ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: DAPK1

DAPK1 Kinase Assay

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Scientific Background:

Death-associated protein kinase 1 (DAPK1) is a positive mediator of apoptosis induced by γ-interferon. Activation of DAPK occurs via dephosphorylation of Ser-308 and subsequent association of calcium/calmodulin (1). DAPK is rapidly dephosphorylated in response to tumor necrosis factor or ceramide and then subsequently degraded via proteasome activity. The decline in DAPK expression is paralleled with increased caspase activity and cell apoptosis. Studies suggest that the apoptosis regulatory activities mediated by DAPK are controlled both by phosphorylation status and protein stability (2).

1. Deiss, L. P. et al: Identification of a novel serine/threonine kinase and a novel 15-kD protein as potential mediators of the gamma interferon-induced cell death. Genes Dev.1995; 9: 15-30.

2. Feinstein, E. et al: Assignment of DAP1 and DAPK: genes that positively mediate programmed cell death triggered by IFN-gamma--to chromosome regions 5p12.2 (sic) and 9q34.1, respectively. Genomics. 1995; 29: 305-307.

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.

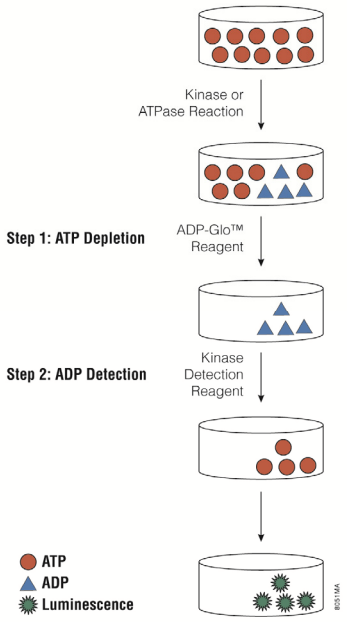

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

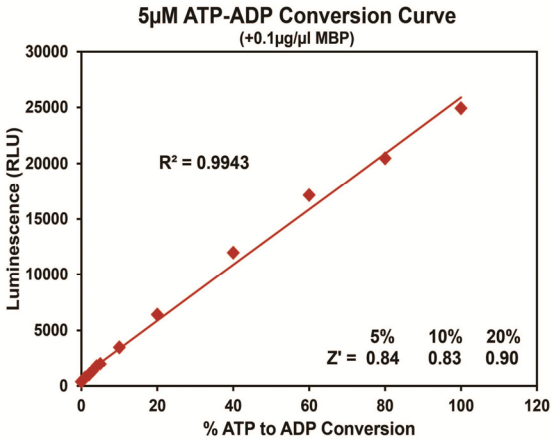

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 5µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 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. DAPK1 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.

DAPK1, ng	100	50	25	13	6	3.1	1.6	0.8	0.4	0.2	0
RLU	33096	27323	23774	17208	11108	5657	3075	1651	1012	658	307
S/B	108	89	77	56	36	18	10	5	3.3	2.1	1
% Conversion	100	90	78	56	36	17	9	4	1.8	0.6	0

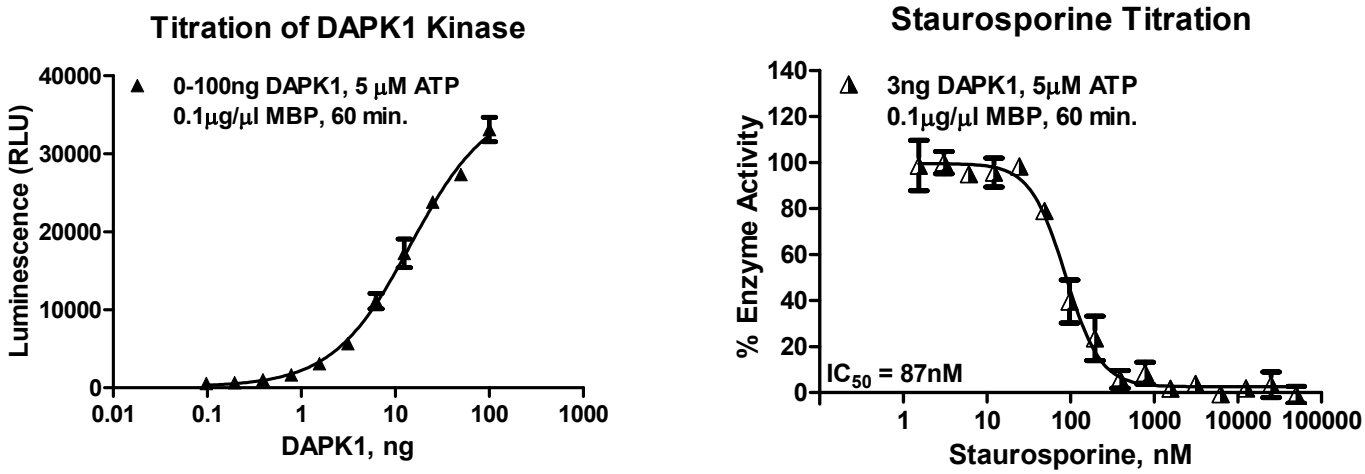

Figure 3. DAPK1 Kinase Assay Development. **(A) DAPK1 enzyme was titrated using 5µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 3ng of DAPK1 to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
DAPK1 Kinase Enzyme System	Promega	V4096
ADP-Glo™ + DAPK1 Kinase Enzyme System	Promega	V4097

DAPK1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT; Ca2+/Calmodulin solution (0.03µg/µl Calmodulin, 1mM Tris, pH 7.3, 0.5mM $CaCl_2$)