ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: ZAK

# ZAK Kinase Assay

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## Scientific Background:

ZAK is a member of the MAPKKK family of signal transduction molecules and mediates gamma radiation signaling leading to cell cycle arrest. The activity of ZAK plays a role in cell cycle checkpoint regulation as well as being involved in regulating actin organization (1). Expression of kinase-dead ZAK in mouse fibroblasts disrupts actin stress fibers and causes morphologic changes. ZAK can activate JNK through MKK4/MKK7 and ERK5/p38-gamma via MKK3/MKK6. Expression of ZAK increases the population of cells in the G2/M phase of the cell cycle, whereas dominant-negative ZAK attenuated the G2 arrest caused by gamma radiation (2).

1. Yang J-J, et al: Mixed lineage kinase ZAK utilizing MKK7 and not MKK4 to activate the c-Jun N-terminal kinase and playing a role in the cell arrest. Biochem. Biophys. Res. Commun. 297: 105-110, 2002.

2. Gross E. A, et al: MRK, a mixed lineage kinase-related molecule that plays a role in gamma-radiation-induced cell cycle arrest. J. Biol. Chem. 277: 13873-13882, 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.

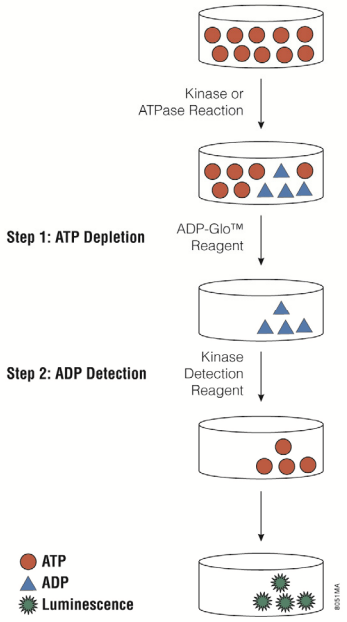


**Figure 1. Principle of the ADP-Glo™ Kinase Assay.** The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

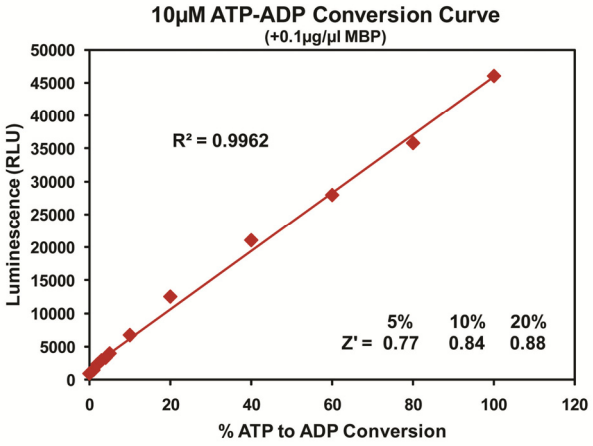


**Figure 2. Linearity of the ADP-Glo Kinase Assay.** ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 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. ZAK 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.

| ZAK, ng | 100 | 50 | 25 | 13 | 6.3 | 3.1 | 1.6 | 0.8 | 0.4 | 0.2 | 0 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| RLU | 74104 | 73035 | 63145 | 49063 | 33371 | 20905 | 11462 | 6536 | 3709 | 2323 | 558 |
| S/B | 133 | 131 | 113 | 88 | 60 | 37 | 21 | 12 | 7 | 4 | 1 |
| % Conversion | 89 | 87 | 75 | 58 | 39 | 24 | 13 | 7 | 3 | 1 | 0 |

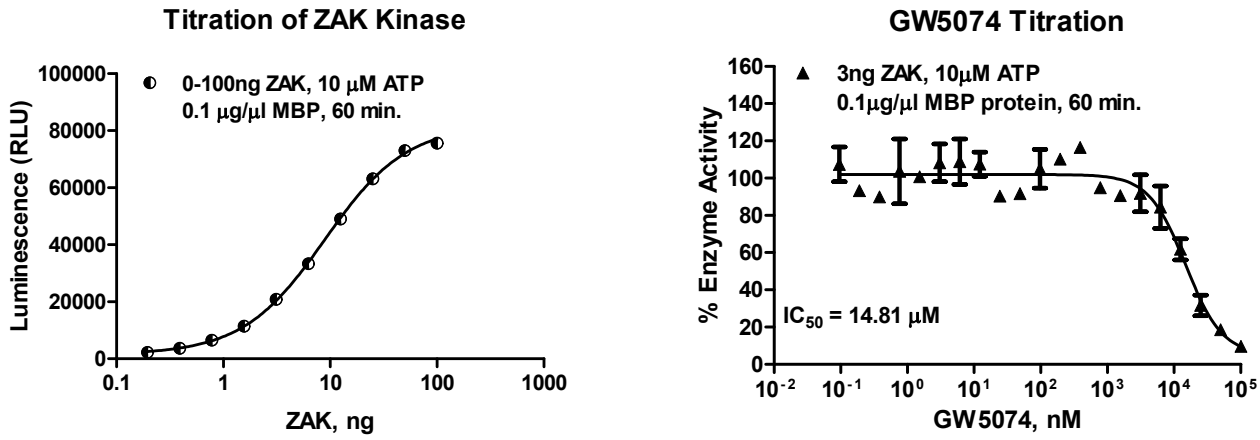


**Figure 3. ZAK Kinase Assay Development.** (A) ZAK enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) GW5074 inhibitor dose response was created using 3ng of ZAK to determine the potency of the inhibitor ($IC_{50}$).

**Assay Components and Ordering Information:**

**Products**

| | Company | Cat.# |
|---|---|---|
| ADP-Glo™ Kinase Assay | Promega | V9101 |
| ZAK Kinase Enzyme System | Promega | V4244 |
| ADP-Glo™ + ZAK Kinase Enzyme System | Promega | V4245 |

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

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