ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: p38β

p38β Kinase Assay

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Scientific Background:

p38β is a member of the p38 MAP kinase family and is activated by both proinflammatory cytokines and environmental stress (1). The p38β is activated through its phosphorylation by MAP kinase kinases (MKKs), preferably by MKK6. Transcription factor ATF2/CREB2 has been shown to be a substrate of this kinase (2). Alternatively spliced transcript variants encoding the same protein have been observed.

1. Jiang, Y. et al: Characterization of the structure and function of a new mitogen-activated protein kinase (p38-beta). J. Biol. Chem. 271: 17920-17926, 1996.

2. Stein, B. et al: p38-2, a novel mitogen-activated protein kinase with distinct properties. J. Biol. Chem. 272: 19509-19517, 1997.

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.

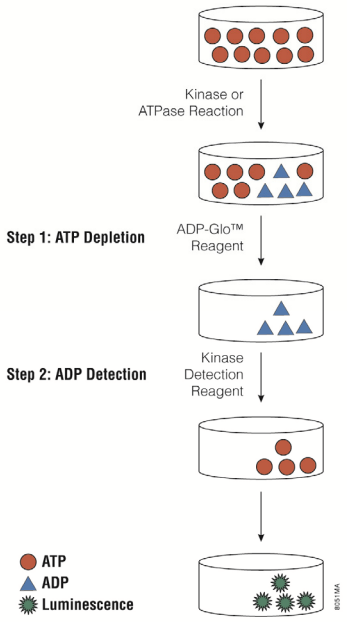


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

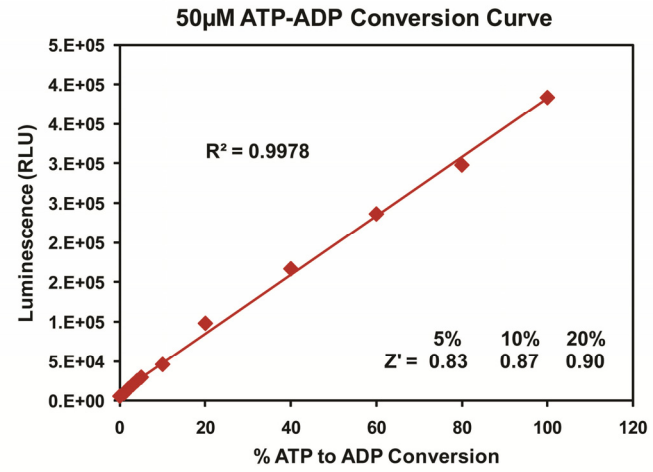


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

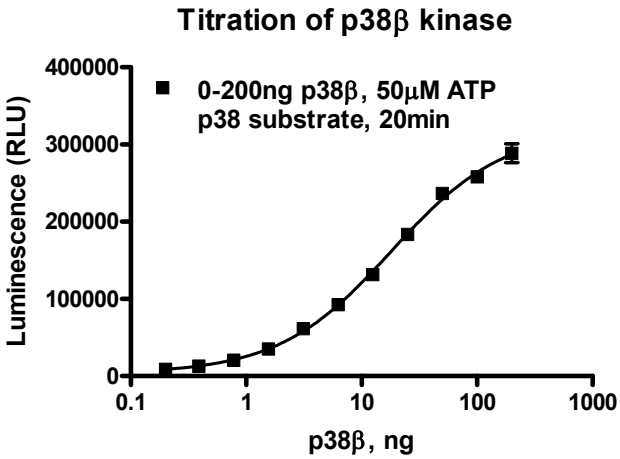
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 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 20 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. p38β 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.

p38β, ng	100	50	25	13	6.3	3.1	1.6	0.8	0.4	0.2	0
RLU	258259	236338	183518	131543	92662	61414	35021	20446	12738	8826	810
S/B	319	292	227	162	114	76	43	25	16	11	1
% Conversion	99	91	70	51	36	24	14	8	5	4	0



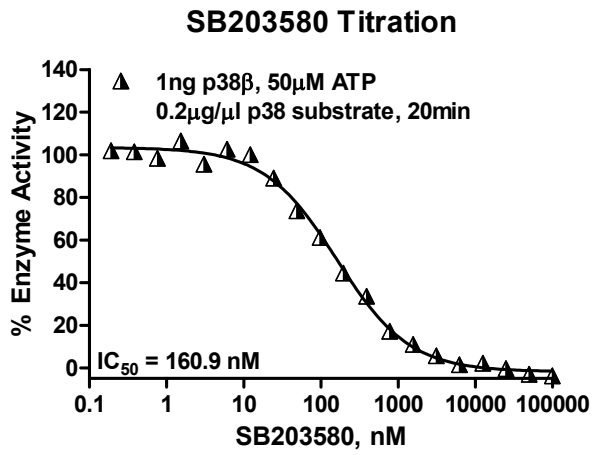


Figure 3. p38β Kinase Assay Development. **(A) p38β enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) SB203580 inhibitor dose response was created using 1ng of p38β to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
p38β Kinase Enzyme System	Promega	V4154
ADP-Glo™ + p38β Kinase Enzyme System	Promega	V4155

p38β Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.