ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: STK33

STK33 Kinase Assay

By Dongping Ma, M.S., Juliano Alves, Ph.D., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

STK33 is a distant member of the CAMK group of serine/threonine kinases but it lacks the calcium/calmodulin-binding domain and the C-terminal regulatory tail. STK33 is highly expressed in testis, lung epithelia, alveolar macrophages, horizontal cells in the retina and in embryonic organs such as heart, brain and spinal cord. STK33 is essential for abnormal cell growth in human cell lines expressing oncogenic mutations in KRAS, but not in human cancer cell lines expressing wildtype KRAS. STK33 is required for survival and proliferation of mutant KRAS-dependent cancer cells, in which it suppresses the S6K1-BAD proapoptotic signaling pathway.

1. Mujica A O, et al: A novel serine/threonine kinase gene, STK33, on human chromosome 11p15.3. Gene 280: 175-181, 2001.

2. Scholl C, et al: Synthetic lethal interaction between oncogenic KRAS dependency and STK33 suppression in human cancer cells. Cell 137: 821-834, 2009.

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.

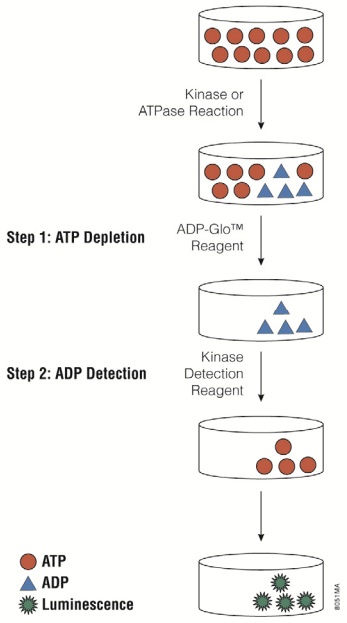

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

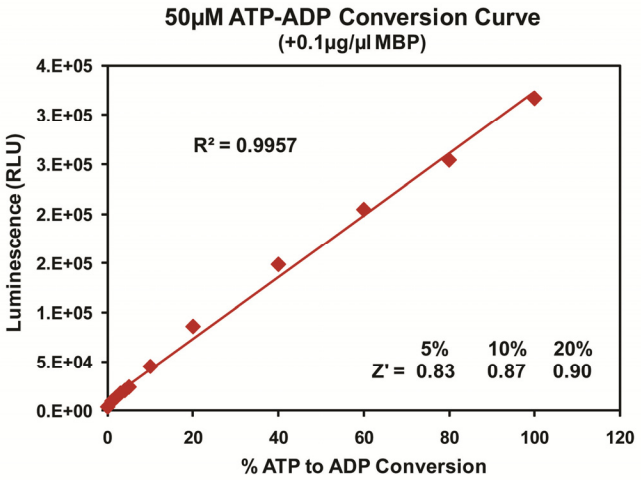

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 120 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. STK33 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.

STK33, ng	200	100	50	25	13	6.3	3.1	1.6	0
RLU	125216	67742	38151	20672	10113	5759	3558	2170	1195
S/B	148	80	45	24	12	7	4	3	1
% Conversion	45	24	13	7	3	2	1.02	0.52	0

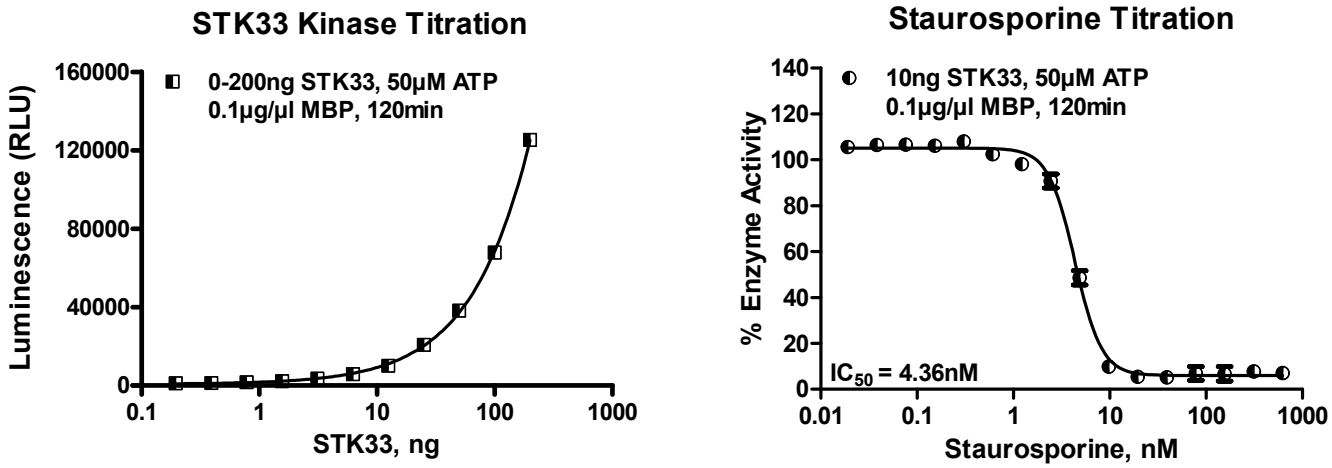

Figure 3. STK33 Kinase Assay Development. (A) STK33 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of STK33 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
STK33 Kinase Enzyme System	Promega	V4086
ADP-Glo™ + STK33 Kinase Enzyme System	Promega	V4087

STK33 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.