ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

ERN1 (IRE1) Kinase Assay

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Scientific Background:

ERN1 or endoplasmic reticulum to nucleus signaling 1 is the ER to nucleus signalling 1 protein, a human homologue of the yeast Ire1 gene product which possesses intrinsic kinase activity and an endoribonuclease activity and it is important in endoplasmic reticulum-based stress signals. ERN1 controls IRE1 proteolysis in mammalian cells (1). The activation of ERN1 through oligomerization expands the mechanistic repertoire of kinase-based signaling receptors. ERNI has a critical function in extraembryonic cells that is essential for fetal viability (2).

1. Niwa, M.et.al: A role for presenilin-1 in nuclear accumulation of Ire1 fragments and induction of the mammalian unfolded protein response. Cell 99: 691-702, 1999.

2. Iwawaki, T.et.al: Function of IRE1 alpha in the placenta is essential for placental development and embryonic viability. Proc. Nat. Acad. Sci. 106: 16657-16662, 2009.

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.

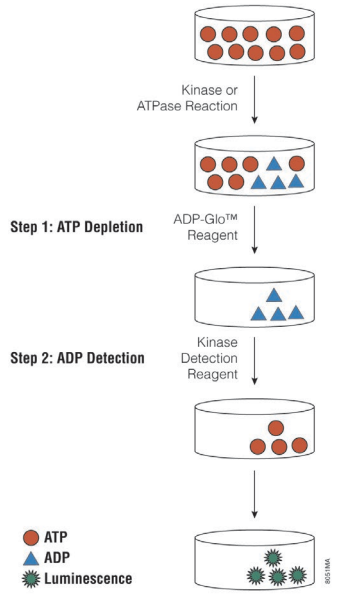

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

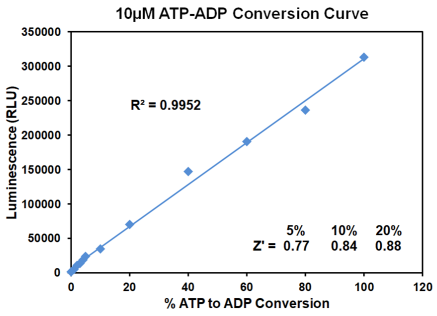

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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
Luminescence	255,017	204,568	164,393	93,613	48,880	19,967	10,304	5,314	2,516
S/B	101	81	65	37	19	8	4	2	1
% Conversion	83	66	53	29	14	4	1	0	0

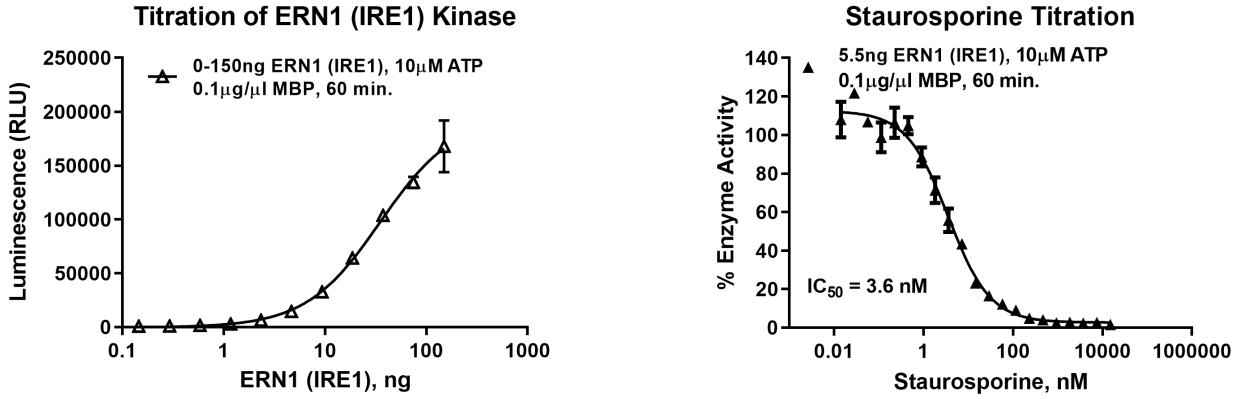

Figure 3. ERN1 (IRE1) Kinase Assay Development. (A) ERN1 (IRE1) enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 5.5ng of ERN1 (IRE1) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
ERN1 (IRE1) Kinase Enzyme System	10µg	VA7144
	1mg	VA7145
ADP-Glo™ + ERN1 (IRE1) Kinase Enzyme System	1 Each	VA7146