ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

DYRK1A Kinase Assay

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Scientific Background:

DYRK1 A or dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A is a member of the Dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) family which contains a nuclear targeting signal sequence, a protein kinase domain, a leucine zipper motif, and a highly conservative 13-consecutive-histidine repeat. DYRK1A participates in various cellular processes. DYRK1A is a highly conserved gene located in the so-called Down syndrome critical region (DSCR) on part of chromosome 21 that is responsible for the majority of phenotypic features in Down syndrome (1). DYRK1A plays a significant role in a signaling pathway regulating cell proliferation and may be involved in brain development. DYRK1A is also contributing to early onset of Alzheimer disease (2).

1. van Bon, B. et al: Intragenic deletion in DYRK1A leads to mental retardation and primary microcephaly. Clin. Genet. 79: 296-299, 2011.

2. Ryoo, S. R. et al: DYRK1A-mediated hyperphosphorylation of tau: a functional link between Down syndrome and Alzheimer disease. J. Biol. Chem. 282: 34850-34857, 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.

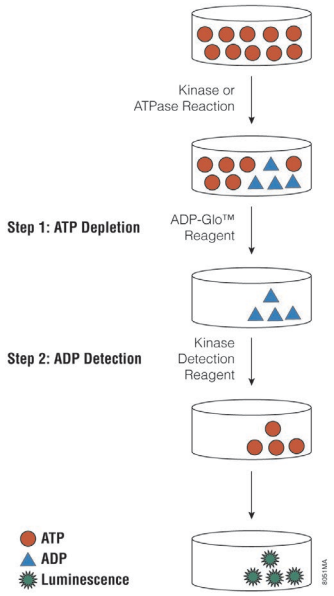

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

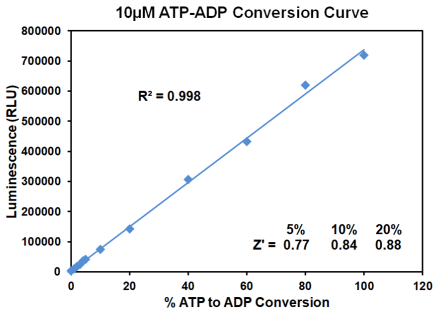

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	180	90	45	22.50	11.25	5.63	2.81	1.41	0.70	0.35	0.18	0
Luminescence	298,689	308,038	302,538	296,734	269,339	188,607	107,692	55,379	28,555	13,845	7,948	2,011
S/B	149	153	150	148	134	94	54	28	14	7	4	1
% Conversion	76	78	76	75	68	47	26	13	6	2	1	0

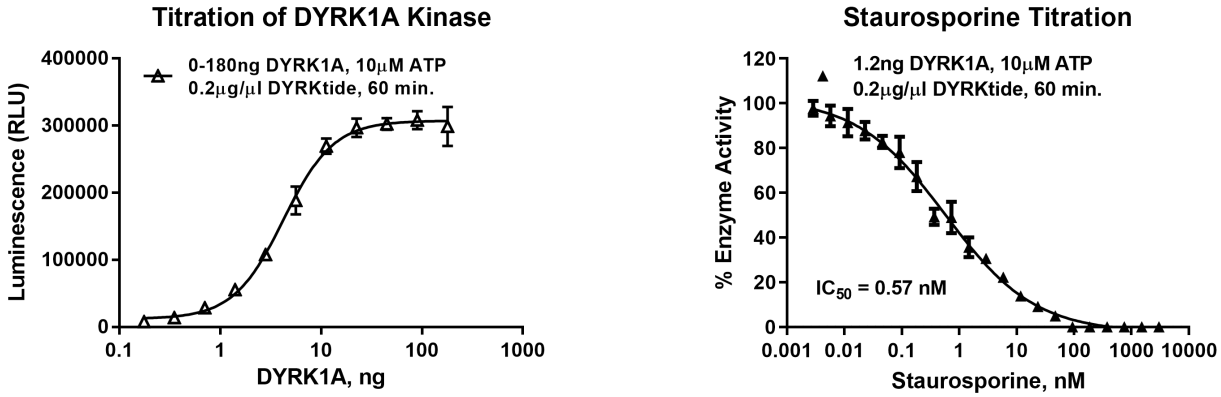

Figure 3. DYRK1A Kinase Assay Development. (A) DYRK1a enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1.2ng of DYRK1A to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
DYRK1A Kinase Enzyme System	10µg	VA7423
	1mg	VA7424
ADP-Glo™ + DYRK1A Kinase Enzyme System	1 Each	VA7425