WNK1 Kinase Assay

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Scientific Background:

WNK1 is a member of the WNK subfamily of serine/threonine protein kinases that is a key regulator of blood pressure by controlling the transport of sodium and chloride ions. Mutations in WNK1 have been associated with pseudohypoaldosteronism type II and hereditary sensory neuropathy type II. WNK1 is a regulator of blood pressure and deficiency of this protein in mice lowers the blood pressure (1). WNK1 can regulate the Ca(2+) sensing and the subsequent Ca(2+)-dependent interactions mediated by synaptotagmin C2 domains and WNK1 exhibits additive CFTR inhibition (2).

1. Zambrowicz, B. P. et.al: Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc. Nat. Acad. Sci. 100: 14109-14114, 2003.

2. Lee, B.-H. et.al: WNK1 phosphorylates synaptotagmin 2 and modulates its membrane binding. Molec. Cell 15: 741-751, 2004.

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.

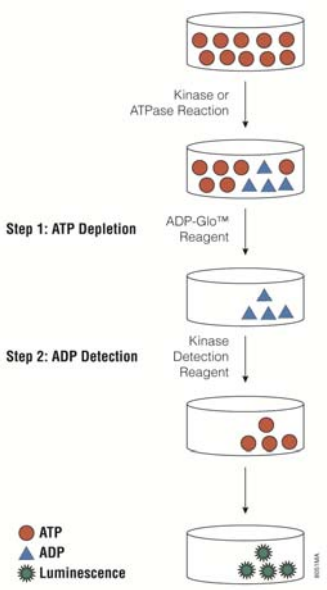


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

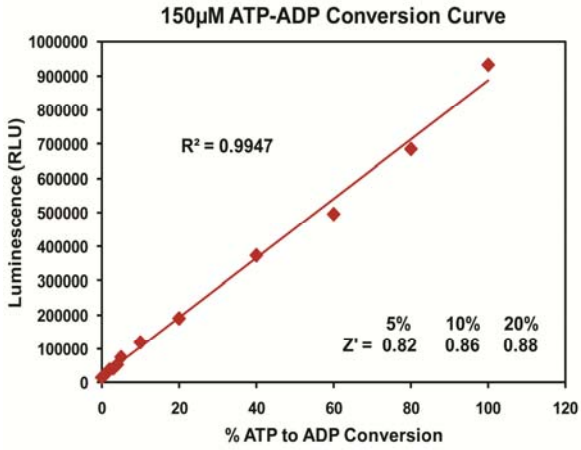


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 150µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

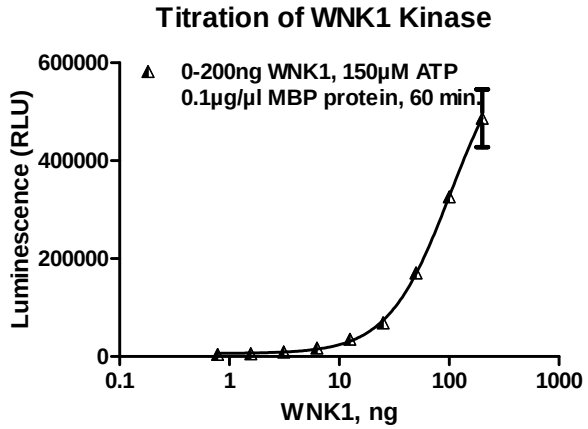
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, and the KES Protocol available at: http://www.promega.com/tbs/tm313/tm313.html, and http://www.promega.com/KESProtocol, respectively.

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. WNK1 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.

WNK1, ng	200	100	50	25	12.5	6.3	3.1	1.6	0
RLU	486863	243523	170794	68739	35427	17334	9497	5781	3504
S/B	139	69	49	20	10	5	3	2	1
% Conversion	58	26	18	5	3	1	0.5	0.1	0



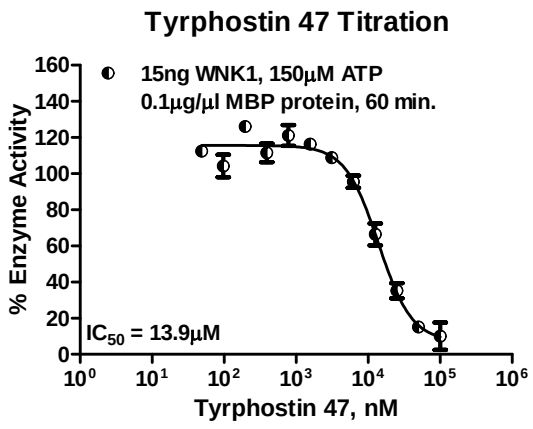


Figure 3. WNK1 Kinase Assay Development. (A) WNK1 enzyme was titrated using 150µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Tyrphostin 47 dose response was created using 15ng of WNK1 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
WNK1 Kinase Enzyme System	Promega	V5084
ADP-Glo™ + WNK1 Kinase Enzyme System	Promega	V5085

WNK1 Kinase Buffer: 40mM Tris, pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 2mM $MnCl_2$; 50µM DTT.