GRK5 Kinase Assay

By Hicham Zegzouti, Ph.D., Mary Sobol, M.S., Jolanta Vidugiriene, Ph.D., and Said A. Goueli, Ph.D., Promega Corporation

Scientific Background:

GRK5 is a member of the guanine nucleotide-binding protein (G protein)-coupled receptor kinase subfamily of the Ser/Thr protein kinase family (1). It phosphorylates the activated forms of G protein-coupled receptors thus initiating their deactivation. GRK5 plays a role in regulating the motility of polymorphonuclear leukocytes (PMNs). Desensitization of G protein-coupled receptors regulates the number of polymorphonuclear leukocytes (PMNs), as well as their motility and ability to stop upon contact with pathogens or target cells, and this desensitization is mediated by GRK5 (2).

1. Haribabu, B. et al: Identification of additional members of human G-protein-coupled receptor kinase multigene family. Proc. Nat. Acad. Sci. 90: 9398-9402, 1993.

2. Fan, J. et al: Toll-like receptor-4 (TLR4) signaling augments chemokine-induced neutrophil migration by modulating cell surface expression of chemokine receptors. Nature Med. 9: 315-321, 2003.

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.

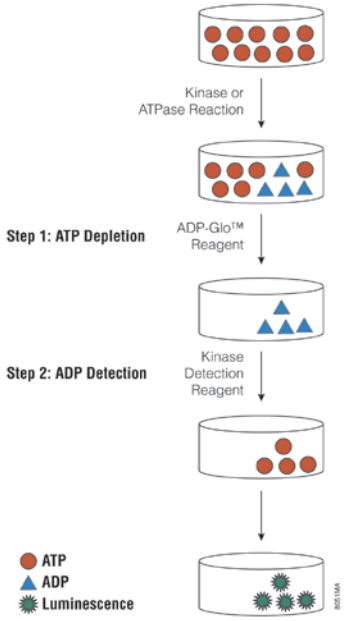

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

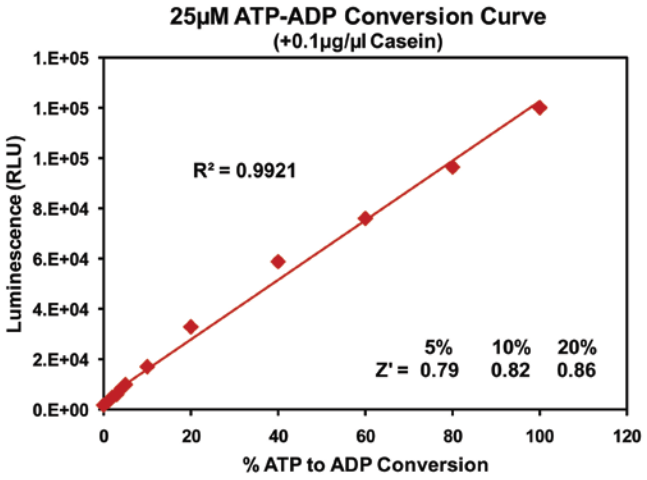

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. GRK5 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.

GRK5, ng	200	100	50	25	12.5	6.3	3.1	1.6	0
RLU	74353	24272	12672	7333	4702	3657	3510	3168	1956
S/B	38.0	12.4	6.5	3.7	2.4	1.9	1.8	1.6	1
% Conversion	40.0	16.8	7.2	3.6	2.5	1.2	1.1	0.9	0

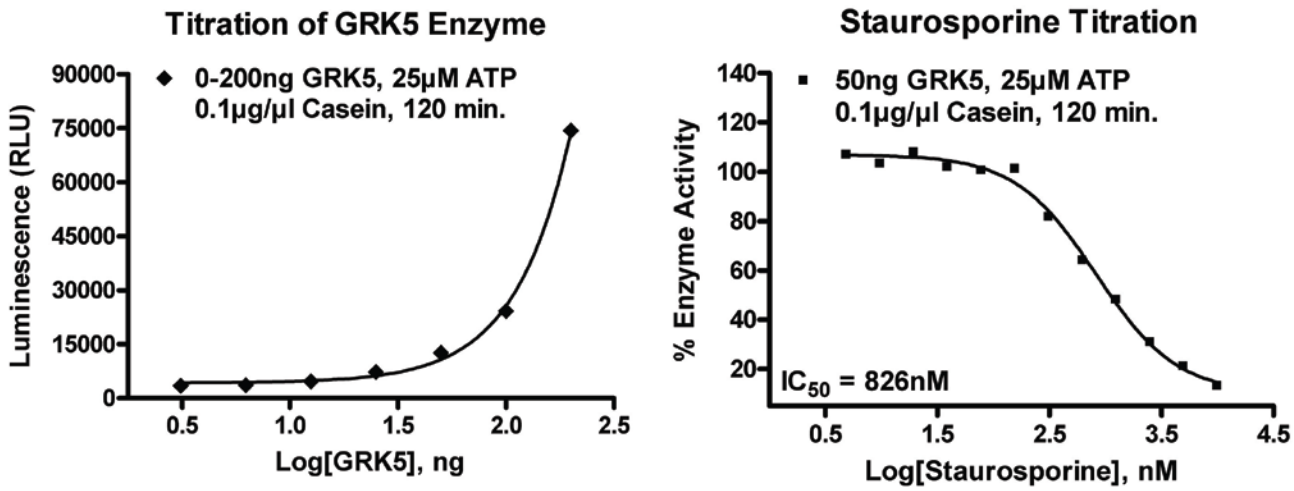

Figure 3. GRK5 Kinase Assay Development. (A) GRK5 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 50ng of GRK5 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
GRK5 Kinase Enzyme System	Promega	V3981
ADP-Glo™ + GRK5 Kinase Enzyme System	Promega	V9351

GRK5 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

Promega Corporation • 2800 Woods Hollow Road • Madison, WI 53711-5399 USA • Telephone 608-274-4330 • Fax 608-277-2601
promega.com