ERK2 Kinase Assay

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

Scientific Background:

ERK2 is a protein serine/threonine kinase that is a member of the extracellular signal-regulated kinases (ERKs) which are activated in response to numerous growth factors and cytokines (1). Activation of ERK2 requires both tyrosine and threonine phosphorylation that is mediated by MEK. ERK2 is ubiquitously distributed in tissues with the highest expression in heart, brain and spinal cord. Activated ERK2 translocates into the nucleus where it phosphorylates various transcription factors (e.g., Elk-1, c-Myc, c-Jun, c-Fos, and C/EBP beta).

1. Boulton, TG. et al: Purification and properties of extracellular signal-regulated kinase 1, an insulin-stimulated microtubule-associated protein 2 kinase. Biochemistry. 1991 Jan 8;30(1):278-86.

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.

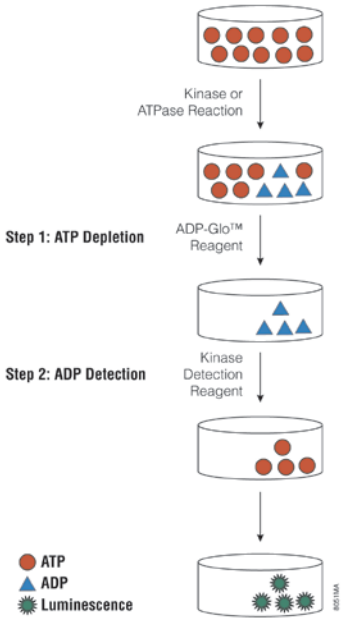

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

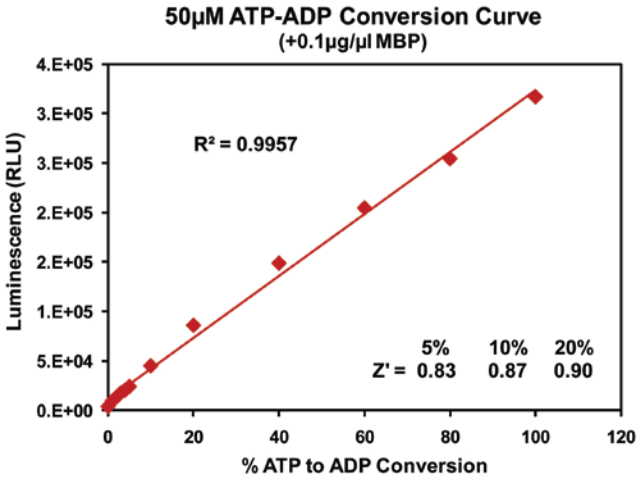

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. ERK2 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.

ERK2, ng	200	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0
RLU	335225	229081	167222	120293	92968	72310	58141	43727	33689	23740	4383
S/B	76	52.3	38.2	27.4	21.2	16.5	13.3	10.0	7.7	5.4	1
% Conversion	104	69.9	50.1	35.1	26.4	19.8	15.2	10.6	7.4	4.2	0

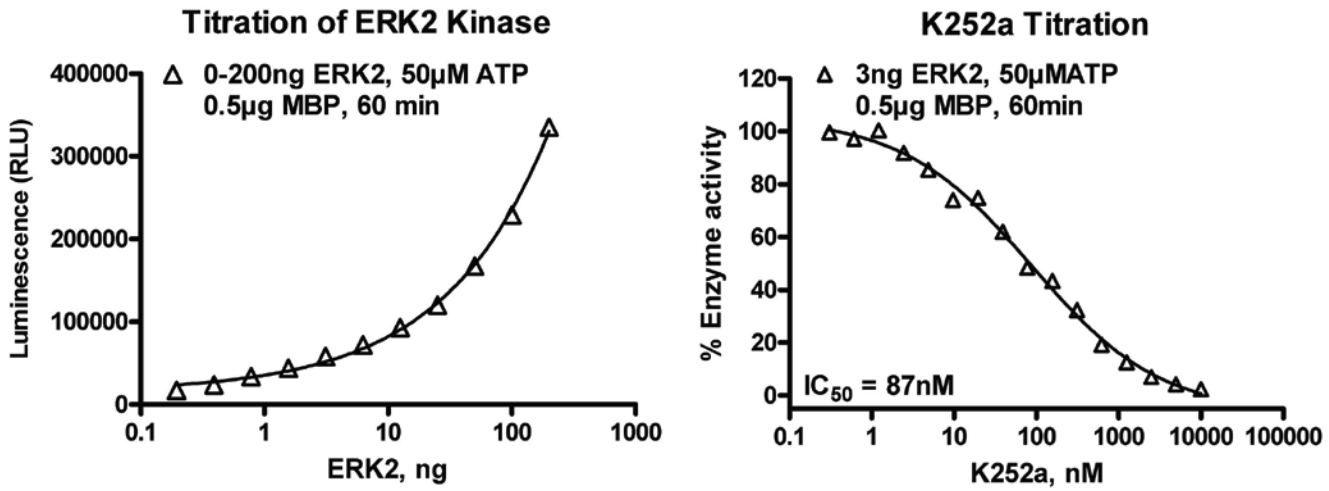

Figure 3. ERK2 Kinase Assay Development. (A) ERK2 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) K252a dose response was created using 3ng of ERK2 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
ERK2 Kinase Enzyme System	Promega	V1961
ADP-Glo™ + ERK2 Kinase Enzyme System	Promega	V9291

ERK2 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.