ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

BLK Kinase Assay

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Scientific Background:

BLK, also known as B lymphoid kinase, is a 55 kDa tyrosine kinase with SH3, SH2 and catalytic domains that contain consensus sequences of the src protein tyrosine kinase family. BLK is expressed specifically in the B cell lineage and plays a role in signal transduction pathway that is restricted to B lymphoid cells (1). Stimulation of resting B-lymphocytes with antibodies to surface immunoglobulin (sIgD or sIgM) induces activation of BLK (2)

1. Dymecki, SM. et al: Specific expression of a tyrosine kinase gene, blk, in B lymphoid cells. Science. 1990 Jan 19;247(4940):332-6.

2. Burkhardt, AL. et al: Anti-immunoglobulin stimulation of B lymphocytes activates src-related protein-tyrosine kinases. Proc Natl Acad Sci U S A. 1991 Aug 15;88(16):7410-4.

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.

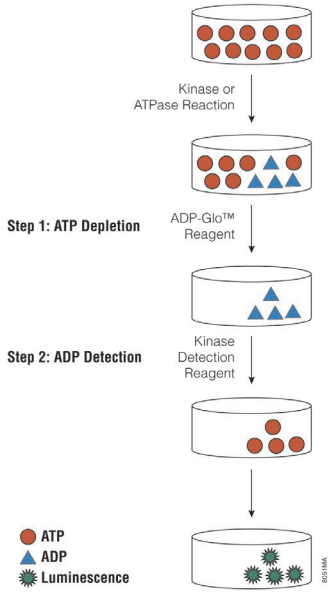

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

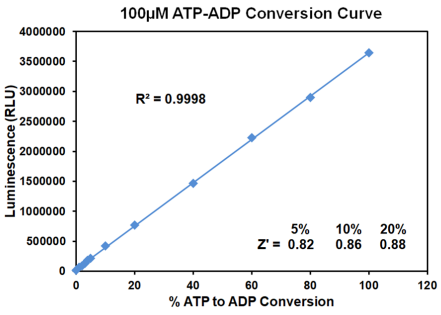

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction 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 indicated time (See Figure 3).
- 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 0.5-1 second).

Table 1. 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.

Enzyme, ng	240	120	60	30	15	7.50	3.75	1.88	0
Luminescence	1,911,390	775,061	473,013	325,519	190,615	107,350	56,995	36,302	9,726
S/B	197	80	49	33	20	11	6	4	1
% Conversion	53	21	12	8	5	2	1	0	0

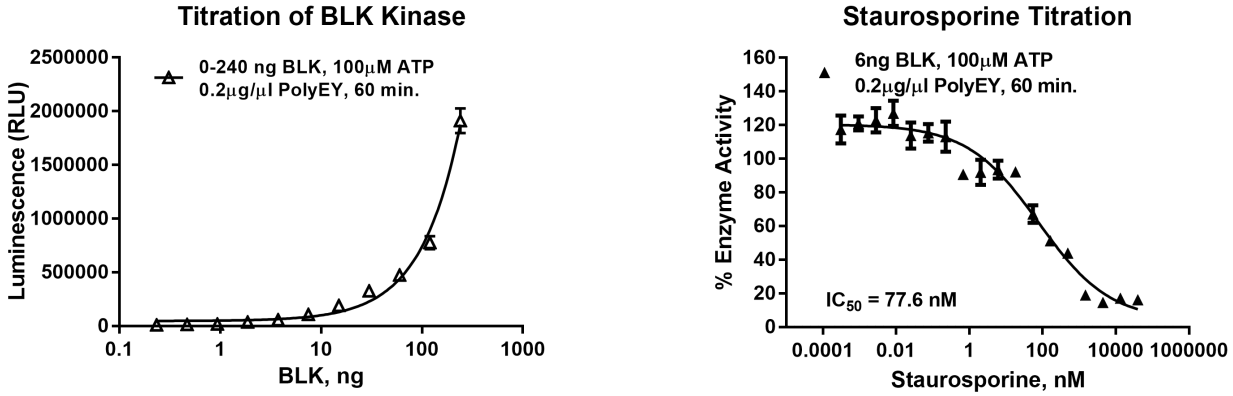

Figure 3. BLK Kinase Assay Development. (A) BLK enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 6ng of BLK to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
BLK Kinase Enzyme System	10µg	VA7384
	1mg	VA7385
ADP-Glo™ + BLK Kinase Enzyme System	1 Each	VA7386