IKKβ Kinase Assay

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Scientific Background:

IKKbeta (IKKβ) 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. The activity of IKKβ is stimulated by TNF and IL1 and IKKβ forms a heterodimer that interacts with NIK (1). Overexpression of catalytically inactive IKKβ blocks cytokine-induced NF-kappa-B activation. Aspirin and sodium salicylate can specifically inhibit IKKβ activity in vitro and in vivo by binding to IKKβ to reduce ATP binding (2).

1. Woronicz, J D. et al: IkappaB kinase-beta: NF-kappa-B activation and complex formation with IkappaB kinase-alpha and NIK. Science 278: 866-869, 1997.

2. Yin, M.-J. et al: The anti-inflammatory agents aspirin and salicylate inhibit the activity of I-kappa-B kinase-beta. Nature 396: 77-80, 1998.

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.

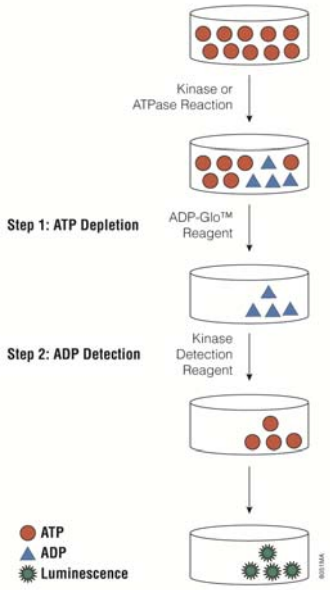


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

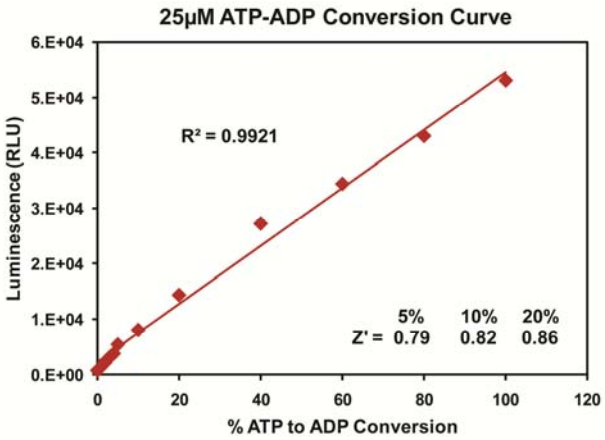


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

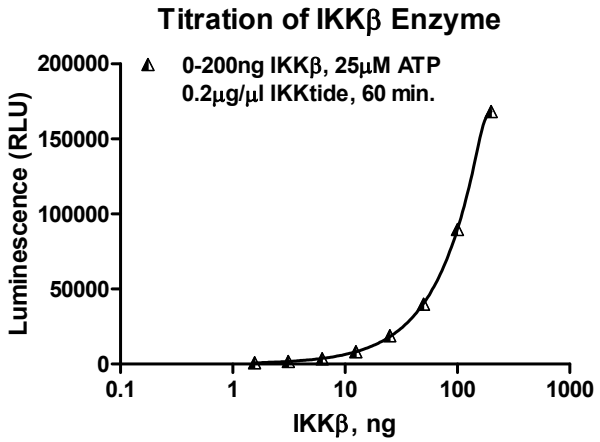
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, and the KES Protocol available at: http://www.promega.com/tbs/tm313/tm313.html, and http://www.promega.com/KESProtocol, respectively.

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 0.5-1sec).

Table 1. IKKβ 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.

IKKβ, ng	200	100	50	25	12.5	6.3	3.1	0
RLU	168316	89715	39842	18993	8188	3569	1788	669
S/B	252	134	60	28	12	5	3	1
% Conversion	69	37	16	7	3	1	0.4	0



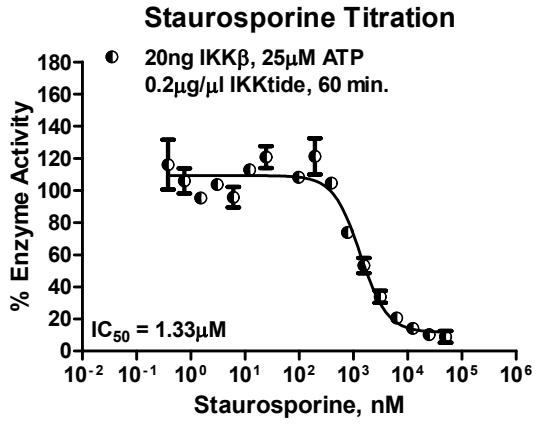


Figure 3. IKKβ Kinase Assay Development. **(A) IKKβ enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 20ng of IKKβ to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
IKKβ Kinase Enzyme System	Promega	V4502
ADP-Glo™ + IKKβ Kinase Enzyme System	Promega	V4503

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