# PKCγ Kinase Assay

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## Scientific Background:

PKCγ is a member of the protein kinase C (PKC) family of serine- and threonine-specific protein kinases that can phosphorylate a wide variety of protein targets known to be involved in diverse cellular signaling pathways. In the brain, PKCγ is translocated to cell membranes during ischemia and is rapidly removed or degraded during the second otherwise lethal ischemic insult in preconditioned brains. This suggest that ischemic preconditioning enhances downregulation of cell signaling mediated by PKCγ and may thereby provide neuroprotection (1).

1. Shamloo, M. et al: Rapid decline in protein kinase Cgamma levels in the synaptosomal fraction of rat hippocampus after ischemic preconditioning. Neuroreport. 1999 Apr 6;10(5):931-5.

## 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.

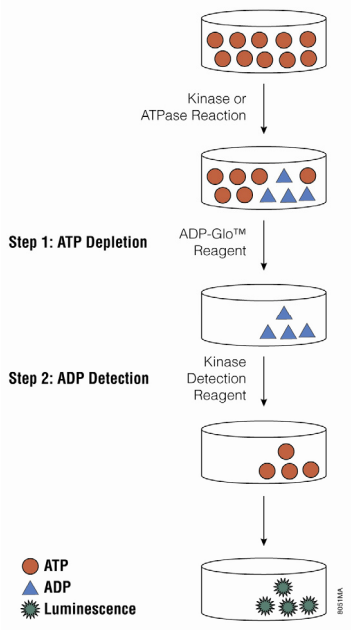


**Figure 1. Principle of the ADP-Glo™ Kinase Assay.** The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

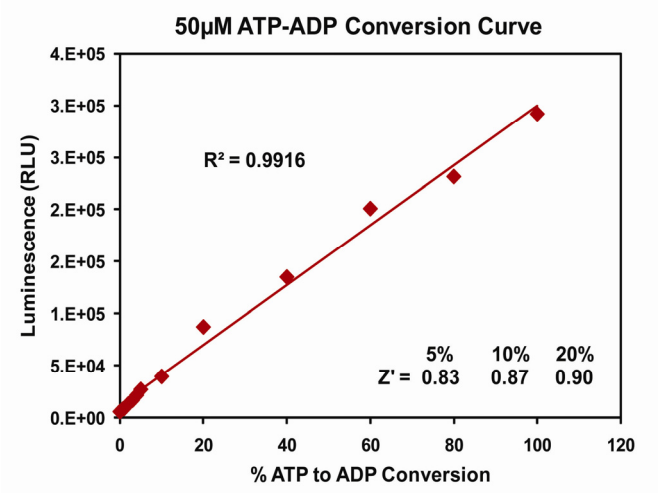


**Figure 2. Linearity of the ADP-Glo Kinase Assay.** ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 20 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. PKCγ 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.

| PKCγ, ng | 21.9 | 10.9 | 5.5 | 2.73 | 1.37 | 0.68 | 0.34 | 0.17 | 0 |
|---|---|---|---|---|---|---|---|---|---|
| RLU | 291849 | 309271 | 293573 | 250211 | 163261 | 102035 | 63244 | 35830 | 6307 |
| S/B | 46.3 | 49.0 | 46.6 | 39.7 | 25.9 | 16.2 | 10.0 | 5.7 | 1 |
| % Conversion | 97.3 | 103.4 | 97.9 | 82.8 | 52.6 | 31.3 | 17.8 | 8.2 | 0 |

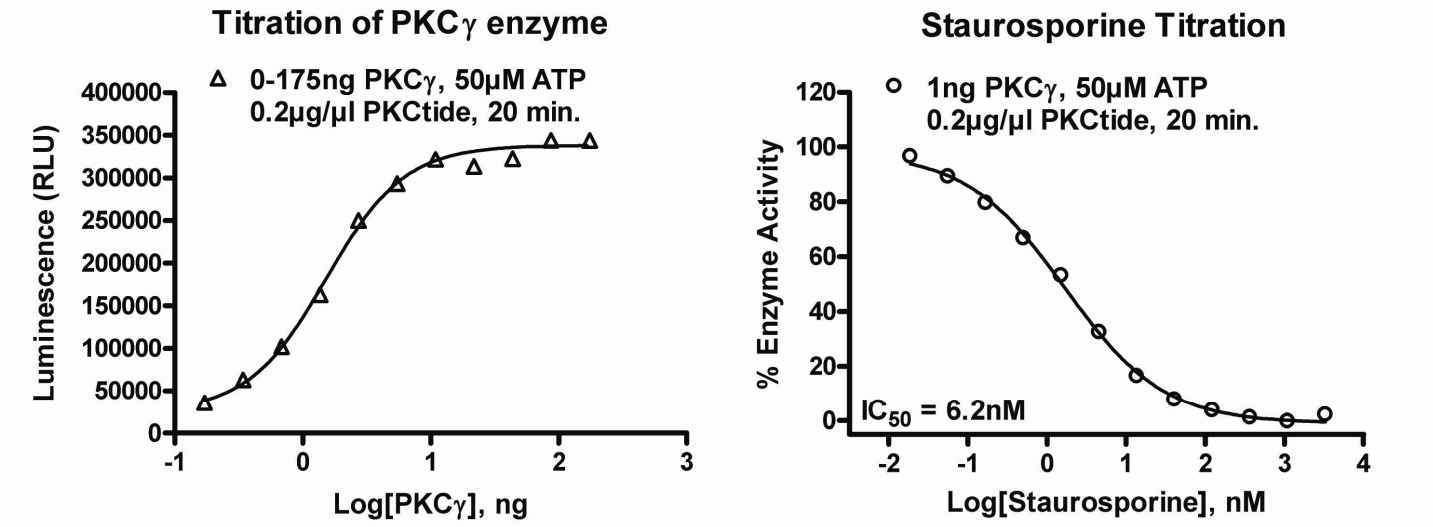


**Figure 3. PKCγ Kinase Assay Development:** (A) PKCγ enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1ng of PKCγ to determine the potency of the inhibitor ($IC_{50}$).

**Assay Components and Ordering Information:**

| Products | Company | Cat.# |
|---|---|---|
| ADP-Glo™ Kinase Assay | Promega | V9101 |
| PKCγ Kinase Enzyme System | Promega | V3391 |
| ADP-Glo + PKCγ Kinase Enzyme System | Promega | V9711 |

PKCγ Kinase Buffer: 40mM Tris, 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT; 1 x PKC Lipid activator mix.

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