

p38 α Kinase Assay

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Scientific Background:

p38 α (SAPK2A) is a member of the p38 MAPK family which are activated by various environmental stresses and proinflammatory cytokines (1). The activation of p38 requires its phosphorylation by MAP kinase kinases (MKKs), or its autophosphorylation triggered by the interaction of MAP3K7IP1/TAB1 protein with this kinase (2). The substrates of p38 include transcription regulator ATF2, MEF2C, and MAX, cell cycle regulator CDC25B, and tumor suppressor p53, which suggest the roles of this kinase in stress related transcription and cell cycle regulation, as well as in genotoxic stress response (5).

1. Han, J. et al: A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* 265: 808-811, 1994.
2. Ge, B. et al: MAPKK-independent activation of p38-alpha mediated by TAB1-dependent autophosphorylation of p38-alpha. *Science* 295: 1291-1294, 2002.

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.

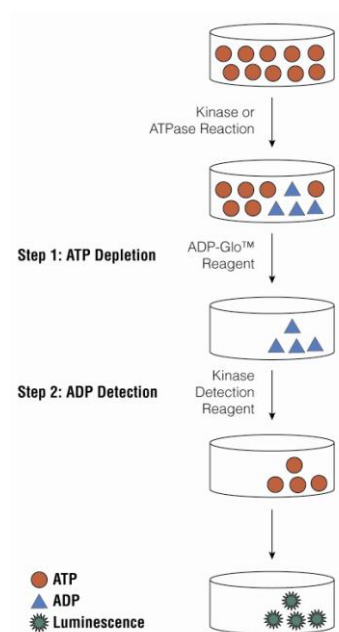


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

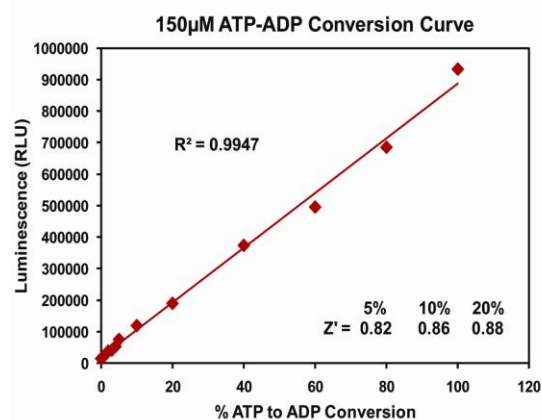


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 150 μ M ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



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 μ l of inhibitor or (5% DMSO)
 - 2 μ l of enzyme (defined from table 1)
 - 2 μ l of substrate/ATP mix
- Incubate at room temperature for 60 minutes.
- Add 5 μ l of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10 μ l of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1second).

Table 1. p38 α 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.

P38 α , ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0
RLU	692806	617694	482472	376125	261190	157428	94726	49838	29893	8477
S/B	82	73	57	44	31	19	11	6	4	1
% Conversion	79	70	54	42	28	16	9	3	2	0

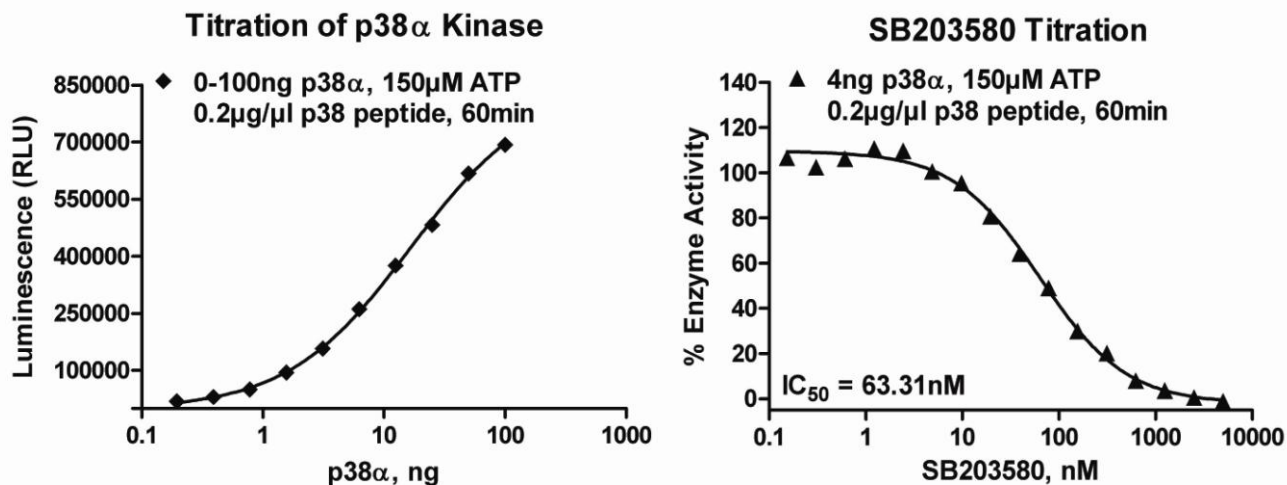


Figure 3. p38 α Kinase Assay Development. (A) p38 α enzyme was titrated using 150 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) SB203580 dose response was created using 4ng of p38 α to determine the potency of the inhibitor (IC₅₀).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
p38 α Kinase Enzyme System	Promega	V2701
ADP-Glo™ + p38 α Kinase Enzyme System	Promega	V9591

p38 α Kinase Buffer: 40mM Tris,7.5; 20mM MgCl₂; 0.1mg/ml BSA; 50 μ M DTT.

