ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

Haspin Kinase Assay

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Scientific Background:

Haspin is a protein kinase that regulates chromosome and spindle function during mitosis and meiosis. Haspin expression is detected in fetal liver, skin, kidney, small intestine and in all proliferating cells. Haspin phosphorylates H3 thr3 (H3T3ph) in human cell lines and depletion of Haspin by RNA interference reveals that Haspin is required for H3 thr3 phosphorylation in mitotic cells (1). Phosphorylation of H3T3ph by Haspin is necessary for chromosomal passenger complex (CPC) accumulation at centromeres. H3T3ph then positions the CPC at centromeres to regulate selected targets of Aurora B during mitosis (2).

1. Dai, J. et al: The kinase haspin is required for mitotic histone H3 Thr 3 phosphorylation and normal metaphase chromosome alignment. Genes Dev. 19: 472-488, 2005.

2. Wang, F. et al: Histone H3 Thr-3 phosphorylation by Haspin positions Aurora B at centromeres in mitosis. Science 330: 231-235, 2010.

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.

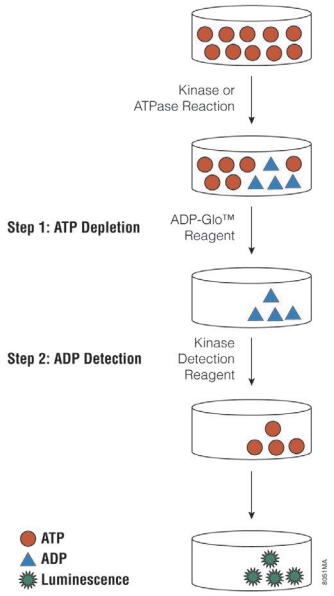

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

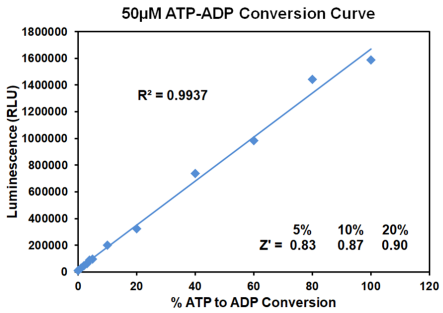

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	180	90	45	22.50	11.25	5.63	2.81	1.41	0.70	0.35	0.18	0
Luminescence	1,014,954	822,397	851,457	728,983	532,279	340,389	233,079	137,672	120,681	76,325	49,306	8,826
S/B	115	93	96	83	60	39	26	16	14	9	6	1
% Conversion	74	60	62	53	38	24	16	9	8	4	2	0

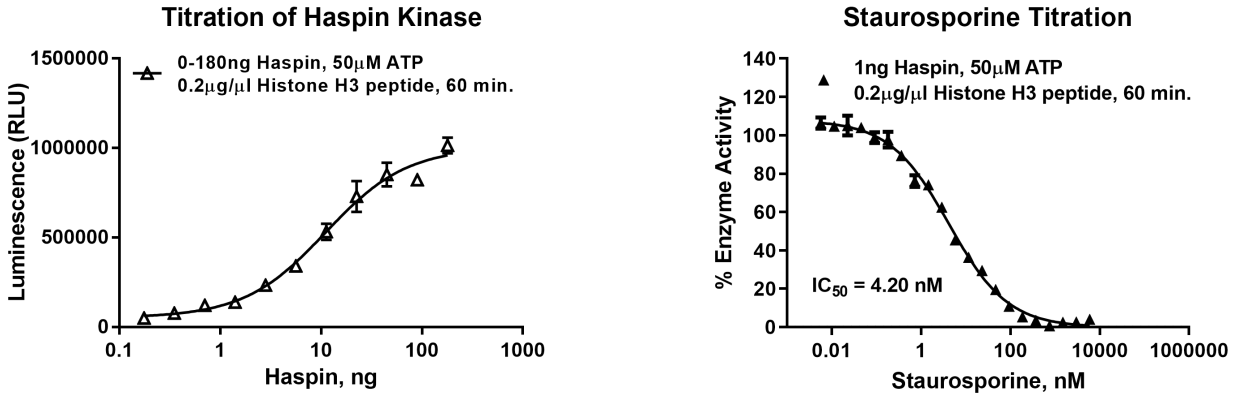

Figure 3. Haspin Kinase Assay Development. (A) Haspin enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1ng of Haspin to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
Haspin Kinase Enzyme System	10µg	VA7468
	1mg	VA7469
ADP-Glo™ + Haspin Kinase Enzyme System	1 Each	VA7470