ADP-Glo™ Kinase Assay Application Notes

TYROSINE KINASE SERIES: PYK2

PYK2 Kinase Assay

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

Scientific Background:

PYK2 (also known as FAK2/RAFTK) is a member of the focal adhesion PTK family. PYK2/FAK2 can be activated by a variety of extracellular signals that elevate intracellular calcium concentration, and by stress signals (1). Unlike FAK, which is widely expressed in various tissues and links transmembrane integrin receptors to intracellular pathways, PYK2/FAK2 is expressed mainly in the central nervous system and in cells derived from hematopoietic lineages. In osteoclasts, although FAK is expressed, PYK2/FAK2 appears to be the predominant mediator of integrin alpha(v)beta3 signaling events that influence osteoclast physiology and pathology (2).

1. Avraham, H. et al: RAFTK/Pyk2-mediated cellular signalling. Cell Signal. 2000 Mar;12(3):123-33.

2. Xiong, W C. et al: PYK2 and FAK in osteoclasts. Front Biosci. 2003 Sep 1;8:d1219-26.

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.

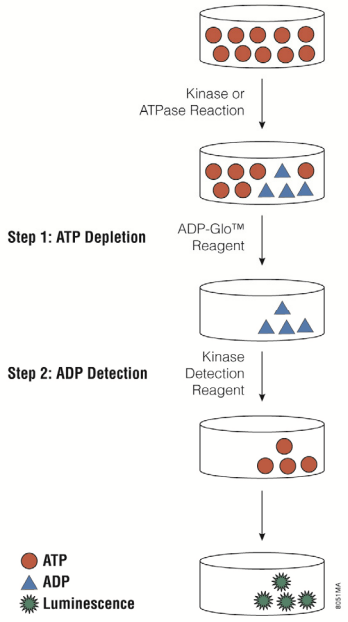

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

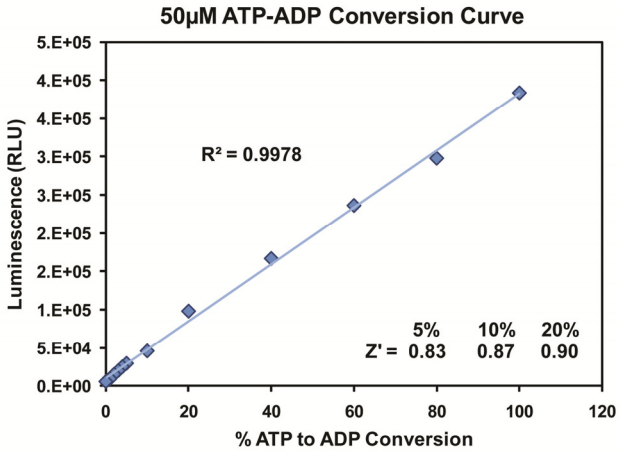

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 Tyrosine 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. PYK2 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.

PYK2, ng	200	100	50	25	13	6.3	3.1	1.6	0.8	0.4	0
Luminescence	89846	45108	31091	17562	11165	10481	8222	5642	3032	2328	624
S/B	144	72	50	28	18	17	13	9	5	4	1
% Conversion	35	18	12	7	5	4	3	2	1.4	1.2	0

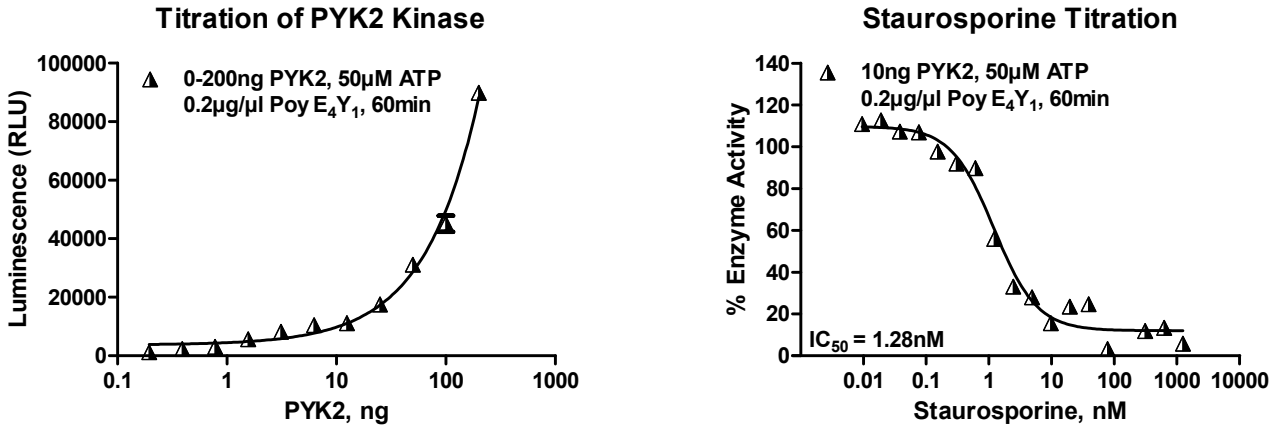

Figure 3. PYK2 Kinase Assay Development. **(A) PYK2 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of PYK2 to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PYK2 Kinase Enzyme System	Promega	V4082
ADP-Glo™ + PYK2 Kinase Enzyme System	Promega	V4083

PYK2 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 2.5mM $MnCl_2$; 50µM DTT.