

PI3K(p110 α [E545K]/p85 α) 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. Hafner C, et al: Oncogenic PIK3CA mutations occur in epidermal nevi and seborrheic keratosis with a characteristic mutation pattern. *Proc Natl Acad Sci U S A*, 2007.

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.

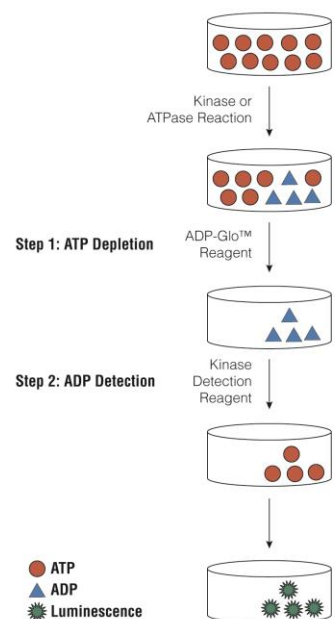


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

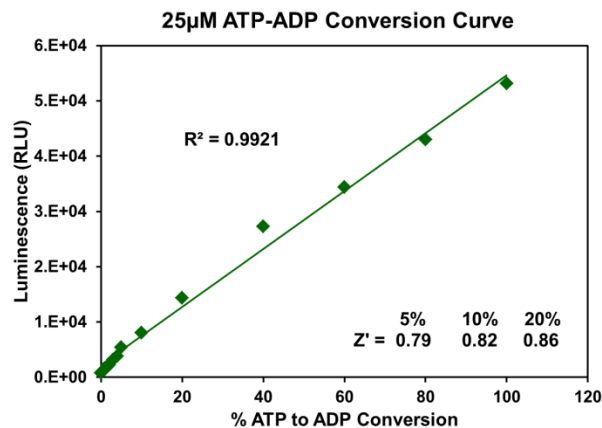


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25 μ M ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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

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 μ l of inhibitor or vehicle
 - 4 μ l of enzyme/Lipid mixture
 - 0.5 μ l of 250 μ M ATP in water
- Incubate at room temperature for 60 minutes.
- Add 5 μ l of ADP-Glo™ Reagent (with 10mM MgCl₂)
- Incubate at room temperature for 40 minutes.
- Add 10 μ l of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1second).

Table 1. PI3K(p110 α [E545K]/p85 α) 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 (p110 α [E545K]/p85 α), ng	40	20	10	5	2.5	1.2	0.6	0.3	0.15	0
RLU	119470	106149	82650	58062	33572	18383	10217	5805	3761	796
S/B	150	130	104	73	42	23	13	7	5	1
% Conversion	90	80	62	43	25	13	7	4	2	0

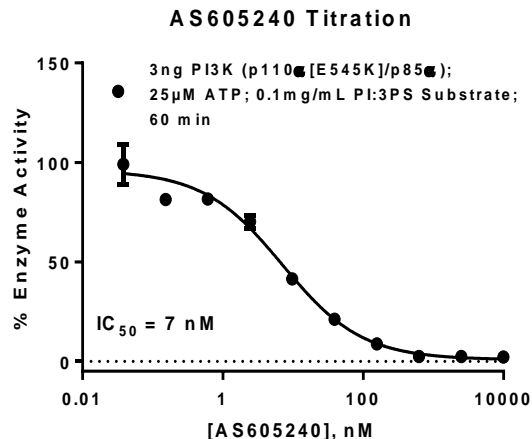
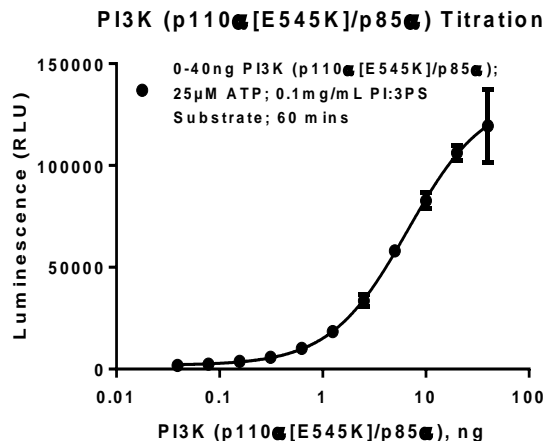


Figure 3. PI3K(p110 α [E545K]/p85 α) Kinase Assay Development. (A) PI3K(p110 α [E545K]/p85 α) enzyme was titrated using 25 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) AS605240 dose response was created using 3 ng of PI3K(p110 α [E545K]/p85 α) to determine the potency of the inhibitor (IC₅₀).

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(p110 α [E545K]/p85 α), 20 μ g	200 μ l	V1731
PI3K-Glo™ Class I Profiling Kit	1 each	V1690

PI3K Kinase Buffer: 50mM HEPES, pH 7.5; 50mM NaCl; 3mM MgCl₂; 0.025mg/ml BSA.