KDR Kinase Assay

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Scientific Background:

KDR (or kinase insert domain receptor) is a growth factor receptor tyrosine kinase that was originally isolated from human endothelial cells where it plays a pivotal role in endothelial cell proliferation and differentiation. KDR and its mouse homolog Flk1 bind VEGF with high affinity and are implicated in the development of new blood vessels (angiogenesis) (1). The expression levels of VEGF and KDR are highly correlated during the normal development of the ocular vasculature in humans (1). Induction of angiogenesis is a critical step in tumor progression, and inhibitors of KDR have been demonstrated both to induce tumor regression and reduce metastatic potential in preclinical models (2).

1. Neufeld, G. et al: Vascular endothelial growth factor (VEGF) and its receptors. FASEB J. 1999 Jan;13(1):9-22.

2. Zhu, Z. et al: Inhibition of tumor growth and metastasis by targeting tumor-associated angiogenesis with antagonists to the receptors of vascular endothelial growth factor. Invest New Drugs. 1999;17(3):195-212.

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.

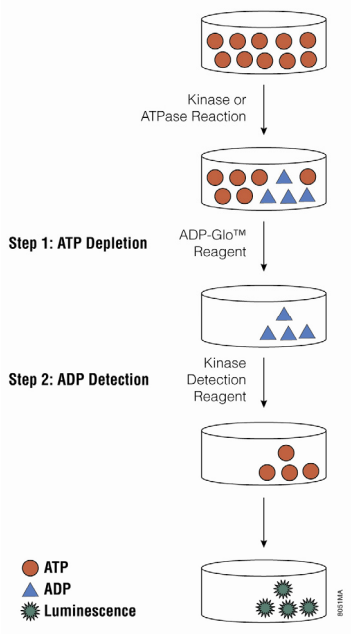

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

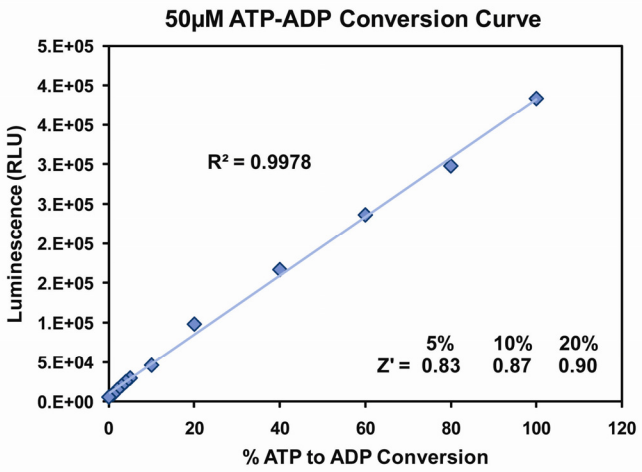

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.

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 60 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. KDR 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.

KDR, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0
Luminescence	308871	259683	223129	180955	144962	111758	68264	39483	23327	4673
S/B	66	56	48	39	31	24	15	8	5	1
% Conversion	80	67	57	46	36	27	16	8	3	0

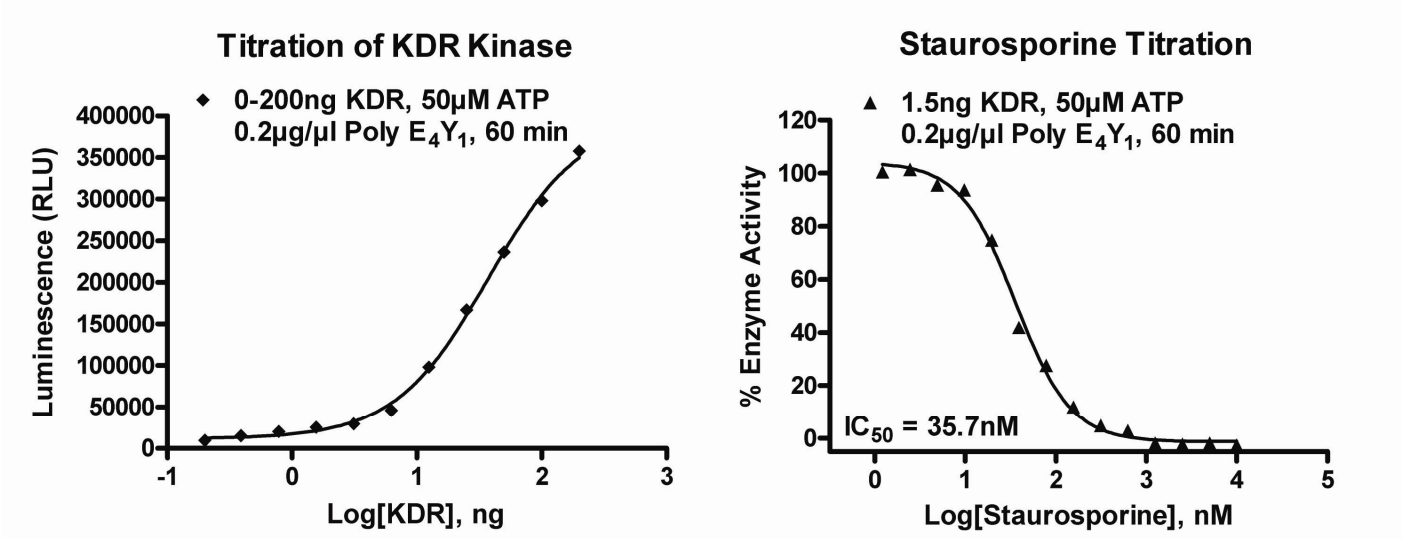

Figure 3. KDR Kinase Assay Development: (A) KDR enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1.5ng of KDR to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
KDR Kinase Enzyme System	Promega	V2681
ADP-Glo + KDR Kinase Enzyme System	Promega	V9471

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