ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

HCK Kinase Assay

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Scientific Background:

HCK, a protein-tyrosine kinase belonging to the Src family, is expressed in certain hemopoietic cells and especially prominent in cells of myeloid lineage, particularly mature granulocytes and monocytes (1). HCK gene is located on chromosome sequence 20q11-q12, a region that is affected by interstitial deletions in some acute myeloid leukemias and myeloproliferative disorders suggesting damage to HCK may contribute to the pathogenesis of these conditions (2).

1. Ziegler, S F. et al: Novel protein-tyrosine kinase gene (hck) preferentially expressed in cells of hematopoietic origin. Molec. Cell. Biol. 7: 2276-2285, 1987.

2. Quintrell, N. et al: Identification of a human gene (HCK) that encodes a protein-tyrosine kinase and is expressed in hemopoietic cells. Molec. Cell. Biol. 7: 2267-2275, 1987

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.

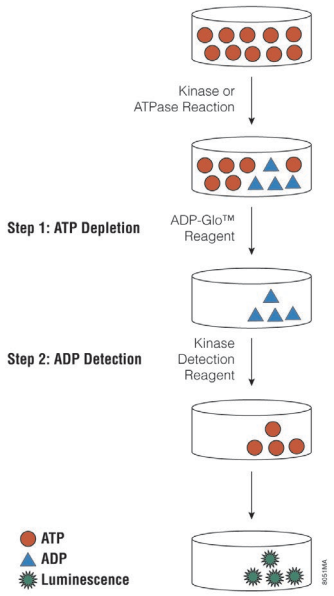

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

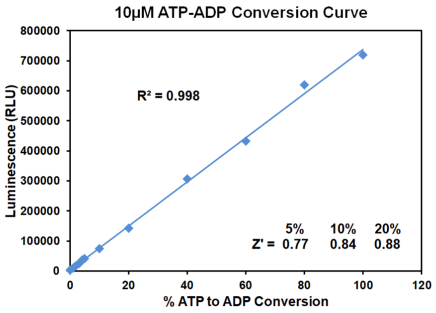

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	0
Luminescence	481,150	470,172	434,880	303,184	260,608	191,879	129,999	88,247	55,428	31,634	20,722	3,885
S/B	124	121	112	78	67	49	33	23	14	8	5	1
% Conversion	69	67	62	43	37	26	17	11	7	3	2	0

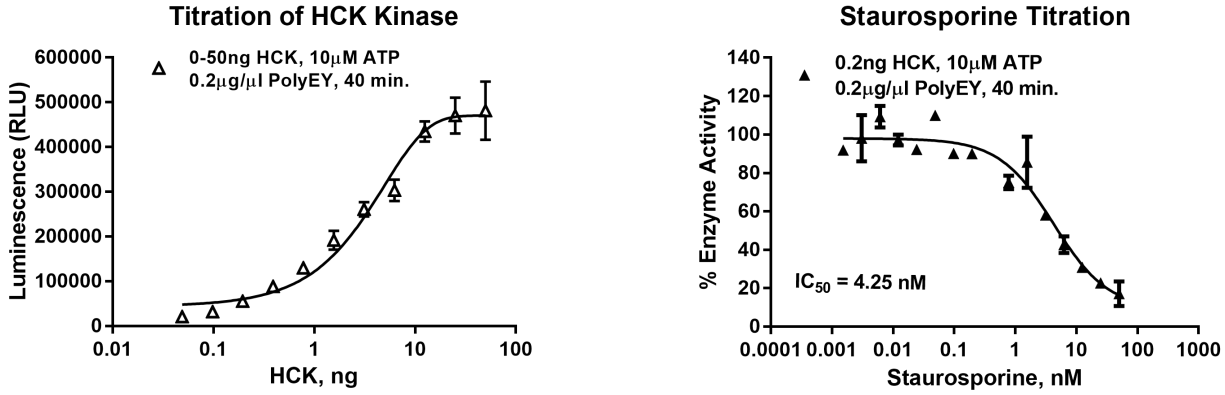

Figure 3. HCK Kinase Assay Development. (A) HCK enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 0.2ng of HCK to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
HCK Kinase Enzyme System	10µg	VA7186
	1mg	VA7187
ADP-Glo™ + HCK Kinase Enzyme System	1 Each	VA7188