ADP-Glo™ Kinase Assay Application Notes

LIPID KINASE SERIES: PI3K(p110α/p85α)

PI3K(p110α/p85α) Kinase Assay

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Scientific Background:

Phosphatidylinositol 3-kinase is composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The catalytic subunit uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns(4,5)P2. This gene has been found to be oncogenic and has been implicated in cervical cancers.

1. **Chomczyk MA, et al: PIK3CA mutations in the most common types of cancer. Postepy Biochem, 2013.**

2. **Jiang G, et al: PIK3CA gene mutations and amplifications in Chinese patients with ovarian clear cell carcinoma. Cancer Invest, 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.

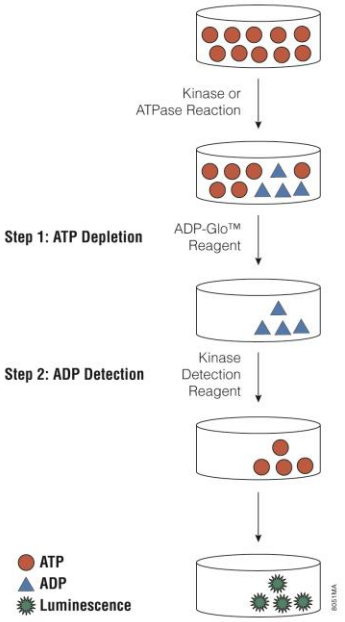

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

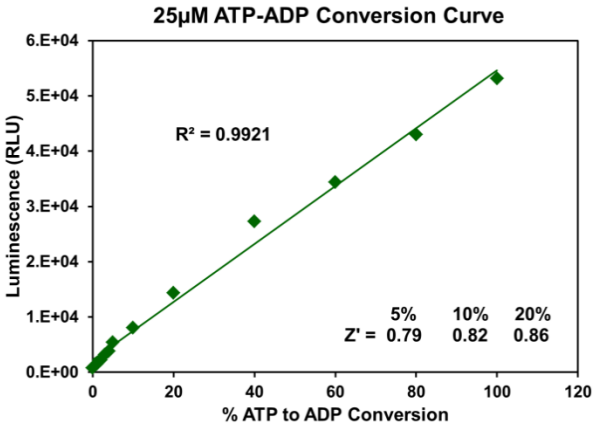

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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

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 µl of inhibitor or vehicle
  - 4 µl of enzyme/Lipid mixture
  - 0.5 µl of 250µM ATP in water
- Incubate at room temperature for 60 minutes.
- Add 5 µl of ADP-Glo™ Reagent (with 10mM MgCl2)
- 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. PI3K(p110α/p85α) 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 (p110α/p85α), ng	32	16	8	4	2	1	0.5	0.25	0.125	0
RLU	121338	96770	65071	32712	16101	7272	3546	1961	1424	734
S/B	165	132	89	45	22	10	5	3	2	1
% Conversion	90	71	48	23	11	4.4	1.6	0.4	0	0

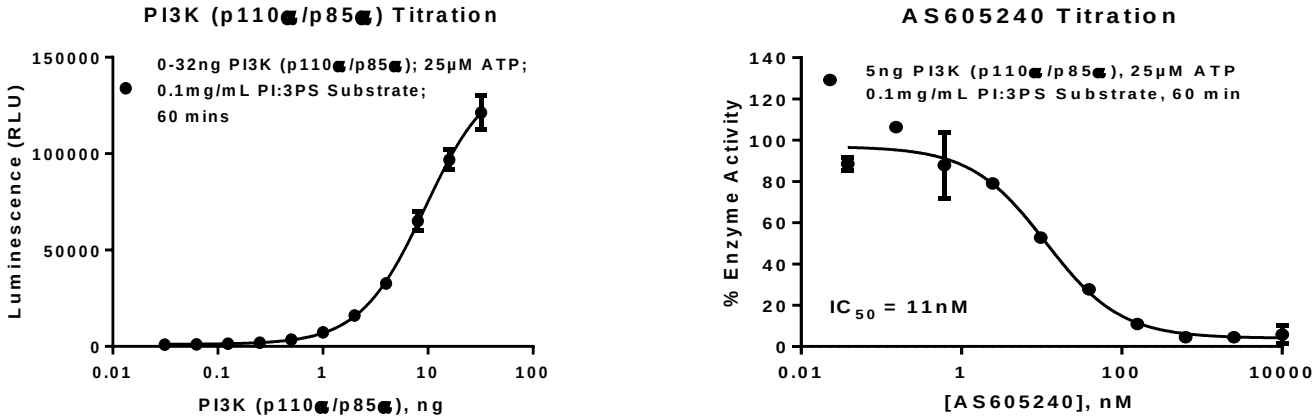

Figure 3. PI3K(p110α/p85α) Kinase Assay Development. **(A) PI3K(p110α/p/85α) enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) AS605240 dose response was created using 5ng of PI3K(p110α/p85α) to determine the potency of the inhibitor (IC_{50}).**

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_2:3PS	1,000 Assays	V1791
PI3K(p110α/p85α), 20µg	200µl	V1721
PI3K-Glo™ Class I Profiling Kit	1 each	V1690

PI3K Kinase Buffer: 50mM HEPES,pH 7.5; 50mM NaCl; 3mM $MgCl_2$; 0.025mg/ml BSA.

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