PDGFRβ Kinase Assay

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Scientific Background:

PDGFRβ (platelet-derived growth factor receptor β) is a member of the PDGFR family of membrane receptors with intrinsic tyrosine kinase activity. PDGFRβ deficient mice are hemorrhagic, severely anemic and exhibit a defect in kidney glomeruli function (1). However, absence of PDGFRβ has no impact on major blood vessels and the heart. PDGFRβ expression and activity is elevated in several cancers and inhibition of PDGFRβ activity blocks progression of renal carcinoma in an animal model (2).

1. Soriano, P: Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. Genes Dev. 1994 Aug 15;8(16):1888-96.

2. Xu, L. et al: Blocking platelet-derived growth factor-D/platelet-derived growth factor receptor beta signaling inhibits human renal cell carcinoma progression in an orthotopic mouse model. Cancer Res. 2005 Jul 1;65(13):5711-9.

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.

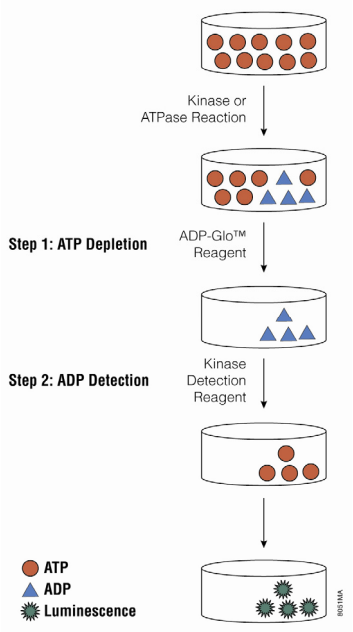

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

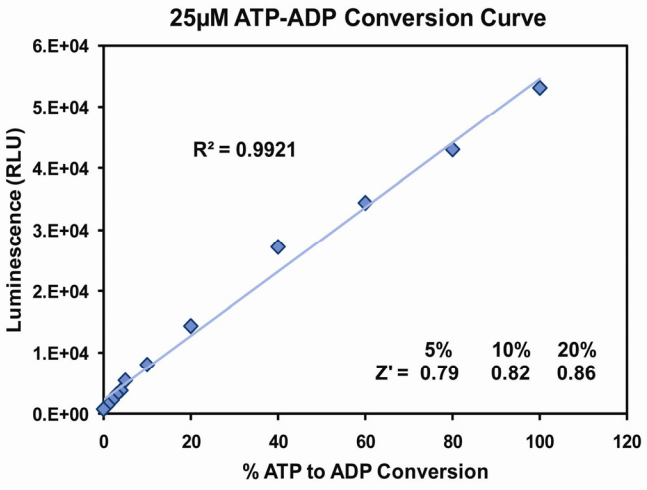

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.

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.PDGFRβ 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.

PDGFRβ, ng	200	100	50	25	12.5	6.25	3.13	1.56	0.78	0
Luminescence	65334	56889	36386	21725	15129	9539	4182	2173	1605	980
S/B	66.7	58.0	37.1	22.2	15.4	9.7	4.3	2.2	1.6	1.0
% Conversion	68.24	59.22	37.34	21.69	14.65	8.69	2.97	0.82	0.22	0

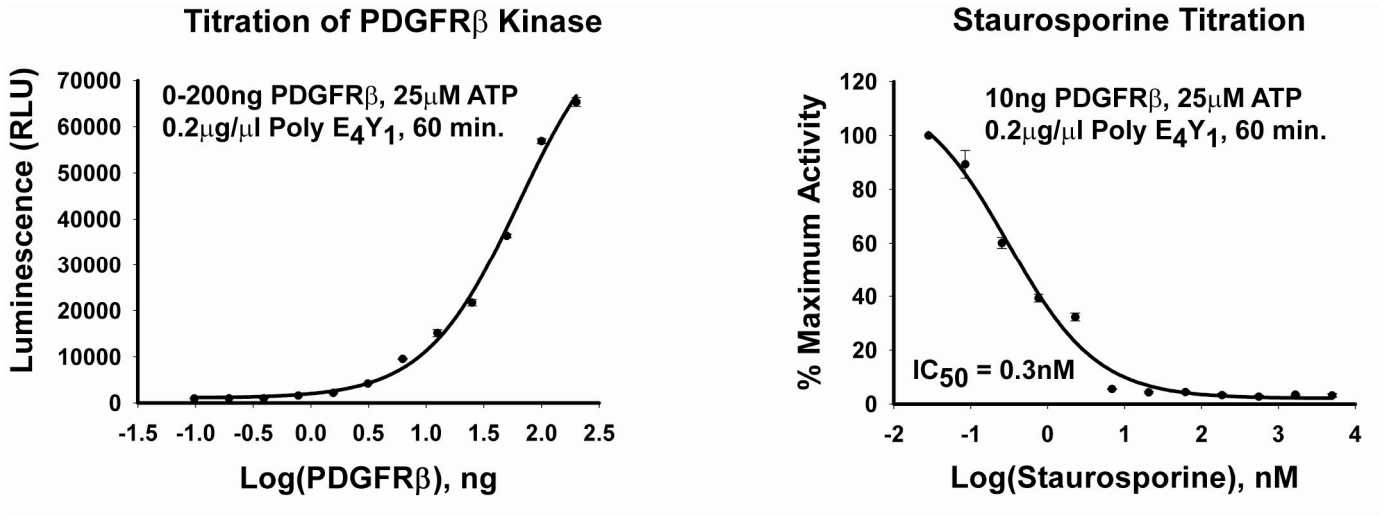

Figure 3. PDGFRβ Kinase Assay Development: (A) PDGFRβ enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of PDGFRβ to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PDGFRβ Kinase Enzyme System	Promega	V3731
ADP-Glo + PDGFRβ Kinase Enzyme System	Promega	V8021

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

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