InsR 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.

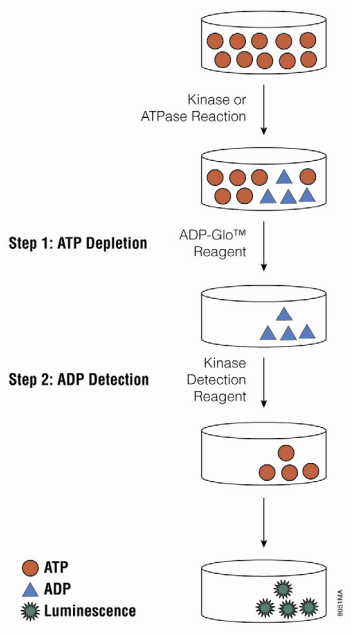

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

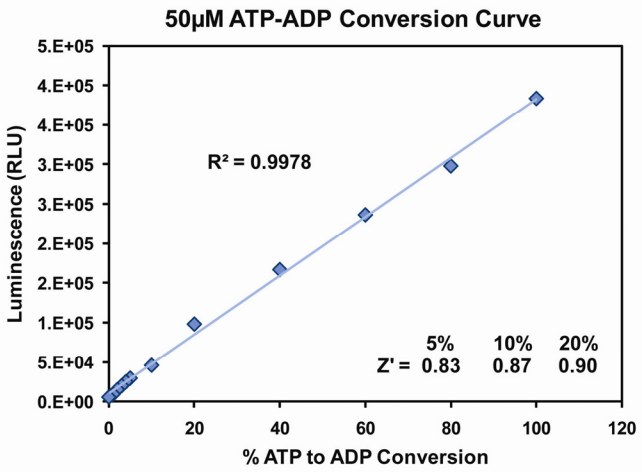

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.

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 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 0.5-1second).

Table 1. InsR 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.

InsR, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0
Luminescence	332499	298051	268202	234368	174531	130018	86380	55293	31400	4138
S/B	80	72	65	57	42	31	21	13	8	1
% Conversion	88	78	71	62	46	35	23	15	9	0

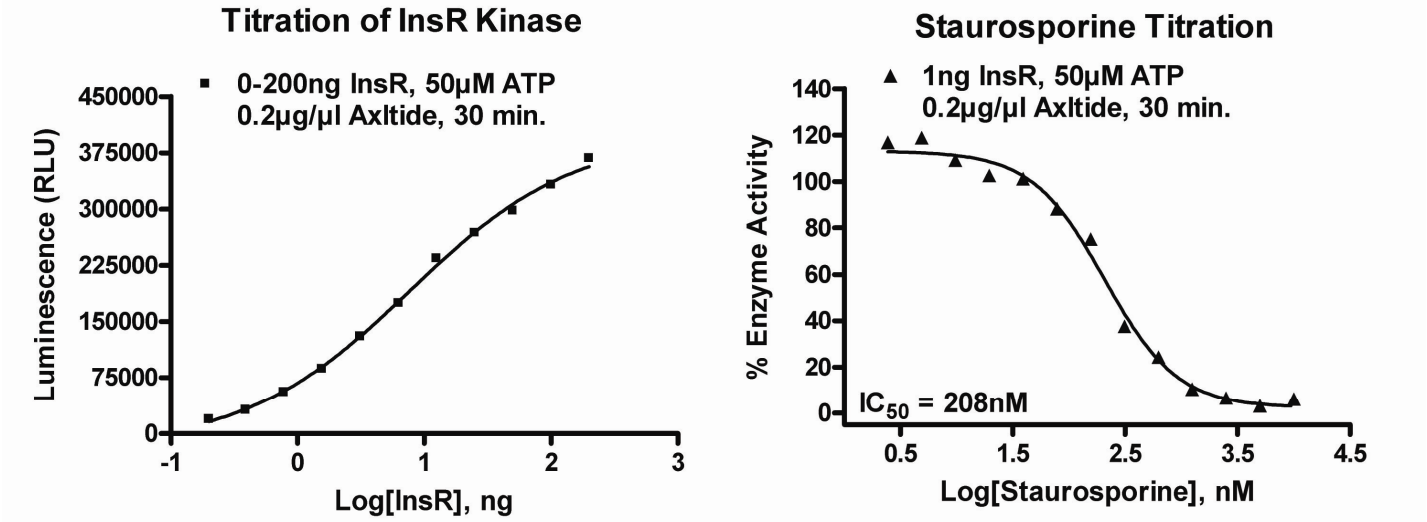

Figure 3. InsR Kinase Assay Development: (A) InsR enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1ng of InsR to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
InsR Kinase Enzyme System	Promega	V3901
ADP-Glo + InsR Kinase Enzyme System	Promega	V9411

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

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