

# AKT1 Kinase Assay

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## Scientific Background:

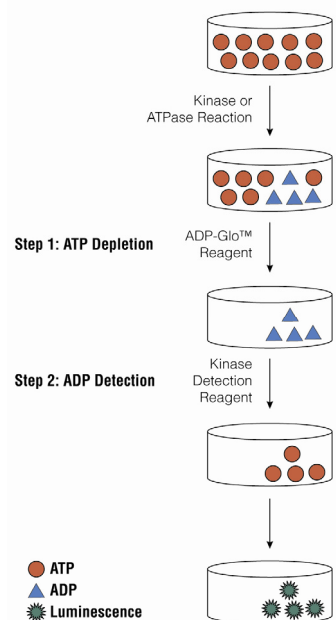
AKT1/PKB $\alpha$  is a serine/threonine kinase that belongs to the AKT family. AKT1 is activated in cells in response to diverse stimuli such as hormones, growth factors and extracellular matrix components and is involved in glucose metabolism, transcription, survival, cell proliferation, angiogenesis, and cell motility (1). AKT1 is frequently overexpressed and active in many types of human cancers including cancers of colon, breast, brain, pancreas and prostate as well as lymphomas and leukemias (2).

1. Coffey, P.J. 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, K.E. 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.

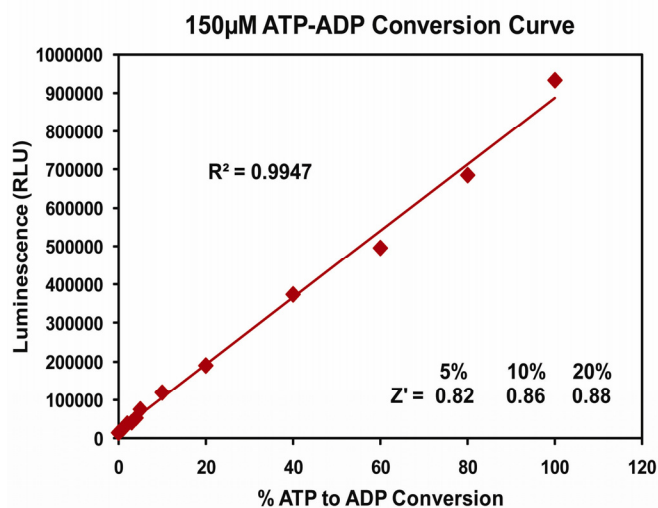
## ADP-Glo™ Kinase Assay

### 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 150 $\mu$ 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.



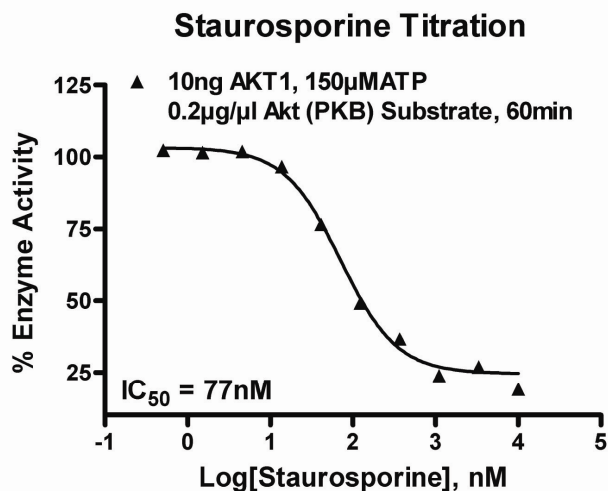
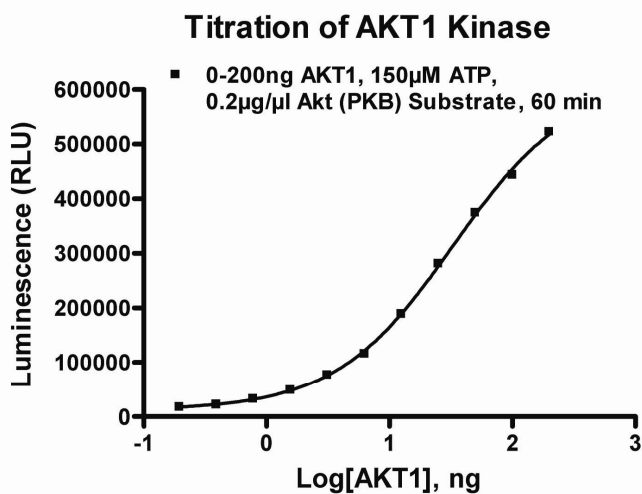
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay Technical Manual #TM313*, available at [www.promega.com/tbs/tm313/tm313.html](http://www.promega.com/tbs/tm313/tm313.html)

## 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. AKT1 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.

AKT1, ng	200	100	50	25	12.5	6.25	3.13	1.56	0.78	0
RLU	522881	443931	374382	281348	188524	115983	76465	48730	32434	13976
S/B	37.4	31.8	26.8	20.1	13.5	8.3	5.5	3.5	2.3	1.0
% Conversion	64.5	54.2	45.2	33.1	21.0	11.5	6.4	2.8	0.7	0.0



**Figure 3. AKT1 Kinase Assay Development:** (A) AKT1 enzyme was titrated using 150µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of AKT1 to determine the potency of the inhibitor ( $IC_{50}$ ).

### Assay Components and Ordering Information:



Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
AKT1 Kinase Enzyme System	Promega	V1911
ADP-Glo + AKT1 Kinase Enzyme System	Promega	V9061

AKT1 Kinase Buffer: 40mM Tris, 7.5; 20mM MgCl<sub>2</sub>; 0.1mg/ml BSA; 50µM DTT.