ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

DRAK1 (STK17A) Kinase Assay

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Scientific Background:

DRAK1 (STK17A) is a member of the DAP kinase-related apoptosis-inducing protein kinase family. DRAK1 encodes an autophosphorylated nuclear protein with a protein kinase domain which has apoptosis-inducing activity. DRAK1 is capable of autophosphorylation and of phosphorylating myosin light chain as an exogenous substrate. The noncatalytic C terminus of DRAK1 is crucial for full kinase activity (1). DRAK1 is highly expressed in placenta, but also in heart, lung, skeletal muscle, kidney, and pancreas. DRAK1 act as a novel direct target of p53 and a modulator of cisplatin toxicity and reactive oxygen species in testicular cancer cells (2).

1. Sanjo, H. et.al: DRAKs, novel serine/threonine kinases related to death-associated protein kinase that trigger apoptosis. J. Biol. Chem. 273: 29066-29071, 1998.

2. Mao P, et.al: Serine/threonine kinase 17A is a novel p53 target gene and modulator of cisplatin toxicity and reactive oxygen species in testicular cancer cells. J Biol Chem. 2011 Jun 3;286(22):19381-91.

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.

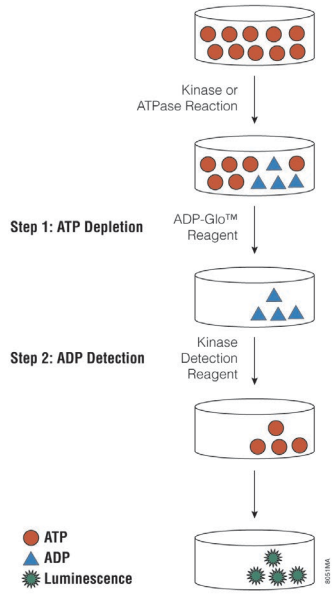

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

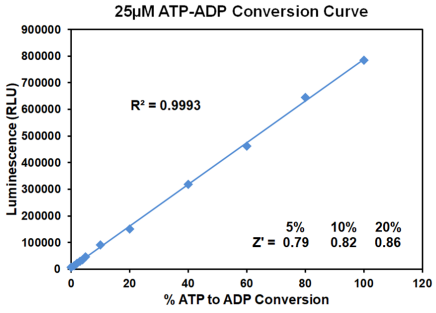

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	300	150	75	37.50	0
Luminescence	749,069	366,259	119,704	43,643	6,458
S/B	116	57	19	7	1
% Conversion	74	35	10	3	0

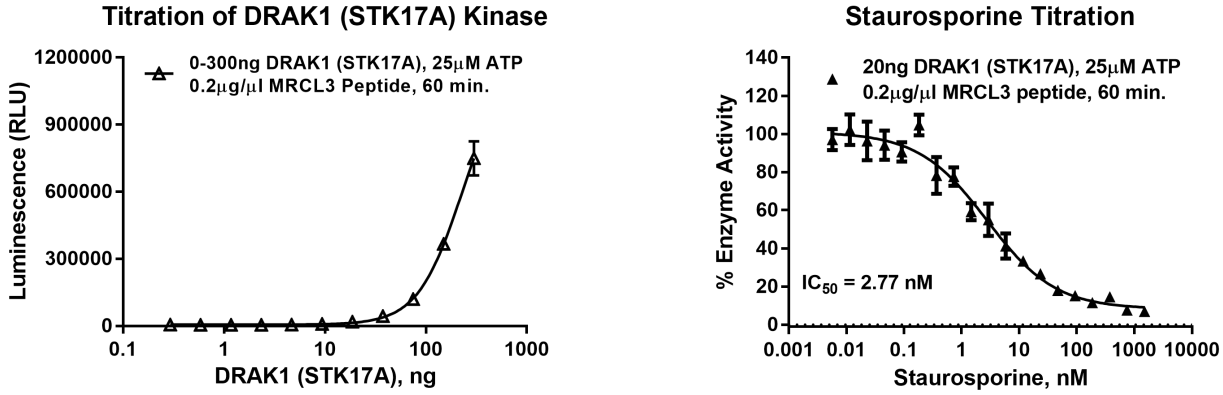

Figure 3. DRAK1 (STK17A) Kinase Assay Development. (A) DRAK1 (STK17A) enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 20ng of DRAK1 (STK17A) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
DRAK1 (STK17A) Kinase Enzyme System	10µg	VA7078
	1mg	VA7079
ADP-Glo™ + DRAK1 (STK17A) Kinase Enzyme System	1 Each	VA7080