ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

DRAK2 Kinase Assay

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Scientific Background:

DRAK2 is a member of the serine/threonine kinase family and is related to death-associated protein kinase that triggers apoptosis (1). DRAK2 is selectively important for T-cell survival and inhibition of DRAK2 has therapeutic potential for autoimmune disease (2). T-cell survival depends on a balance of T-cell receptor and co-stimulatory signals and deficiency of DRAK2 can affect autoimmune disease susceptibility without generalized suppression of the immune system.

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. Ramos, S. J. et.al: Enhanced T cell apoptosis within Drak2-deficient mice promotes resistance to autoimmunity. J. Immun. 181: 7606-7616, 2008.

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.

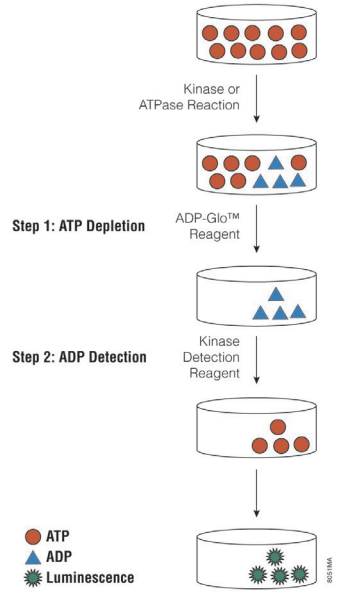

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

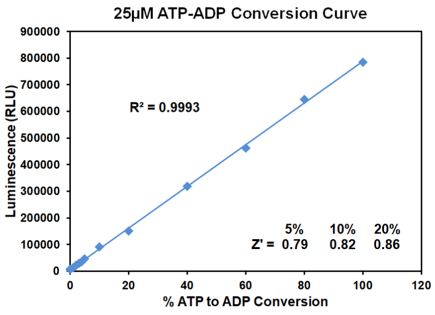

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	18.75	9.38	4.69	2.34	0
Luminescence	876,749	709,599	438,670	210,188	86,127	33,602	16,932	9,485	4,037
S/B	217	176	109	52	21	8	4	2	1
% Conversion	87	70	42	19	6	1	0	0	0

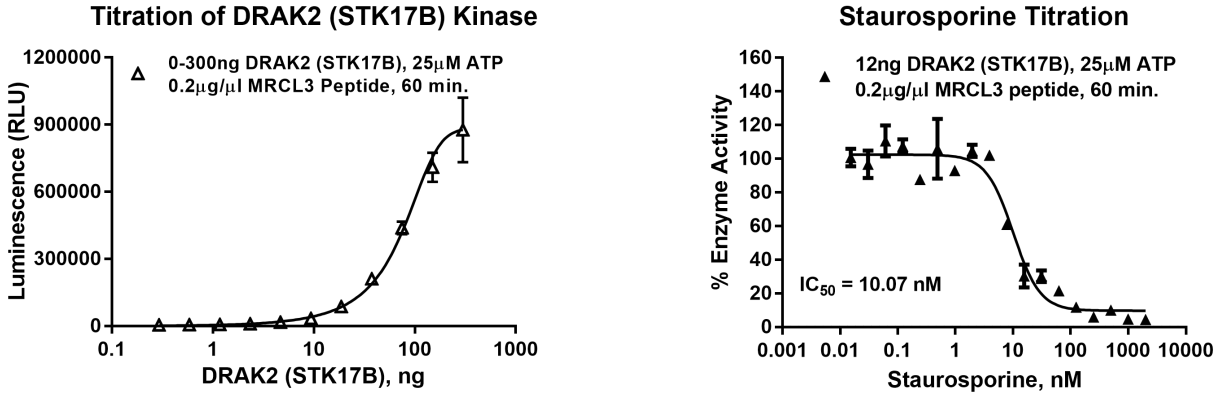

Figure 3. DRAK2 Kinase Assay Development. (A) DRAK2 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 12ng of DRAK2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
DRAK2 Kinase Enzyme System	10µg	VA7081
	1mg	VA7082
ADP-Glo™ + DRAK2 Kinase Enzyme System	1 Each	VA7083