PDGFRα Kinase Assay

By Jolanta Vidugiriene, Ph.D., Hicham Zegzouti, Ph.D., and Said A. Goueli, Ph.D., Promega Corporation

Scientific Background:

PDGFRα (platelet-derived growth factor receptor α) is a member of the PDGFR family of membrane receptors with intrinsic tyrosine kinase activity. Aberrant expression of PDGFRα has been linked to developmental abnormalities in vertebrate models and has been implicated in multiple disease states in humans. There is widespread expression of PDGFRα in renal cell types involved in fibrotic and sclerosing processes (1). PDGF and its receptor PDGFRα are inducers of fibrosis in the repair phase of inflammatory bowel disease and they may also be involved in the active inflammatory phase (2).

1. Floege, J. et al: Expression of PDGF alpha-receptor in renal arteriosclerosis and rejecting renal transplants. J Am Soc Nephrol. 1998 Feb;9(2):211-23.

2. Kumagai, S. et al: Platelet-derived growth factor and its receptors are expressed in areas of both active inflammation and active fibrosis in inflammatory bowel disease. Tohoku J Exp Med. 2001 Sep;195(1):21-33.

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.

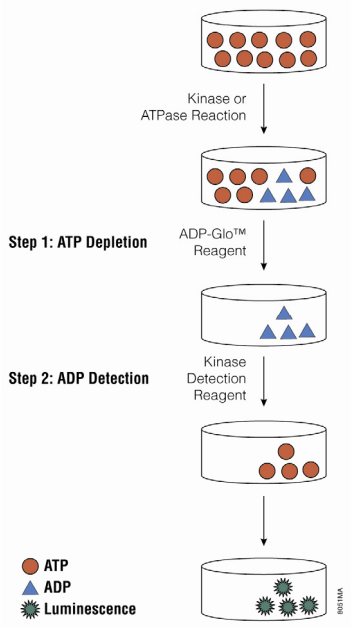

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

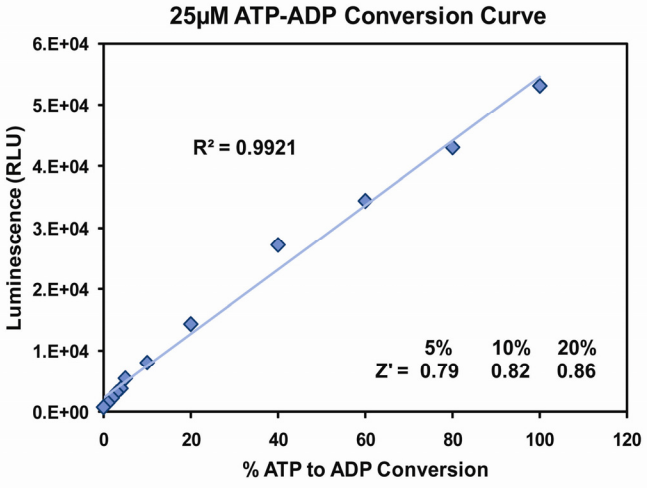

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.

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 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. PDGFRα 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.

PDGFRα, ng	200	100	50	25	12.5	6.25	3.13	1.56	0.78	0
Luminescence	41469	35595	23334	15126	10561	7634	4044	2498	1702	938
S/B	44.2	37.9	24.9	16.1	11.3	8.1	4.3	2.7	1.8	1
% Conversion	42.77	36.50	23.41	14.65	9.78	6.65	2.82	1.17	0.32	0

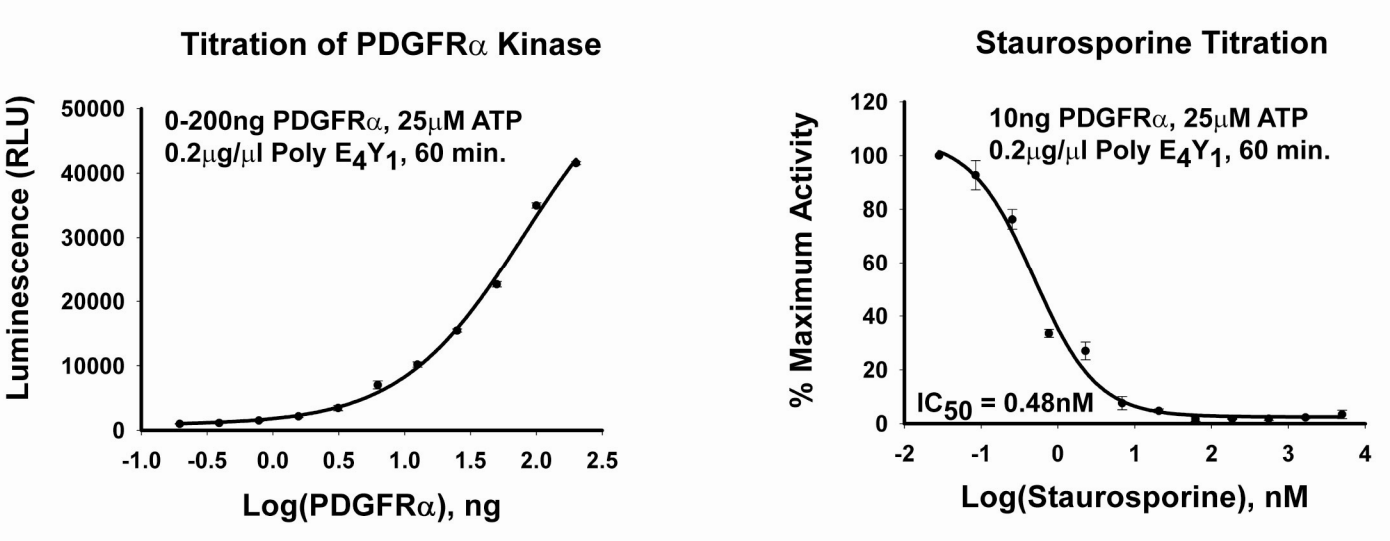

Figure 3. PDGFRα Kinase Assay Development: (A) PDGFRα enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of PDGFRα to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PDGFRα Kinase Enzyme System	Promega	V3721
ADP-Glo + PDGFRα Kinase Enzyme System	Promega	V8011

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

Promega Corporation • 2800 Woods Hollow Road • Madison, WI 53711-5399 USA • Telephone 608-274-4330 • Fax 608-277-2601
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