ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

InsR (R1201Q) Kinase Assay

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Scientific Background:

InsR is the insulin receptor tyrosine kinase that is involved in insulin signaling. InsR is post-translationally cleaved into two chains, α and β that are covalently linked. Binding of insulin to the InsR stimulates glucose uptake (1). Insulin receptor signaling helps to maintain fuel homeostasis and prevent diabetes. Studies have shown that a conditional knockout of insulin receptor substrate 2 (IRS2) in mouse pancreas β cells and parts of the brain--including the hypothalamus--increased appetite, lean and fat body mass, linear growth, and insulin resistance that progressed to diabetes. InsR signaling also increases the regeneration of adult β cells and the central control of nutrient homeostasis (2).

1. Okamoto, H. et al: Transgenic rescue of insulin receptor-deficient mice. J. Clin. Invest. 2004;114(2):214-23.

2. Lin, X. et al: Dysregulation of insulin receptor substrate 2 in beta cells and brain causes obesity and diabetes. J. Clin. Invest. 2004;114(7):886-8.

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.

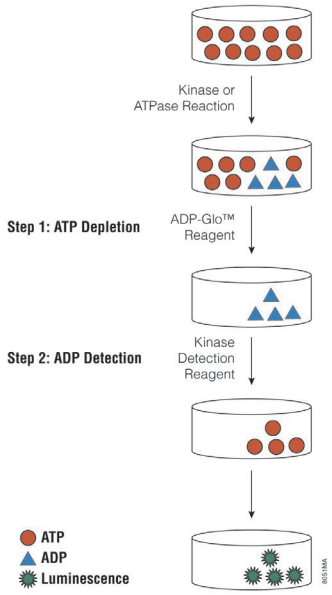

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

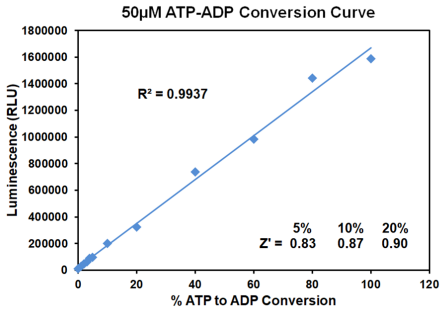

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	1.17	0.59	0
Luminescence	1,705,235	1,203,155	790,824	427,829	209,380	87,261	47,075	17,220	11,450	7,373
S/B	231	163	107	58	28	12	6	2	2	1
% Conversion	96	67	44	23	10	3	1	0	0	0

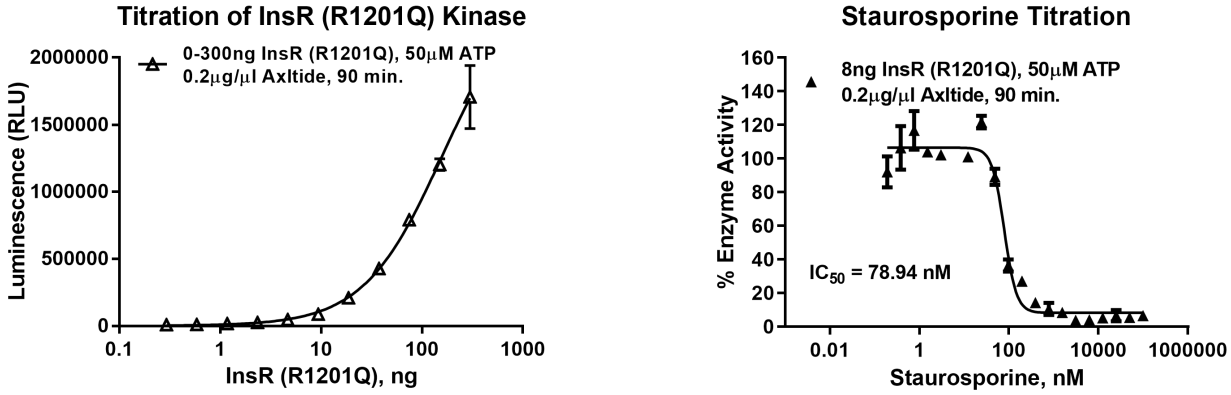

Figure 3. InsR (R1201Q) Kinase Assay Development. (A) InsR (R1201Q) enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 8ng of InsR (R1201Q) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
InsR (R1201Q) Kinase Enzyme System	10µg	VA7474
	1mg	VA7475
ADP-Glo™ + InsR (R1201Q) Kinase Enzyme System	1 Each	VA7476