ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

IKKε Kinase Assay

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Scientific Background:

IKK epsilon (IKKε) is a noncanonical I-kappa-B kinase (IKK) that is essential for regulating antiviral signaling pathways that has also been identified as a breast cancer oncogene and is amplified and overexpressed in over 30% of breast carcinomas and breast cancer cell lines (1). IKKε is a part of a novel PMA-inducible I-kappa-B kinase complex which play an essential role for an in triggering the host antiviral response to viral infection. IKKε plays a critical role in the IFN-inducible antiviral transcriptional response (2).

1. Hutti, J. E.et.al: Phosphorylation of the tumor suppressor CYLD by the breast cancer oncogene IKK-epsilon promotes cell transformation. Molec. Cell 34: 461-472, 2009.

2. tenOever, B. R. et.al: Multiple functions of the IKK-related kinase IKK-epsilon in interferon-mediated antiviral immunity. Science 315: 1274-1278, 2007.

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.

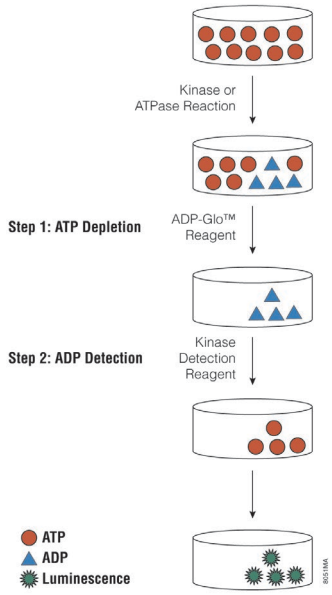

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

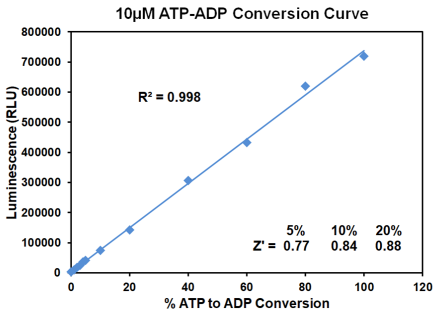

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction 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 indicated time (See Figure 3).
- 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 0.5-1 second).

Table 1. 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.

Enzyme, ng	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0.59	0.29	0.15	0
Luminescence	341,805	316,362	315,171	272,556	172,933	80,034	36,936	18,508	9,484	5,210	3,984	1,981
S/B	173	160	159	138	87	40	19	9	5	3	2	1
% Conversion	81	75	75	65	41	19	8	4	2	1	1	0

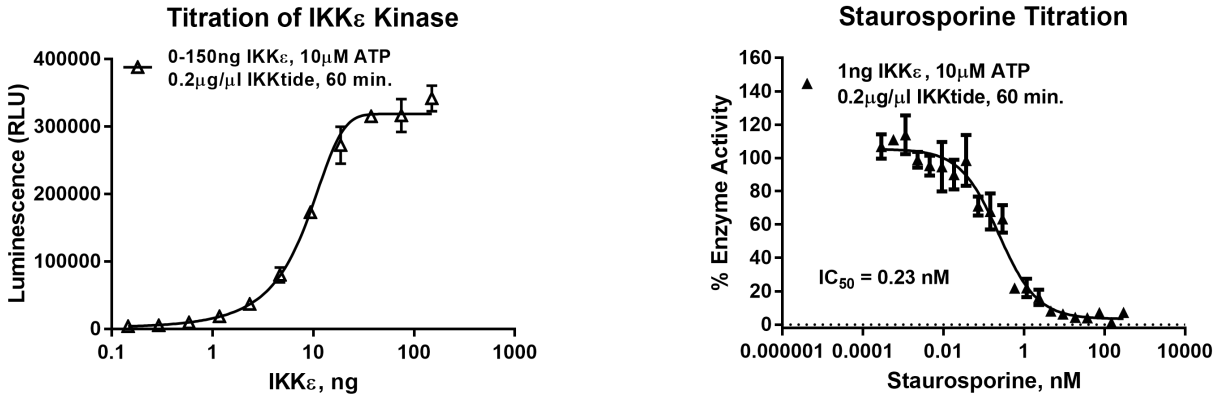

Figure 3. IKKε Kinase Assay Development. (A) IKKε enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1ng of IKKε to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
IKKε Kinase Enzyme System	10µg	VA7201
	1mg	VA7202
ADP-Glo™ + IKKε Kinase Enzyme System	1 Each	VA7203