

ERN2 (IRE2) Kinase Assay

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Scientific Background:

ERN2 (IRE2) or endoplasmic reticulum to nucleus signaling 2 protein, is a human homologue of the yeast Ire1 gene product which plays a role in multiple facets of the ER stress response in mammalian cells (1). This protein belongs to the protein kinase superfamily, Ser/Thr protein kinase family.

1. Wang, X.-Z.et.al: Cloning of mammalian Ire1 reveals diversity in the ER stress responses. EMBO J. 17: 5708-5717, 1998.

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.

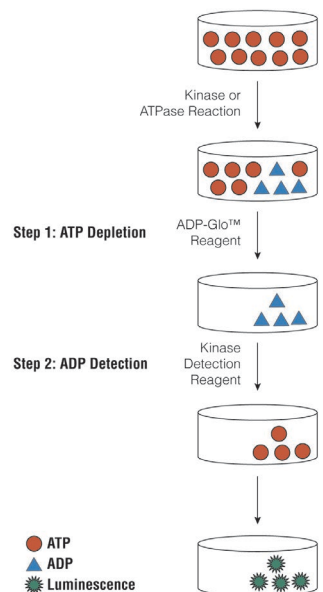


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

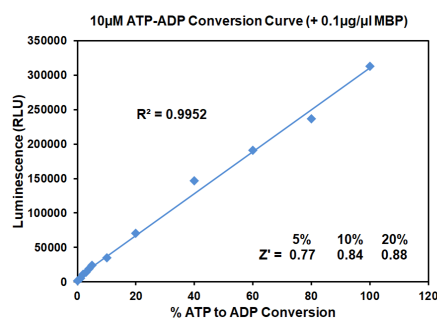


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



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	300	150	75	37.50	18.75	9.38	2.34	0
Luminescence	263,842	134,207	60,047	21,374	9,322	4,456	1,434	606
S/B	435	221	99	35	15	7	2	1
% Conversion	151	76	33	10	4	1	0	0

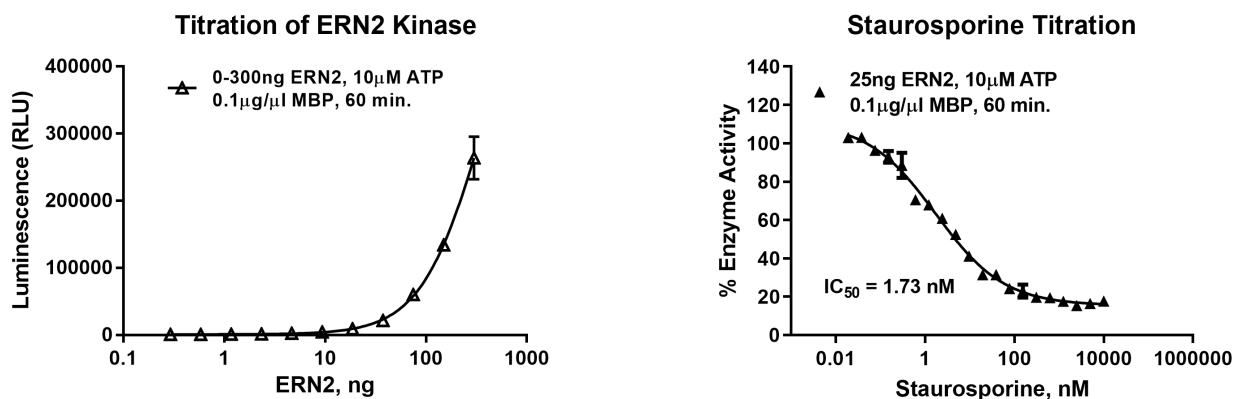


Figure 3. ERN2 (IRE2) Kinase Assay Development. (A) ERN2 (IRE2) enzyme was titrated using 10 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 25ng of ERN2 (IRE2) to determine the potency of the inhibitor (IC₅₀).



Ordering Information:

Products	Size	Cat. #
ERN2 (IRE2) Kinase Enzyme System	10 μ g	VA7360
	1mg	VA7361
ADP-Glo™ + ERN2 (IRE2) Kinase Enzyme System	1 Each	VA7362