

## PI3K(p120 $\gamma$ ) Kinase Assay

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### Scientific Background:

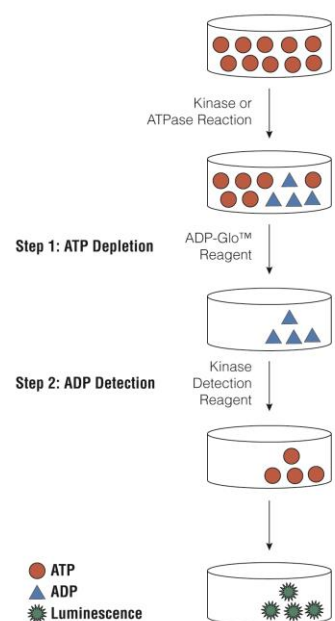
PI3K(p120 $\gamma$ ) is a member of the PI3K kinase family that phosphorylates phosphoinositides on the 3-hydroxyl group of the inositol ring. It is an important modulator of extracellular signals, including those elicited by E-cadherin-mediated cell-cell adhesion, which plays an important role in maintenance of the structural and functional integrity of epithelia. In addition to its role in promoting assembly of adherens junctions, PI3K (p120 $\gamma$ ) is thought to play a pivotal role in the regulation of cytotoxicity in NK cells.

1. Vadas O, et al: Molecular determinants of PI3K $\gamma$ -mediated activation downstream of G-protein-coupled receptors (GPCRs). *Proc Natl Acad Sci USA*, 2013.
2. Xie Y, et al: Identification of upregulated phosphoinositide 3-kinase  $\gamma$  as a target to suppress breast cancer cell migration and invasion. *Biochem Pharmacol*, 2013.

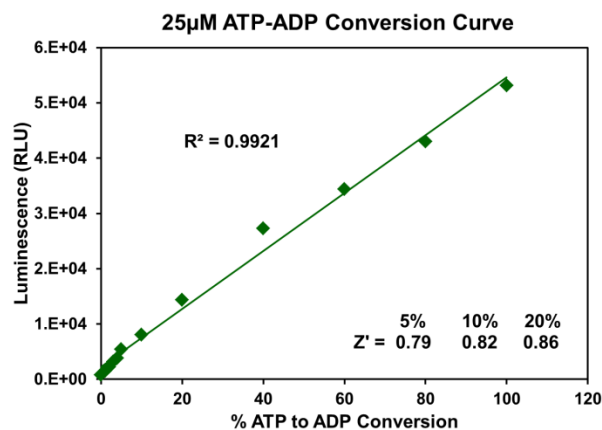
### ADP-Glo™ Kinase Assay

#### 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 25 $\mu$ 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.

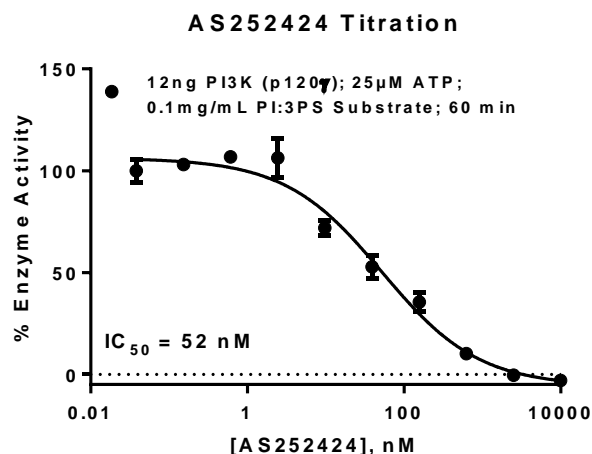
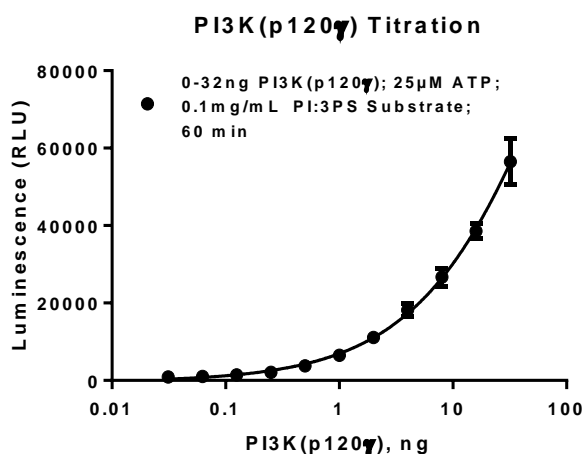
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Lipid Kinase Systems Technical Manual* #TM365, available at [www.promega.com/protocols/tm365](http://www.promega.com/protocols/tm365)

## Protocol

- Prepare PI3K Reaction Buffer/Lipid Substrate mixture.
- Dilute PI3K Enzyme into prepared PI3K Reaction Buffer/Lipid Substrate mixture (amount defined from table 1).
- Add to the wells of 384 low volume plate:
  - 0.5  $\mu$ l of inhibitor or vehicle
  - 4  $\mu$ l of enzyme/Lipid mixture
  - 0.5  $\mu$ l of 250 $\mu$ M ATP in water
- Incubate at room temperature for 60 minutes.
- Add 5  $\mu$ l of ADP-Glo™ Reagent (with 10mM MgCl<sub>2</sub>)
- Incubate at room temperature for 40 minutes.
- Add 10  $\mu$ l of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1second).

**Table 1. PI3K(p120 $\gamma$ ) 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.

PI3K (p120 $\gamma$ ), ng	32	16	8	4	2	1	0.5	0.25	0.125	0
RLU	56495	38548	26692	18152	11131	6533	3765	2108	1424	672
S/B	84	57	40	27	17	9.7	5.6	3.1	2.1	1
% Conversion	41	28	19	13	7.3	3.8	1.7	0.5	0.0	0



**Figure 3. PI3K(p120 $\gamma$ ) Kinase Assay Development.** (A) PI3K(p120 $\gamma$ ) enzyme was titrated using 25 $\mu$ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) AS252454 dose response was created using 12ng of PI3K(p120 $\gamma$ ) to determine the potency of the inhibitor (IC<sub>50</sub>).

### Assay Components and Ordering Information:



Products	Size	Cat.#
ADP-Glo™ Kinase Assay with PI:3PS	1,000 Assays	V1781
ADP-Glo™ Kinase Assay with PIP <sub>2</sub> :3PS	1,000 Assays	V1791
PI3K(p120 $\gamma$ ), 20 $\mu$ g	200 $\mu$ l	V1761
PI3K-Glo™ Class I Profiling Kit	1 each	V1690

PI3K Kinase Buffer: 50mM HEPES, pH 7.5; 50mM NaCl; 3mM MgCl<sub>2</sub>; 0.025mg/ml BSA.