ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: PASK

PASK Kinase Assay

By Juliano Alves, Ph.D., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

PASK or PAS domain containing serine/threonine kinase regulates the function of many intracellular signaling pathways involved in stress. PASK is involved in sensing environmental changes in light intensity, oxygen concentration and redox potential. Through interaction with IRS-1, PASK has been proposed as a counter-regulatory mechanism in insulin and cytokine signaling (1). PASK can phosphorylate and inactivate glycogen synthase in vitro. Efficient phosphorylation requires residues 444 to 955 of PASK between the PAS and catalytic kinase domain and this interaction is inhibited by the PAS domain.

1. Nagase, T. et al: Prediction of the coding sequences of unidentified human genes. IV. The coding sequences of 40 new genes (KIAA0121-KIAA0160) deduced by analysis of cDNA clones from human cell line KG-1. DNA Res. 1996; 2 (4): 167–74, 199–210.

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.

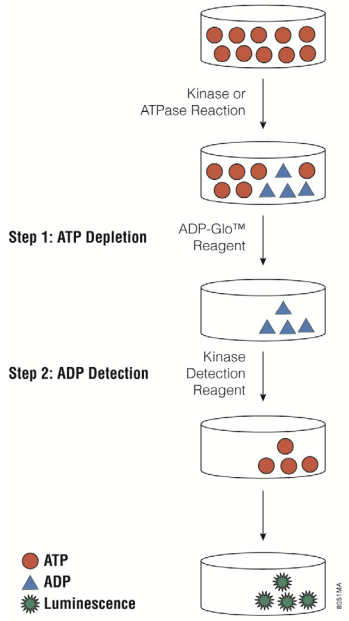

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

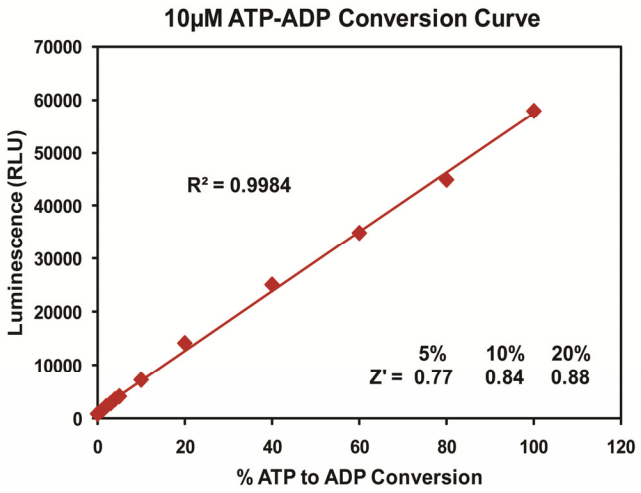

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. PASK 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.

PASK, ng	200	100	50	25	13	6.3	3.1	1.6	0.8	0.4	0
RLU	72583	55846	43188	27959	18313	11515	4882	3278	1626	1148	392
S/B	185	142	110	71	47	29	12	8	4	3	1
% Conversion	95	73	56	35	22	13	4	3.0	1.4	1.0	0

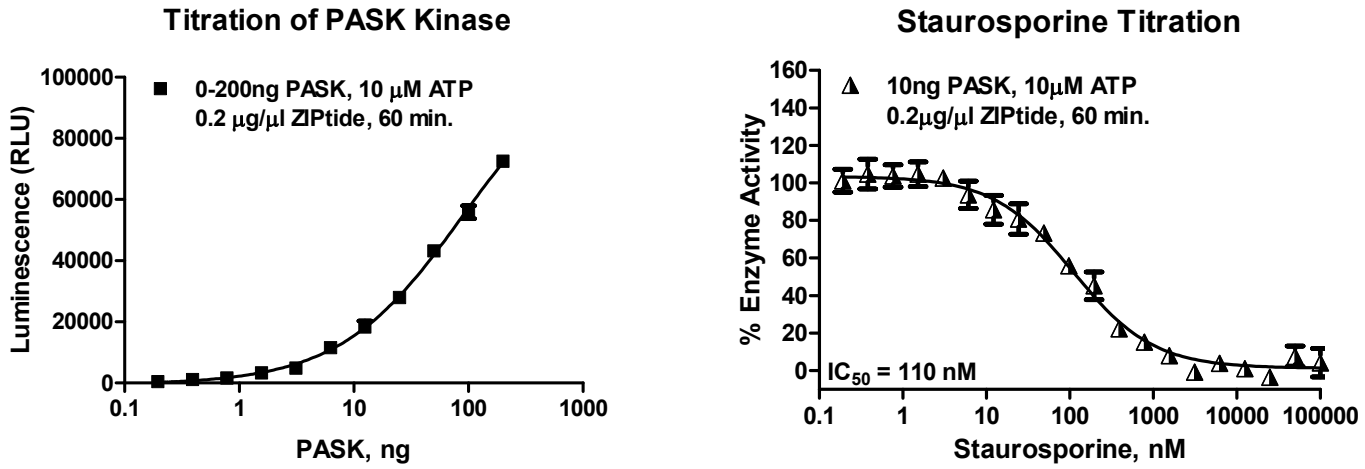

Figure 3. PASK Kinase Assay Development. (A) PASK enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of PASK to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PASK Kinase Enzyme System	Promega	V4240
ADP-Glo™ + PASK Kinase Enzyme System	Promega	V4241

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