ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: AKT3

AKT3 Kinase Assay

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Scientific Background:

AKT3 or Protein Kinase B γ (PKBγ) is a serine/threonine kinase that is a member of the AGC family. AKT 3 is activated in cells exposed to diverse stimuli such as hormones, growth factors, and extracellular matrix components (1). AKT3 phosphorylates and regulates the function of many cellular proteins involved in processes including cellular metabolism, survival/apoptosis, and proliferation. Recent evidence indicates that AKT3 is frequently overexpressed in many types of human cancers including breast and prostate (2).

1. Coffer, PJ. et al: Protein kinase B (c-Akt): a multifunctional mediator of phosphatidylinositol 3-kinase activation. Biochem J. 1998 Oct 1; 335 (Pt 1): 1-13.

2. Anderson, KE. et al: Translocation of PDK-1 to the plasma membrane is important in allowing PDK-1 to activate protein kinase B. Curr Biol. 1998 Jun 4;8(12): 684-91.

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.

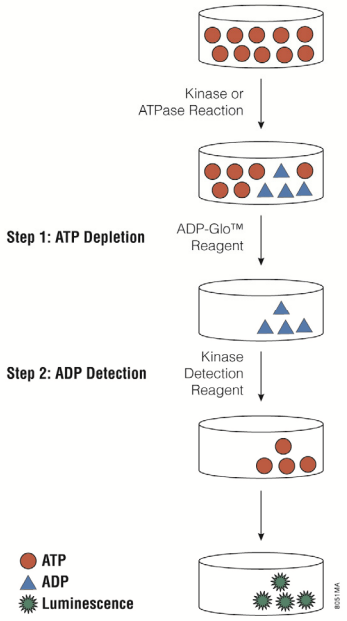

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

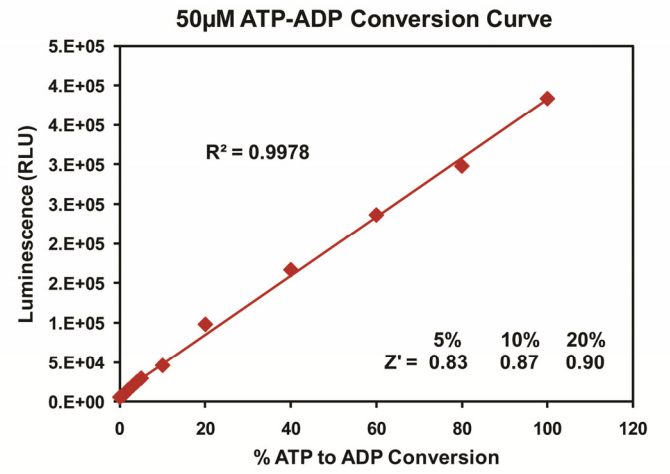

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 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. AKT3 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.

AKT3, ng	200	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0
RLU	315804	278996	255349	234719	171919	114126	48292	11259	4985	3380	2426
S/B	130	115	105	97	71	47	20	5	2.1	1.4	1
% Conversion	93	82	75	69	51	34	14	3	1.5	1.0	0

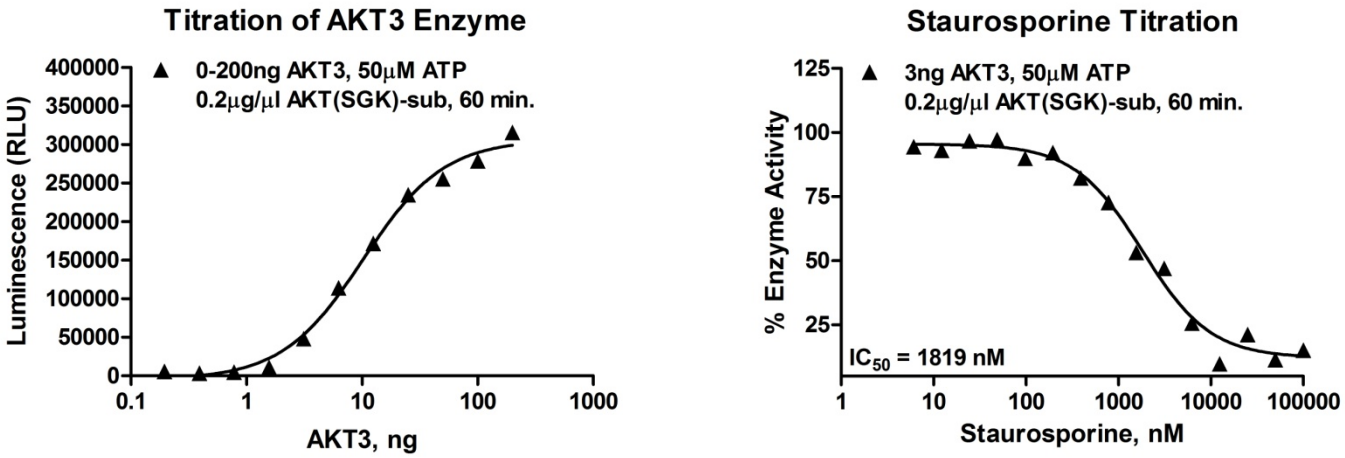

Figure 3. AKT3 Kinase Assay Development. (A) AKT3 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 3ng of AKT3 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
AKT3 Kinase Enzyme System	Promega	V4010
ADP-Glo™ + AKT3 Kinase Enzyme System	Promega	V4011

AKT3 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.