ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

ULK3 Kinase Assay

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

Scientific Background:

ULK3 or unc-51-like kinase 3 is a member of the serine/threonine kinase family that is involved in the SHH signaling pathway as a positive regulator of GLI proteins (1). ULK3 enhances endogenous and over-expressed GLI transcriptional activity in cultured cells and alters its subcellular localization. Furthermore, ULK3 phosphorylates GLI proteins in vitro. ULK3 is widely expressed and its expression is higher in a number of tissues where SHH signaling is known to be active, again suggesting that ULK3 is involved in the SHH pathway as a positive regulator of GLI proteins.

1. Maloverjan, A. et al: Identification of a novel serine/threonine kinase ULK3 as a positive regulator of Hedgehog pathway. Exp Cell Res. 2010 Feb 15;316(4):627-37.

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.

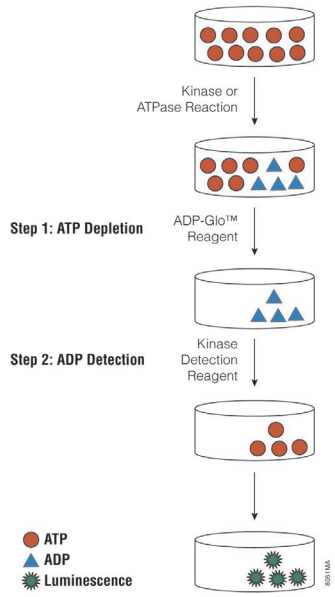

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

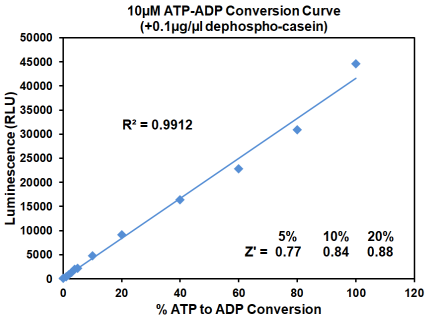

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	200	100	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0
Luminescence	53,761	34,963	24,221	14,824	9,796	5,494	2,745	1,186	749	426	374	159
S/B	339	221	153	94	62	35	17	7	5	3	2	1
% Conversion	129	84	58	36	23	13	6	3	2	1	1	0

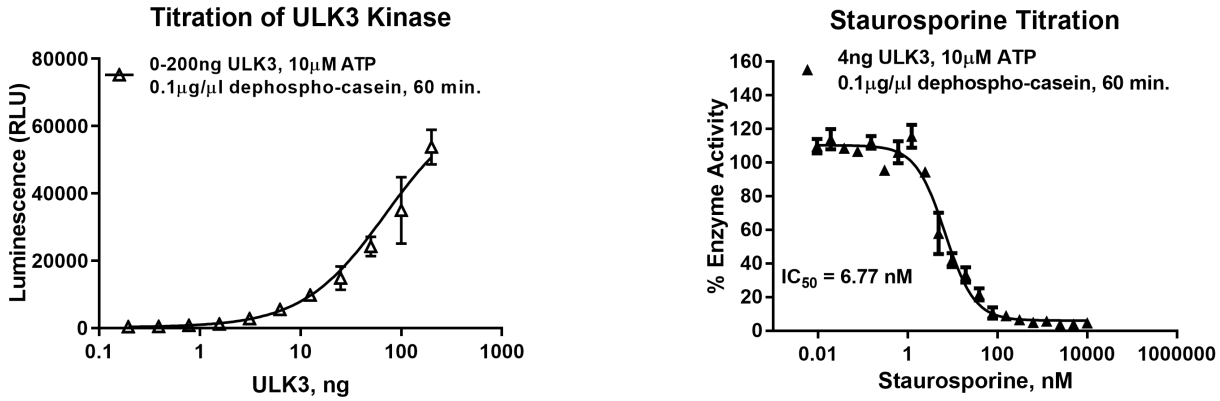

Figure 3. ULK3 Kinase Assay Development. (A) ULK3 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 4ng of ULK3 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
ULK3 Kinase Enzyme System	10µg	VA7585
	1mg	VA7586
ADP-Glo™ + ULK3 Kinase Enzyme System	1 Each	VA7587