DYRK2 Kinase Assay

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Scientific Background:

DYRK2 (also known as dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2) belongs to a family of protein kinases whose members are presumed to be involved in cellular growth and/or development and is defined by structural similarity of their kinase domains and their capability to autophosphorylate on tyrosine residues. DYRK2 expressed in E. coli demonstrated tyrosine autophosphorylation and catalyzed phosphorylation of histones H3 and H2B in vitro (1). DYRK2 also regulates p53 to induce apoptosis in response to DNA damage (2).

1. Becker, W. et.al: Sequence characteristics, subcellular localization, and substrate specificity of DYRK-related kinases, a novel family of dual specificity protein kinases. J. Biol. Chem. 273: 25893-25902, 1998.

2. Taira, N. et.al: DYRK2 is targeted to the nucleus and controls p53 via Ser46 phosphorylation in the apoptotic response to DNA damage. Molec. Cell 25: 725-738, 2007.

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.

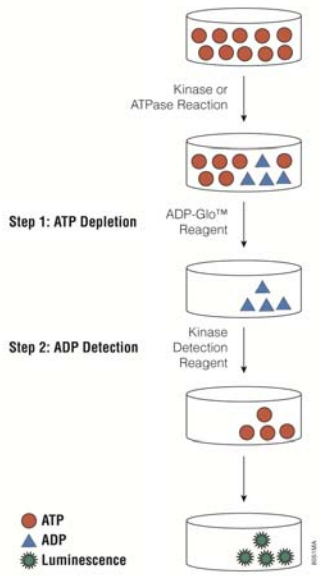

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

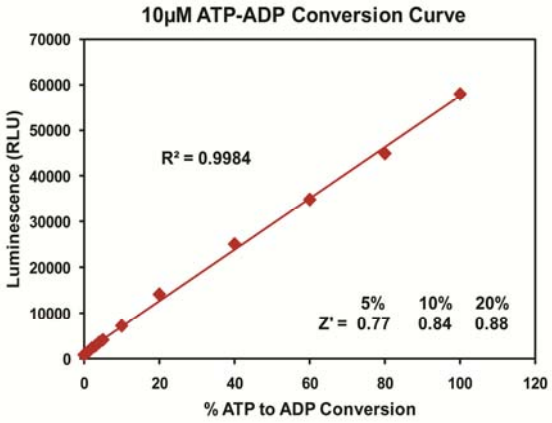

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, and the KES Protocol available at: http://www.promega.com/tbs/tm313/tm313.html, and http://www.promega.com/KESProtocol, respectively.

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 0.5-1sec).

Table 1. DYRK2 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.

DYRK2, ng	24	12	6	3	1.5	0.8	0.4	0.2	0.1	0.05	0.02	0
RLU	58927	58031	54259	50067	38432	24028	13570	7091	3483	1885	966	203
S/B	290	286	267	247	189	118	67	35	17	9	5	1
% Conversion	76	75	70	65	51	27	13	4	3	2	1	0

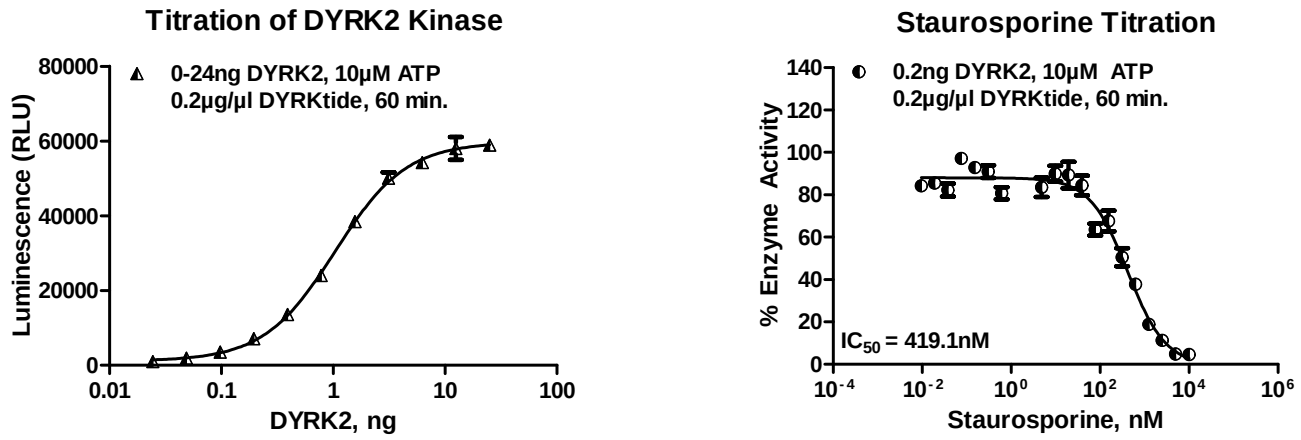

Figure 3. DYRK2 Kinase Assay Development. **(A) DYRK2 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 0.2ng of DYRK2 to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
DYRK2 Kinase Enzyme System	Promega	V5090
ADP-Glo™ + DYRK2 Kinase Enzyme System	Promega	V5091

DYRK2 Kinase Buffer: 40mM Tris, pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.