ADP-Glo™ Kinase Assay Application Note

Ser-Thr Kinase Series

DYRK1B Kinase Assay

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Scientific Background:

DYRK1B (Dual specificity tyrosine-phosphorylation-regulated kinase 1B) is a member of the evolutionarily conserved DYRK family with key roles in the control of cell proliferation and differentiation. DYRK1B acts in G0/G1 to maintain cells in growth arrest and quiescence by targeting cyclin D1 for proteasomal degradation by phosphorylating a threonine residue close to the C terminus. Paradoxically, DYRK1B can also stabilize other proteins by phosphorylation as illustrated by the phosphorylation by DYRK1B of p27Kip1 on Ser10 during the G0 phase of the cell cycle. Deregulation of DYRK1B such as gene amplification, overexpression and/or constitutive activation has been observed in multiple types of cancer.

1. Becker W. Emerging role of DYRK family protein kinases as regulators of protein stability in cell cycle control. Cell Cycle. 2012 11:3389-94.

2. Deng X, Mercer SE, Shah S, Ewton DZ, Friedman E. The cyclin-dependent kinase inhibitor p27Kip1 is stabilized in G(0) by Mirk/dyrk1B kinase. J Biol Chem. 2004 279:22498–504.

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.

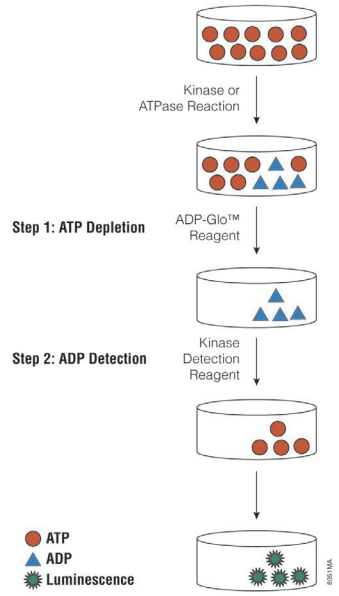

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

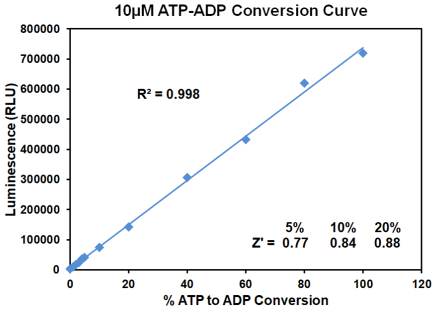

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	15	7.50	3.75	1.88	0.94	0.47	0.23	0.12	0.06	0.03	0.01	0
Luminescence	209,493	208,145	190,926	167,939	127,859	82,747	54,379	28,164	15,664	8,780	5,147	1,993
S/B	105	104	96	84	64	42	27	14	8	4	3	1
% Conversion	65	64	59	52	39	25	16	8	4	1	0	0

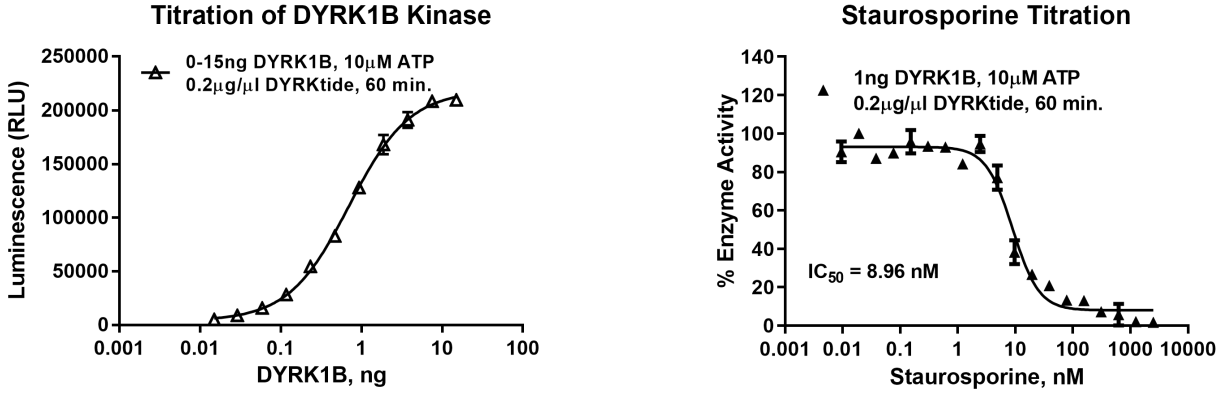

Figure 3. DYRK1B Kinase Assay Development. (A) DYRK1B enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1ng of DYRK1B to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
DYRK1B Kinase Enzyme System	10µg	VA7426
	1mg	VA7427
ADP-Glo™ + DYRK1B Kinase Enzyme System	1 Each	VA7428