ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

DYRK3 Kinase Assay

By Juliano Alves, Laurie Engel, Said A. Goueli, and Hicham Zegzouti, Promega Corporation

Scientific Background:

DYRK3 or dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3 is belongs to the DYRK family of dual-specificity protein kinases that catalyze autophosphorylation on serine/threonine and tyrosine residues. DYRK3 expressed in E. coli undergoes tyrosine autophosphorylation and catalyzes phosphorylation of histones H3 and H2B in vitro (1). DYRK3 regulate different steps of the caveolar cycle (2). DYRK3 can promote cell survival through phosphorylation and activation of SIRT1. DYRK3 directly phosphorylate SIRT1 at Thr (522), promoting deacetylation of p53.

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. Pelkmans. L. et.al: Kinase-regulated quantal assemblies and kiss-and-run recycling of caveolae. Nature 436: 128-133, 2005.

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.

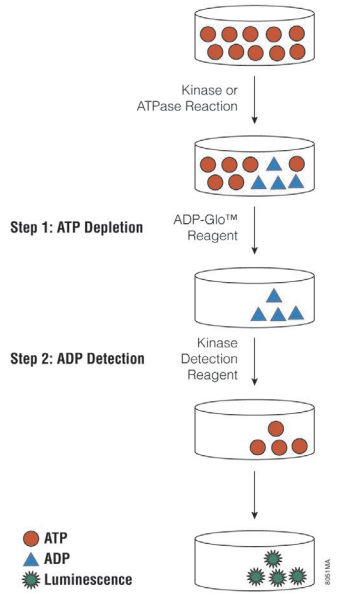

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

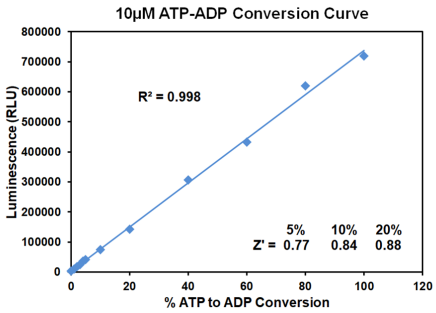

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	300	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0.59	0.29	0
Luminescence	313,211	310,934	294,020	289,740	258,770	173,977	97,858	50,749	22,889	10,342	6,189	2,440
S/B	128	127	120	119	106	71	40	21	9	4	3	1
% Conversion	79	79	74	73	65	43	24	12	5	1	0	0

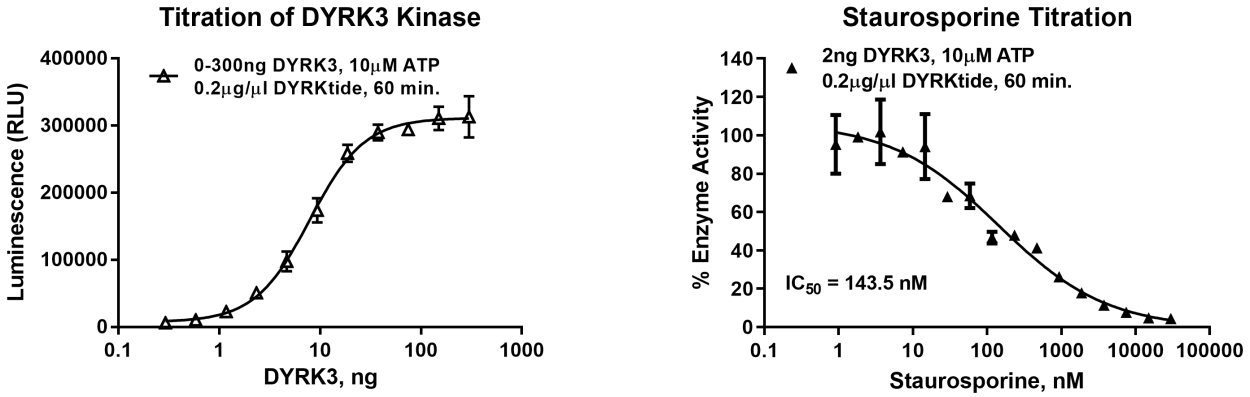

Figure 3. DYRK3 Kinase Assay Development. (A) DYRK3 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2ng of DYRK3 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
DYRK3 Kinase Enzyme System	10µg	VA7429
	1mg	VA7430
ADP-Glo™ + DYRK3 Kinase Enzyme System	1 Each	VA7431