

DDR2 Kinase Assay

By Juliano Alves, Ph.D., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

DDR2 is a member of a novel subclass of RTKs containing a distinct extracellular region encompassing a factor VIII-like domain (1) and is thought to be involved in the regulation of cell growth, differentiation, and metabolism. DDR2 plays a role in the regulation of collagen turnover mediated by smooth muscle cells in obstructive diseases of blood vessels and the lung (2).

1. Karn, T. et al: Structure, expression and chromosomal mapping of TKT from man and mouse: a new subclass of receptor tyrosine kinases with a factor VIII-like domain. *Oncogene* 8: 3433-3440, 1993.
2. Ferri, N. et al: Role of discoidin domain receptors 1 and 2 in human smooth muscle cell-mediated collagen remodeling: potential implications in atherosclerosis and lymphangioliomyomatosis. *Am J Pathol.* 2004 May;164(5):1575-85.

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.

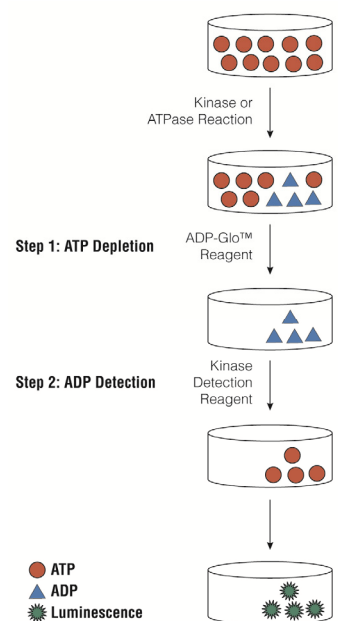


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

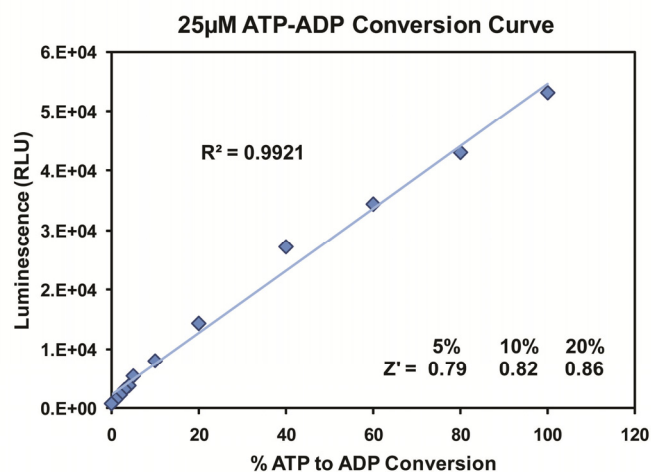
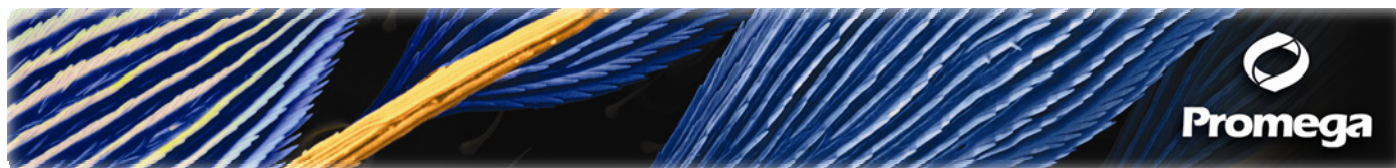


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



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 Tyrosine Kinase Buffer.
- Add to the wells of 384 low volume plate:
 - 1 μ l of inhibitor or (5% DMSO)
 - 2 μ l of enzyme (defined from table 1)
 - 2 μ l of substrate/ATP mix
- Incubate at room temperature for 60 minutes.
- Add 5 μ l of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10 μ l of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1second).

Table 1. DDR2 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.

DDR2, ng	100	50	25	12.5	6.3	3.1	1.56	0.78	0
Luminescence	43823	29682	16039	9019	4427	2953	1955	1750	1268
S/B	35	23	13	7	3	2.3	1.5	1.4	1.0
% Conversion	22	15	8	4	2	1.3	0.8	0.7	0.0

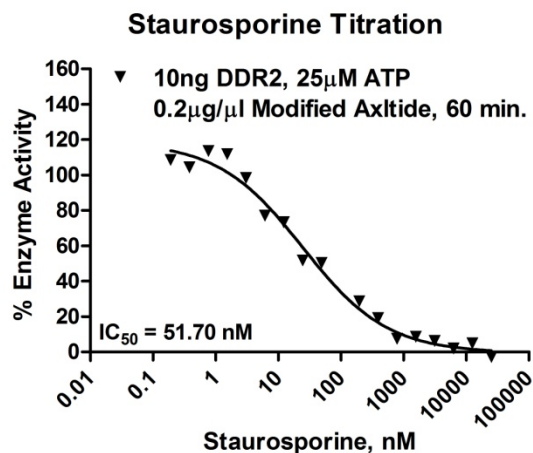
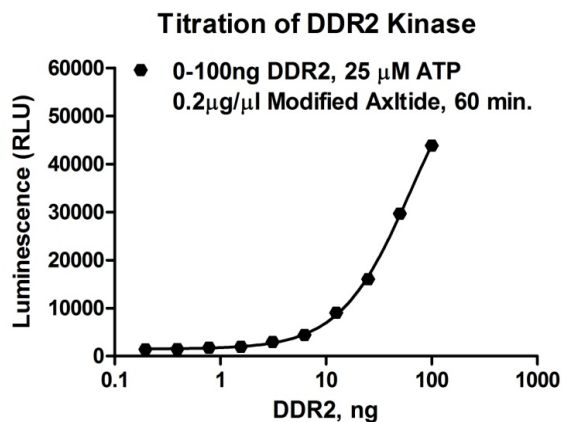


Figure 3. DDR2 Kinase Assay Development. (A) DDR2 enzyme was titrated using 25 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of DDR2 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:		
Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
DDR2 Kinase Enzyme System	Promega	V4058
ADP-Glo™ + DDR2 Kinase Enzyme System	Promega	V4059

DDR2 Kinase Buffer: 40mM Tris,7.5; 20mM MgCl₂; 0.1mg/ml BSA; 2.5mM MnCl₂, 50 μ M DTT.