ADP-Glo™ Kinase Assay Application Notes

TYROSINE KINASE SERIES: IGF1R

IGF1R Kinase Assay

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Scientific Background:

IGF1R (Insulin-like Growth Factor 1) is a transmembrane tyrosine kinase receptor that is activated by IGF-1 and by the related growth factor IGF-2 (1). IGF1R mediates the effects of IGF-1 and plays an important role in growth and anabolic effects in adults. The IGF1R is implicated in several cancers, most notably breast cancer where it is highly overexpressed and functions as an anti-apoptotic agent by enhancing cell survival. In many instances, the anti-apoptotic properties of IGF1R overexpression allows cancerous cells to resist the cytotoxic properties of chemotheraputic drugs or radiotherapy (2).

1. Yaghmaie F, et al:. "Tracking changes in hypothalamic IGF-1 sensitivity with aging and caloric restriction".. Experimental Gerontology, 2007; 42 (1-2): 148-149.

2. Jones, H. et al (2004). "Insulin-like growth factor-I receptor signalling and acquired resistance to gefitinib (ZD1839; Iressa) in human breast and prostate cancer cells". Endocr. Relat. Cancer, 2004 11 (4): 793-814.

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.

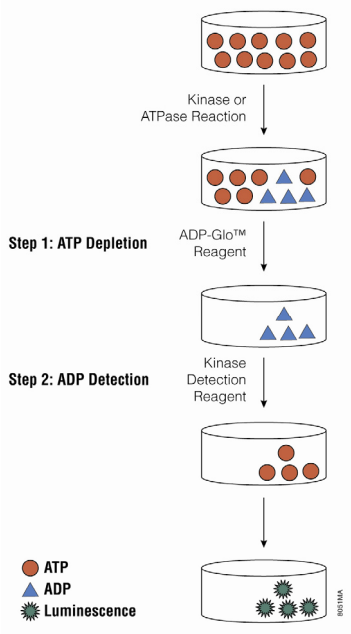

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

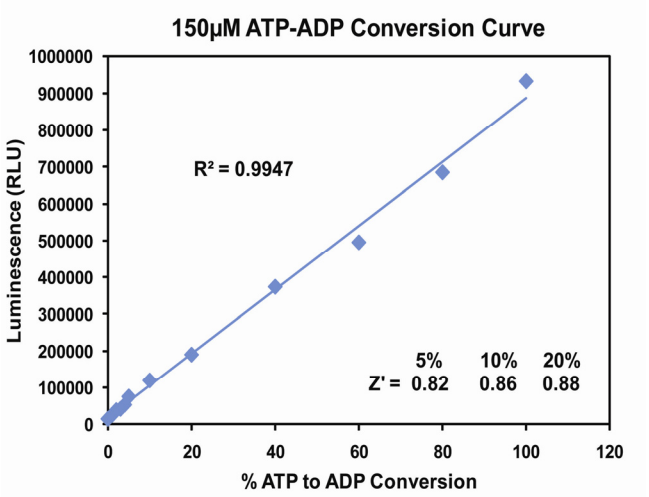

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 150µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.

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. IGF1R 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.

IGF1R, ng	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0
Luminescence	442720	413308	354681	295389	261372	208595	154884	91835	58307	9096
S/B	48.7	45.4	39.0	32.5	28.7	22.9	17.0	10.1	6.4	1
% Conversion	82.8	77.2	65.8	54.4	47.8	37.7	27.3	15.1	8.7	0

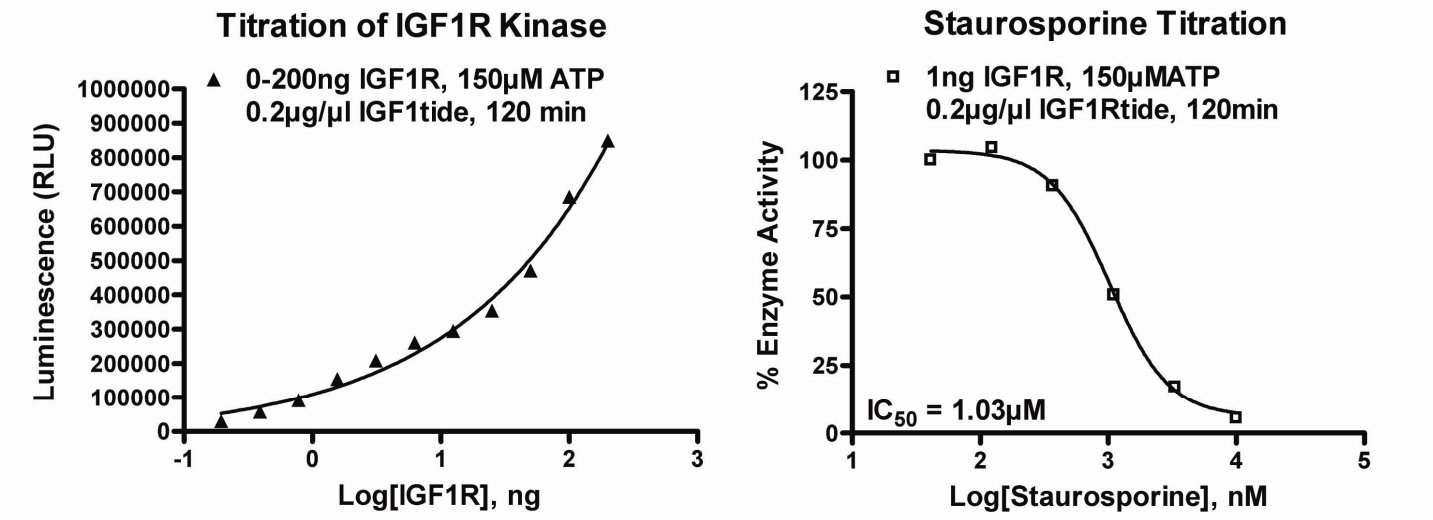

Figure 3. IGF1R Kinase Assay Development: (A) IGF1R enzyme was titrated using 150µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1ng of IGF1R to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
IGF1R Kinase Enzyme System	Promega	V3581
ADP-Glo + IGF1R Kinase Enzyme System	Promega	V9401

IGF1R Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 2mM $MnCl_2$; 50µM DTT.