

ADP-Glo™ Kinase Assay Application Notes

TYROSINE KINASE SERIES: RON



RON Kinase Assay

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Scientific Background:

RON is a macrophage stimulating 1 receptor c-met-related tyrosine kinase (1). RON receptor tyrosine kinase interacts with HYAL2 receptor protein, rendering it functionally inactive. HYAL2 is a candidate tumor suppressor GPI-anchored cell-surface protein that serves as an entry receptor for jaagsiekte sheep retrovirus, a virus that causes contagious lung cancer in sheep that is morphologically similar to human bronchioloalveolar carcinoma. It was shown that RON liberated from the association with HYAL2 becomes functionally active and activates the Akt and mitogen-activated protein kinase pathways (2).

1. Wang, M.H. et al: Identification of the ron gene product as the receptor for the human macrophage stimulating protein. *Science*, 1994; 266 (5182): 117-9.
2. Li, B,Q, et al: "Macrophage-stimulating protein activates Ras by both activation and translocation of SOS nucleotide exchange factor. *Biochem. Biophys. Res. Commun.*1995; 216 (1): 110-8.

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.

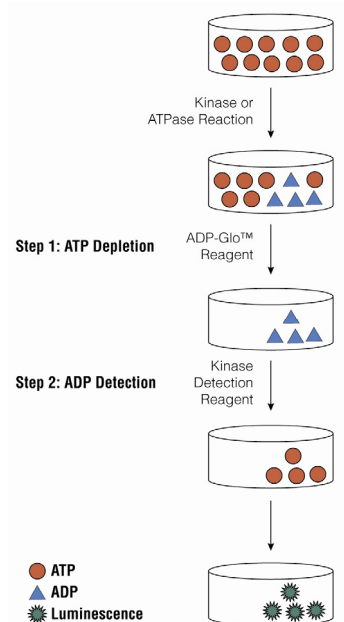


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

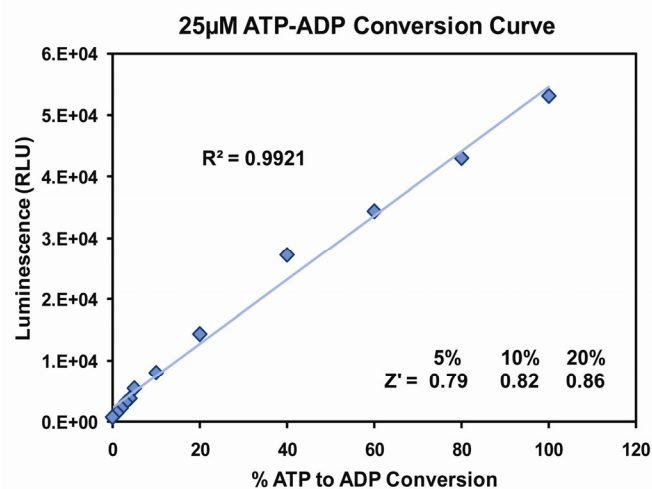


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.



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 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. RON 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.

RON, ng	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0
Luminescence	95173	92924	88870	74568	57225	41059	23417	11142	5067	1823
S/B	52	51	49	41	31	23	13	6	3	1
% Conversion	81	79	75	62	47	32	17	6	0.3	0

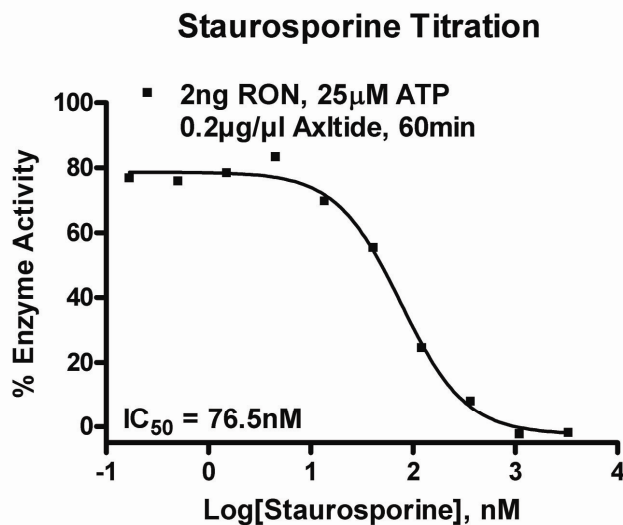
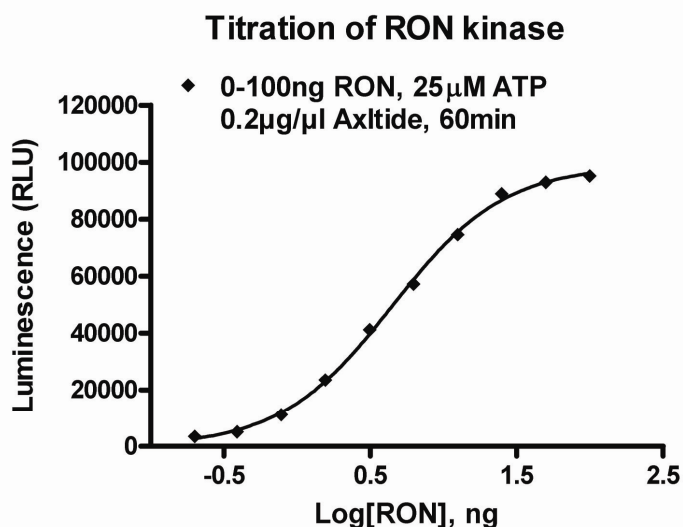


Figure 3. RON Kinase Assay Development: (A) RON enzyme was titrated using 25 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 2ng of RON to determine the potency of the inhibitor (IC₅₀).

Assay Components and Ordering Information:



Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
RON Kinase Enzyme System	Promega	V3921
ADP-Glo + RON Kinase Enzyme System	Promega	V8071

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