

IKK α Kinase Assay

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Scientific Background:

IKK α is a serine/threonine protein kinase that phosphorylates the I-kappa-B protein which is an inhibitor of the transcription factor NF-kappa-B complex. Phosphorylation of I-kappa-B protein triggers the degradation of the inhibitor via the ubiquitination pathway, thereby activating NF-kappa-B complex (1). IKK α is an essential regulator of NF-kappa-B-dependent gene expression through control of promoter-associated histone phosphorylation after cytokine exposure (2). IKK α is a critical component of the cytoplasmic transductional-transcriptional processor leading to induction of IFN α production. IKK α is also involved in the epidermis where it antagonizes mitogenic and angiogenic signals and represses tumor progression and metastases.

1. Anest, V. et.al: A nucleosomal function for I-kappa-B kinase-alpha in NF-kappa-B-dependent gene expression. *Nature* 423: 659-663, 2003.
2. Hoshino, K. et.al: I-kappa-B kinase-alpha is critical for interferon-alpha production induced by Toll-like receptors 7 and 9. *Nature* 440: 949-953, 2006.

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.

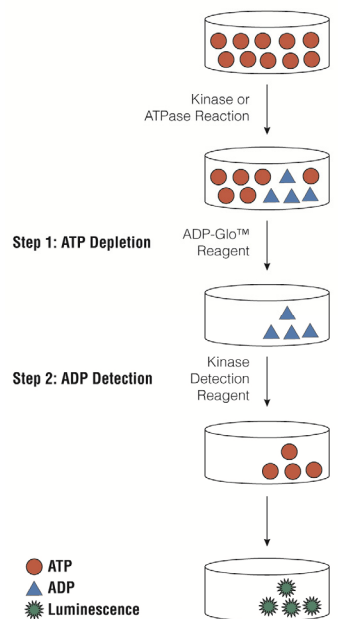


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

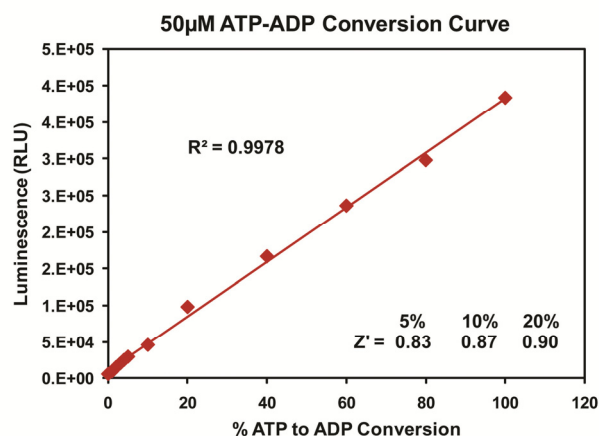
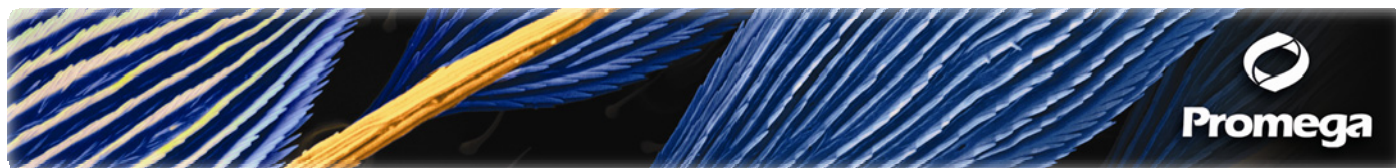


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50 μ M ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



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 120 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. IKK 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.

IKK α , ng	200	100	50	25	13	6.3	3.1	0
RLU	104769	53440	23687	13966	7440	4339	2364	912
S/B	115	59	26	15	8	5	3	1
% Conversion	34	17	7	4	2	1	0.25	0

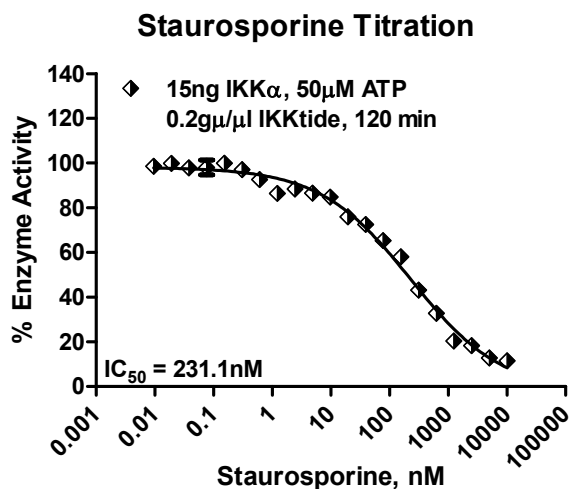
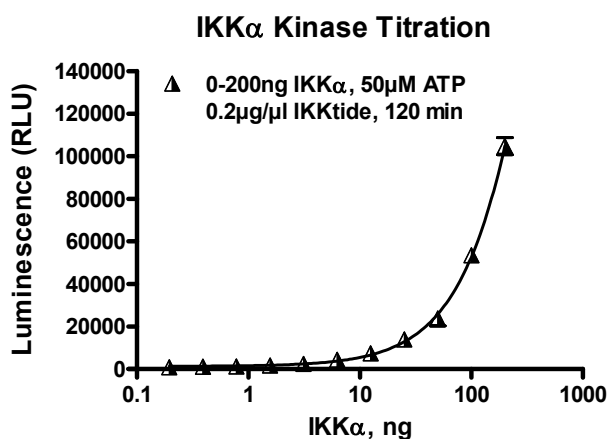


Figure 3. IKK α Kinase Assay Development. (A) IKK α enzyme was titrated using 50 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 15ng of IKK α to determine the potency of the inhibitor (IC₅₀).

Assay Components and Ordering Information:		
Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
IKK α Kinase Enzyme System	Promega	V4068
ADP-Glo™ + IKK α Kinase Enzyme System	Promega	V4069

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