

EIF2AK1 (HRI) Kinase Assay

By Juliano Alves, Laurie Engel, Said A. Goueli, and Hicham Zegzouti, Promega Corporation

Scientific Background:

EIF2AK1 (HRI) or eukaryotic translation initiation factor 2-alpha kinase 1 acts at the level of translation initiation to downregulate protein synthesis in response to stress. EIF2AK1 (HRI) is a kinase that can be inactivated by heme and is activated by heme deficiency and other stimuli and is a major protein kinase that phosphorylates EIF2-alpha (1). EIF2AK1 (HRI) is downregulated in the majority of ovarian cancers compared with normal ovarian tissues. EIF2AK1 (HRI) functions in iron homeostasis and may play a role in hemolytic and inflammatory anemia (2).

1. Hwang, et.al: Cloning of hHRI, human heme-regulated eukaryotic initiation factor 2-alpha kinase: down-regulated in epithelial ovarian cancers. *Molec. Cells* 10: 584-591, 2000.
2. Liu, S. et.al: The function of heme-regulated eIF2-alpha kinase in murine iron homeostasis and macrophage maturation. *J. Clin. Invest.* 117: 3296-3305, 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.

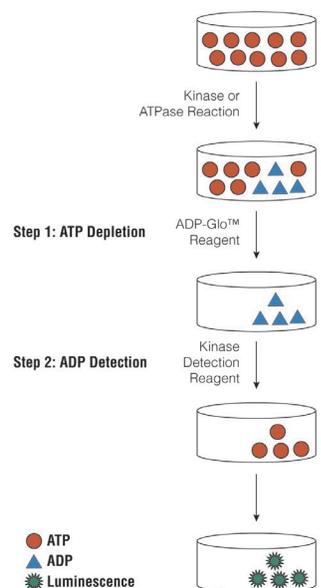


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

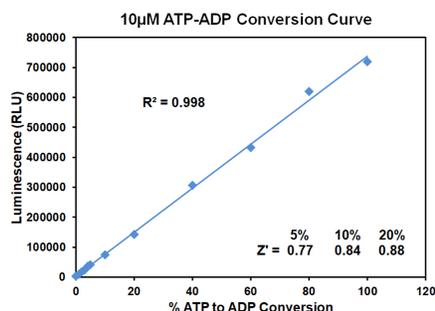


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



ADP-Glo™ Kinase Assay Application Note Ser/Thr Kinase Series

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 μ l of inhibitor or (5% DMSO)
 - ✓ 2 μ l of enzyme (defined from table 1)
 - ✓ 2 μ l of substrate/ATP mix
- Incubate at room temperature for indicated time (See Figure 3).
- Add 5 μ l of ADP-Glo™ Reagent.
- Incubate at room temperature for 40 minutes.
- Add 10 μ l 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
Luminescence	382,259	323,063	252,292	160,113	96,073	49,356	21,541	11,084	5,806	3,605	1,751
S/B	218	184	144	91	55	28	12	6	3	2	1
% Conversion	76	64	50	32	19	10	5	2	1	1	0

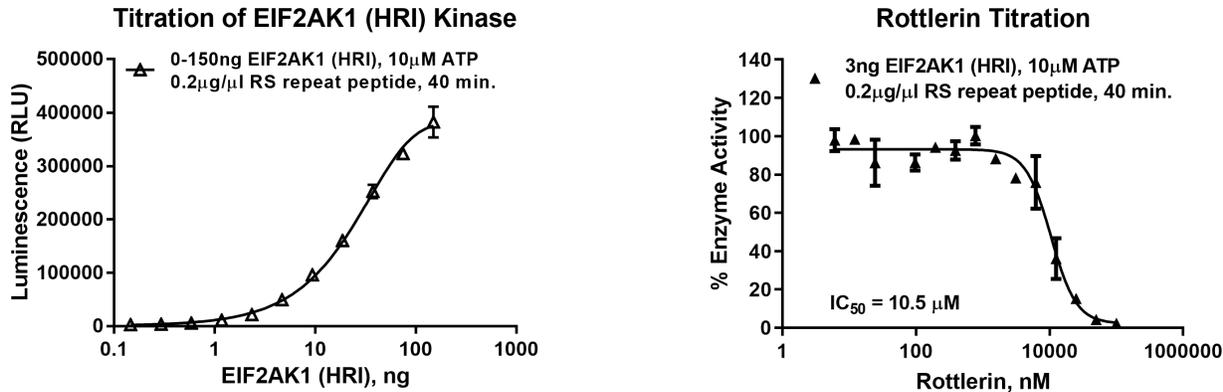


Figure 3. EIF2AK1 (HRI) Kinase Assay Development. (A) EIF2AK1 (HRI) enzyme was titrated using 10 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 3ng of EIF2AK1 (HRI) to determine the potency of the inhibitor (IC₅₀).



Ordering Information:

Products	Size	Cat. #
EIF2AK1 (HRI) Kinase Enzyme System	10 μ g	VA7132
	1mg	VA7133
ADP-Glo™ + EIF2AK1 (HRI) Kinase Enzyme System	1 Each	VA7134