

PDGFR α (T674I) Kinase Assay

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Scientific Background:

PDGFR α (platelet-derived growth factor receptor α) is a member of the PDGFR family of membrane receptors with intrinsic tyrosine kinase activity. Aberrant expression of PDGFR α has been linked to developmental abnormalities in vertebrate models and has been implicated in multiple disease states in humans. There is widespread expression of PDGFR α in renal cell types involved in fibrotic and sclerosing processes(1). PDGF and its receptor PDGFR α 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.

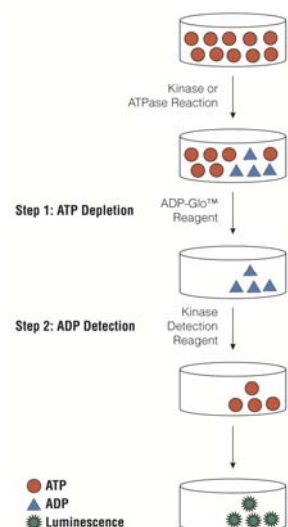


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

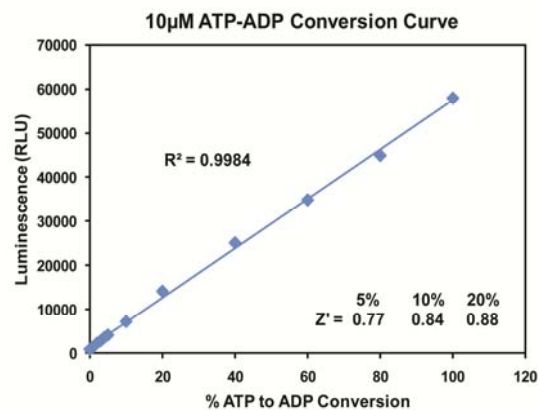
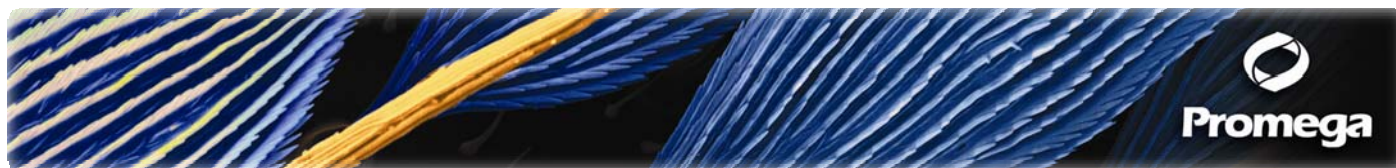


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10 μ M ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



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 Tyrosine Kinase Buffer.
- Add to the wells of 384 low volume plate:
 - 1 μ l of inhibitor or (5% DMSO)
 - 2 μ l of enzyme (defined from table 1)
 - 2 μ l of substrate/ATP mix
- Incubate at room temperature for 120 minutes.
- Add 5 μ l of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10 μ l of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1sec).

Table 1. PDGFR α (T674I) 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.

PDGFR α (T674I), ng	200	100	50	25	12.5	6.3	3.1	1.6	0
Luminescence	37282	24485	15754	8065	3633	2141	1348	948	546
S/B	68	45	29	15	7	4	2	2	1
% Conversion	84	55	35	17	7	4	2	1	0

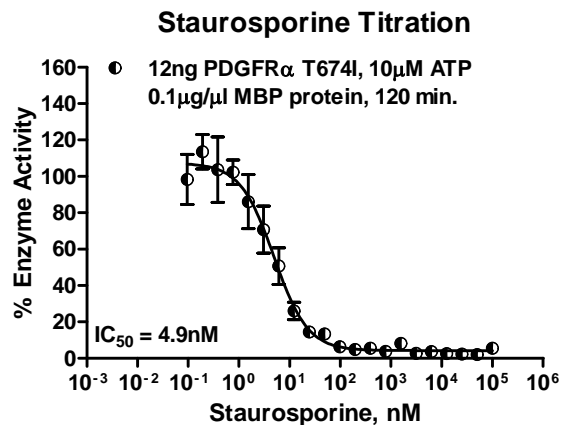
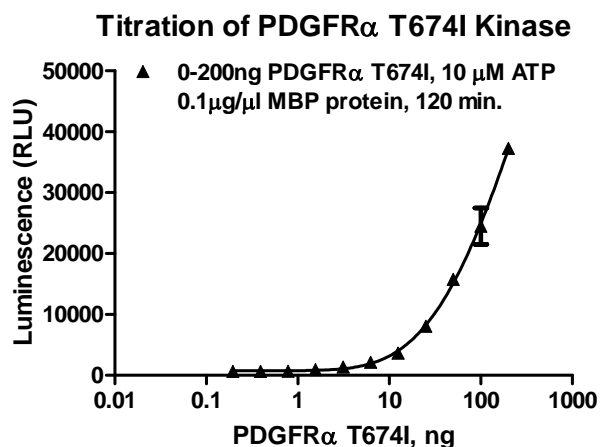


Figure 3. PDGFR α (T674I) Kinase Assay Development. (A) PDGFR α (T674I) enzyme was titrated using 10 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 12ng of PDGFR α (T674I) to determine the potency of the inhibitor (IC₅₀).

Assay Components and Ordering Information:



Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PDGFR α (T674I) Kinase Enzyme System	Promega	V4486
ADP-Glo™ + PDGFR α (T674I) Kinase Enzyme System	Promega	V4487

PDGFR α (T674I) Kinase Buffer: 40mM Tris, pH 7.5; 20mM MgCl₂; 0.1mg/ml BSA; 2mM MnCl₂; 50 μ M DTT.