ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: NIK

NIK Kinase Assay

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Scientific Background:

NIK is a mitogen-activated protein kinase kinase kinase 14 (MAP3K14), which binds to TRAF2 and stimulates NF-kappaB activity. NIK shares sequence similarity with several other MAPKK kinases and participates in NF-kappaB-inducing signalling cascade common to receptors of the tumour-necrosis/nerve-growth factor (TNF/NGF) family and to the interleukin-1 type-I receptor. (1) NIK is expressed in primary human cells and in inflamed rheumatoid arthritis tissue and plays a selective role in signaling by the lymphotoxin-beta receptor (2). NIK is a therapeutic target in the immune and bone-destructive components of inflammatory arthritis.

1. Smith, C. et.al: NF-kappa-B-inducing kinase is dispensable for activation of NF-kappa-B in inflammatory settings but essential for lymphotoxin beta receptor activation of NF-kappa-B in primary human fibroblasts. J. Immun. 167: 5895-5903, 2001.

2. Yin, L. et.al: Defective lymphotoxin-beta receptor-induced NF-kappa-B transcriptional activity in NIK-deficient mice. Science 291: 2162-2165, 2001.

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.

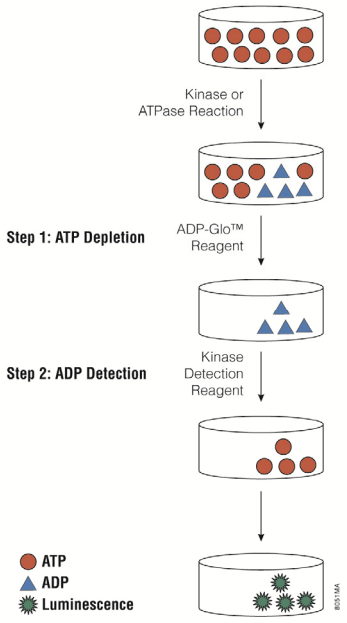

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

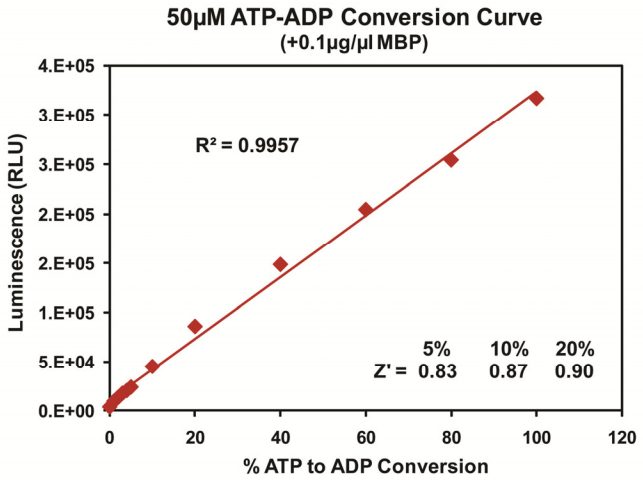

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. NIK 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.

NIK, ng	200	100	50	25	13	6.3	3.1	1.6	0.8	0
RLU	277885	209640	142090	81911	42126	19388	8602	4413	2474	1078
S/B	258	194	132	76	39	18	8	4	2	1
% Conversion	83	63	42	24	12	6	2.3	1.1	0.5	0

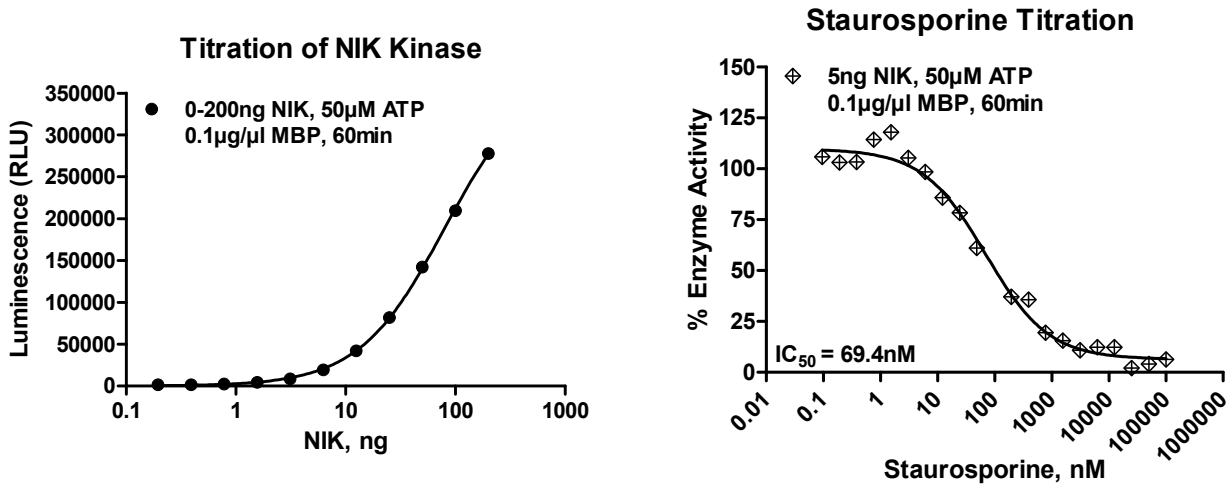

Figure 3. NIK Kinase Assay Development. **(A) NIK enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staursoporine dose response was created using 5ng of NIK to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
NIK Kinase Enzyme System	Promega	V4076
ADP-Glo™ + NIK Kinase Enzyme System	Promega	V4077

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