ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

TNK1 Kinase Assay

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Scientific Background:

TNK1 or tyrosine kinase, non-receptor 1 belongs to the tyrosine protein kinase family which are important regulators of intracellular signal transduction pathways mediating cellular proliferation, survival, and development. TNK1 is highly expressed in fetal tissues and at lower levels in few adult tissues. TNK1 may function in signaling pathways utilized broadly during fetal development, and more selectively in adult tissues. TNK1 plays a negative regulatory role in the Ras-Raf1-MAPK pathway, and knockout mice have been shown to develop spontaneous tumors, suggesting a role as a tumor suppressor gene (1).

1. Hoehn, G. T. et.al: Tnk1: a novel intracellular tyrosine kinase gene isolated from human umbilical cord blood CD34(+)/Lin(-)/CD38(+) stem/progenitor cells. Oncogene 12: 903-913, 1996.

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.

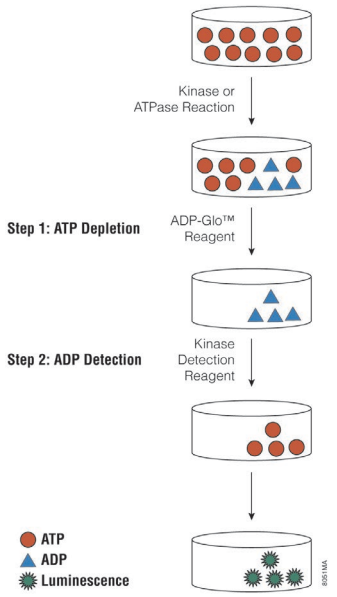

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

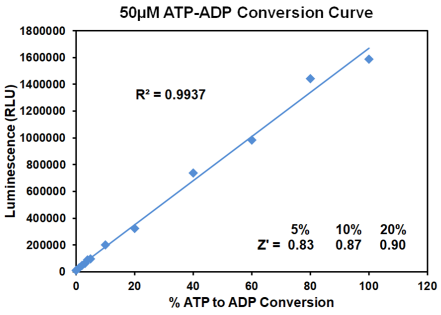

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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
Luminescence	1,900,490	1,346,470	884,238	552,429	298,828	145,436	79,622	38,597	21,637	5,868
S/B	324	229	151	94	51	25	14	7	4	1
% Conversion	111	78	51	32	17	8	4	1	0	0

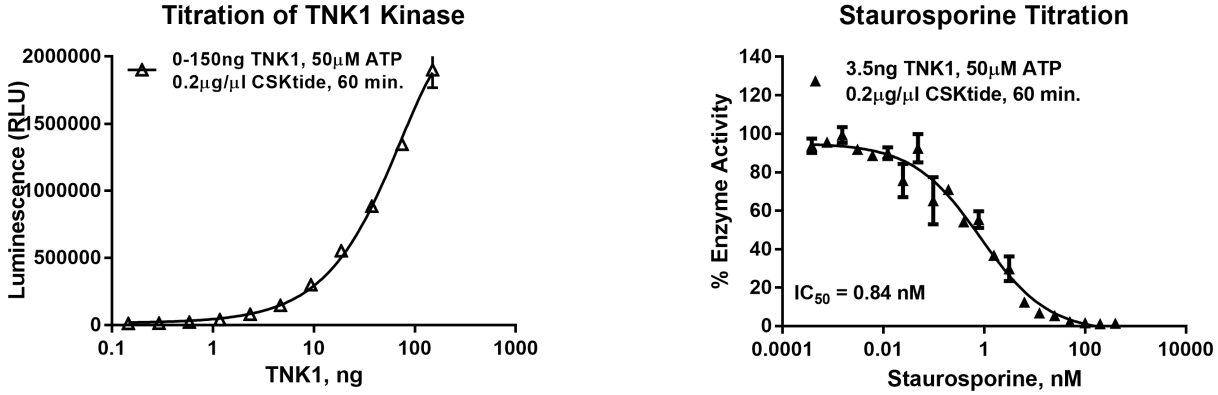

Figure 3. TNK1 Kinase Assay Development. (A) TNK1 enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 3.5ng of TNK1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
TNK1 Kinase Enzyme System	10µg	VA7321
	1mg	VA7322
ADP-Glo™ + TNK1 Kinase Enzyme System	1 Each	VA7323