# PRKG1 Kinase Assay

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## Scientific Background:

PRKG1 is a homodimer, with each monomer containing a regulatory cGMP-binding domain and a catalytic domain (1). By Northern blot analysis PRKG1 was shown to be expressed at highest levels in bladder, uterus, adrenal gland, and fallopian tube. PRKG1 plays an important stimulatory role in platelet activation (2). Expression of recombinant PRKG1 in a reconstituted cell model enhanced von Willebrand factor-induced activation of the platelet integrin alpha-IIb/beta-3. Prkg1 knockout mice showed impaired platelet responses to VWF or low doses of thrombin and prolonged bleeding time. Human platelet aggregation induced by VWF or low-dose thrombin was inhibited by PRKG1 inhibitors but enhanced by cGMP.

1. Orstavik, S. et al: Characterization of the human gene encoding the type I-alpha and type I-beta cGMP-dependent protein kinase (PRKG1). Genomics 42: 311-318, 1997.

2. Li, Z. et al: A stimulatory role for cGMP-dependent protein kinase in platelet activation. Cell 112: 77-86, 2003.

## 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.

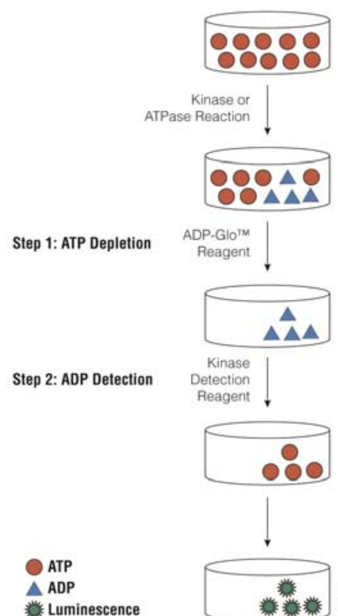



**Figure 1. Principle of the ADP-Glo™ Kinase Assay.** The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

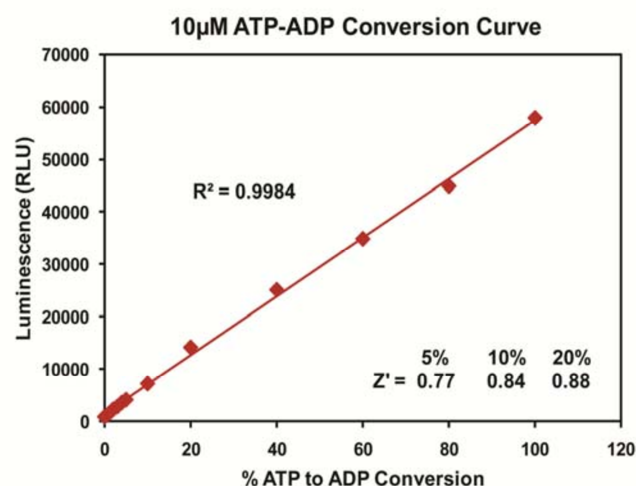



**Figure 2. Linearity of the ADP-Glo Kinase Assay.** ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

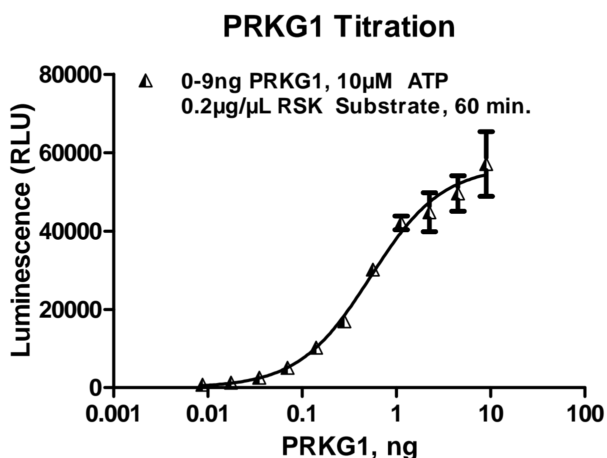
## Protocol

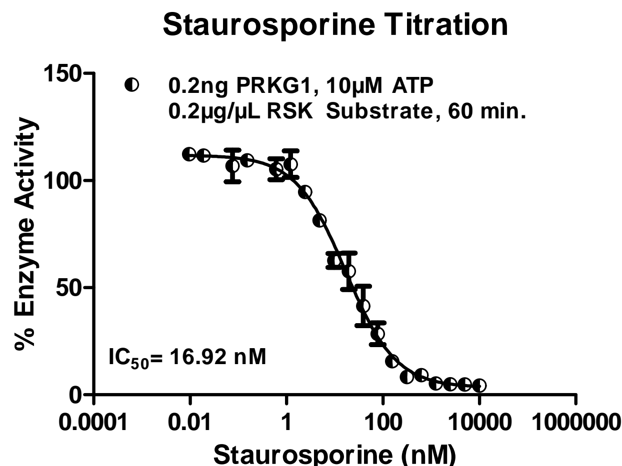
- Dilute enzyme, substrate, ATP and inhibitors in Kinase Buffer.
- Add to the wells of 384 low volume plate:
  - 1 µl of inhibitor or (5% DMSO)
  - 2 µl of enzyme (defined from table 1)
  - 2 µl of substrate/ATP mix
- Incubate at room temperature for 60 minutes.
- Add 5 µl of ADP-Glo™ Reagent
- Incubate 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. PRKG1 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.

| PRKG1, ng | 9 | 4.5 | 2.25 | 1.13 | 0.56 | 0.28 | 0.14 | 0.07 | 0.035 | 0.018 | 0 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| RLU | 57091 | 49575 | 44795 | 42074 | 30199 | 16973 | 10202 | 5112 | 2538 | 1308 | 212 |
| S/B | 269 | 234 | 211 | 199 | 142 | 80 | 48 | 24 | 12 | 6.2 | 1 |
| % Conversion | 92 | 81 | 73 | 69 | 50 | 25 | 14 | 5.8 | 3.2 | 1.4 | 0 |

**Figure 3. PRKG1 Kinase Assay Development.** (A) PRKG1 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 0.2ng of PRKG1 to determine the potency of the inhibitor ($IC_{50}$).




Assay Components and Ordering Information:

| Products | Company | Cat.# |
|---|---|---|
| ADP-Glo™ Kinase Assay | Promega | V9101 |
| PRKG1 Kinase Enzyme System | Promega | V4248 |
| ADP-Glo™ + PRKG1 Kinase Enzyme System | Promega | V4249 |

PRKG1 Kinase Buffer: 40mM Tris,pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT, 10µM cGMP.

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