ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: MAPKAPK5

MAPKAPK5 Kinase Assay

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Scientific Background:

MAPKAPK5 is a member of the serine/threonine kinase family that responds to cellular stress and proinflammatory cytokines. MAPKAPK5 is activated through its phosphorylation by MAP kinases including MAPK1/ERK, MAPK14/p38-alpha, and MAPK11/p38-beta (1). MAPKAPK5 is activated in HeLa cells in response to cellular stress and proinflammatory cytokines. MAPKAPK5 activity is regulated by p38-alpha and p38-beta both in vitro and in vivo, and thr-182 is the regulatory phosphorylation site of MAPKAPK5 (2). In vitro, MAPKAPK5 kinase phosphorylates heat shock protein HSP27 at its physiologically relevant sites.

1. New, L.; Jiang, Y. et al: PRAK, a novel protein kinase regulated by the p38 MAP kinase. EMBO J. 17: 3372-3384, 1998.

2. Ni, H.; Wang. et al: MAPKAPK5, a novel mitogen-activated protein kinase (MAPK)-activated protein kinase, is a substrate of the extracellular-regulated kinase (ERK) and p38 kinase. Biochem. Biophys. Res. Commun. 243: 492-496, 1998.

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.

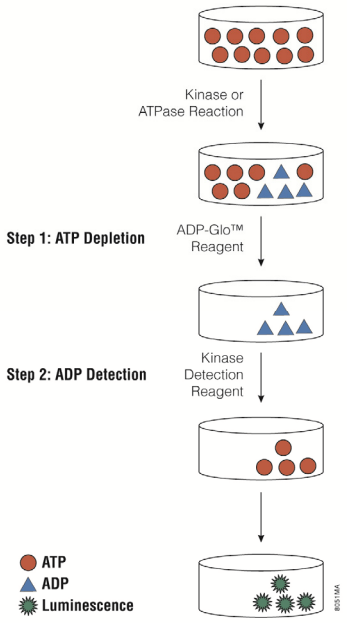

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

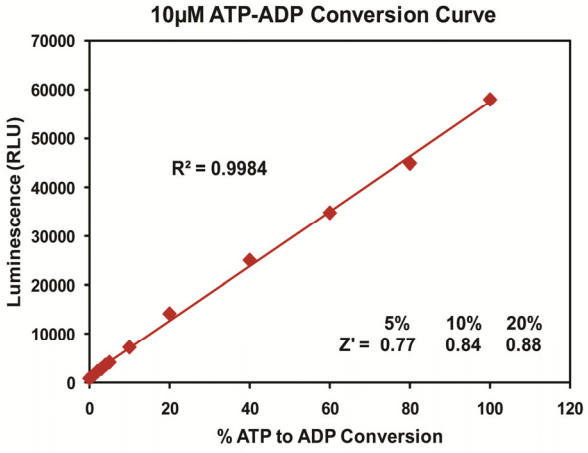

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. MAPKAPK5 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.

MAPKAPK5, ng	200	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0
RLU	69779	61228	63527	52594	37396	20818	8853	3644	1370	560	266
S/B	262	230	239	198	141	78	33	14	5	2	1
% Conversion	92	80	83	68	48	26	9	3	1.1	0.3	0

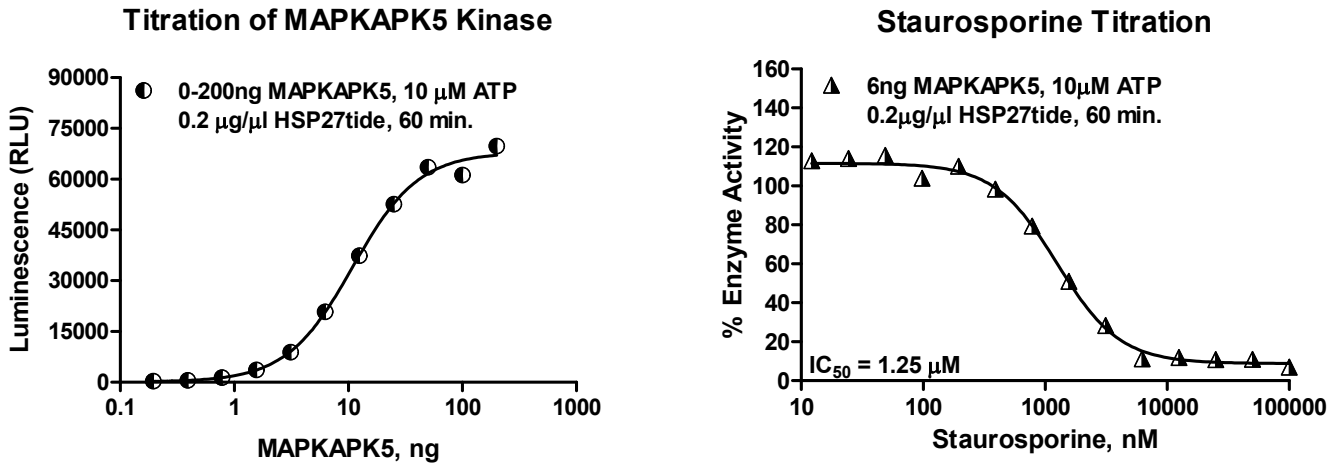

Figure 3. MAPKAPK5 Kinase Assay Development. **(A) MAPKAPK5 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 6ng of MAPKAPK5 to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
MAPKAPK5 Kinase Enzyme System	Promega	V4166
ADP-Glo™ + MAPKAPK5 Kinase Enzyme System	Promega	V4167

MAPKAPK5 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.