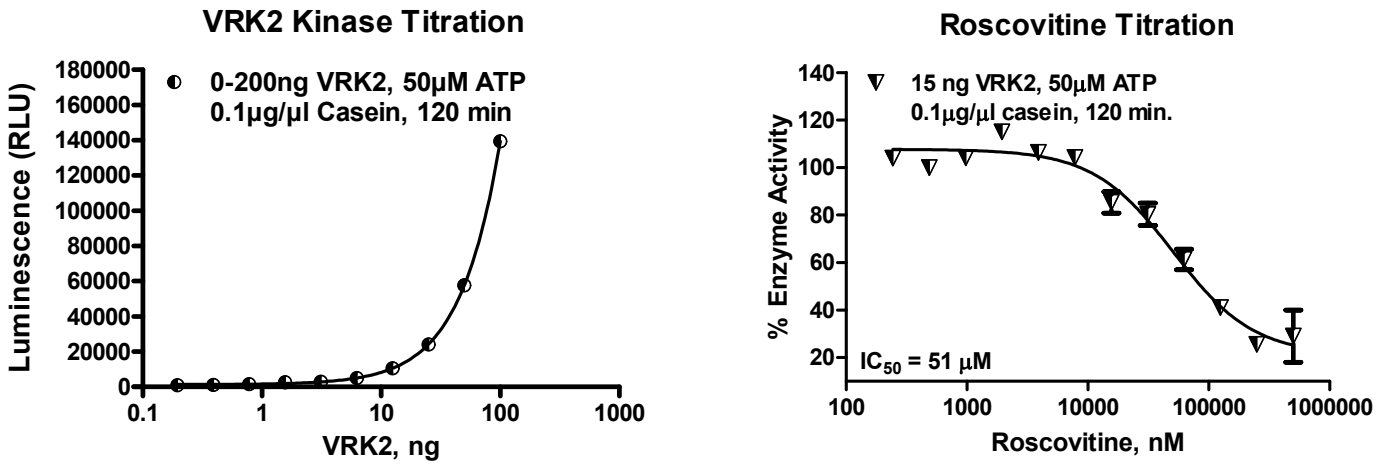

Figure 3. VRK2 Kinase Assay Development. (A) VRK2 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Roscovitine dose response was created using 15ng of VRK2 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
VRK2 Kinase Enzyme System	Promega	V4494
ADP-Glo™ + VRK2 Kinase Enzyme System	Promega	V4495

VRK2 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 2.5mM $MnCl_2$; 50µM DTT.

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