ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

ULK2 Kinase Assay

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Scientific Background:

ULK2 is a serine/threonine protein kinase that plays critical role during initial stages of autophagy which is a vital response to nutrient starvation. Mammalian Atg13 binds ULK2 and mediates the interaction of the ULK protein with FIP200. The binding of Atg13 stabilizes and activates ULK and facilitates the phosphorylation of FIP200 by ULK. The ULK-Atg13-FIP200 complex is a direct target of mTOR and important regulator of autophagy in response to mTOR signaling (1). Yeast 2-hybrid assays showed the C-terminus of ULK2 binds the C-terminus of SynGAP, a negative regulator of Ras that is associated with neural development (2).

1. Jung C H, et al: ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. Mol Biol Cell. 2009 Apr;20(7):1992-2003.

2. Tomoda T, et al: Role of Unc51.1 and its binding partners in CNS axon outgrowth. Genes Dev. 18: 541-558, 2004.

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.

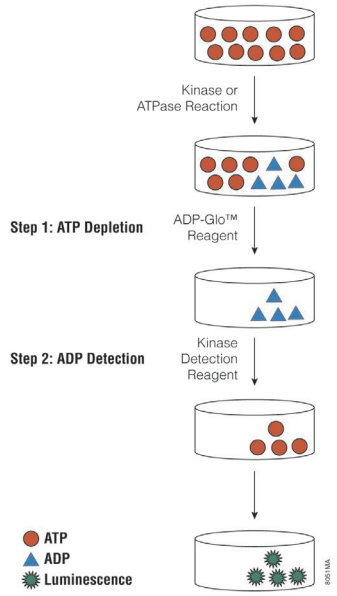

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

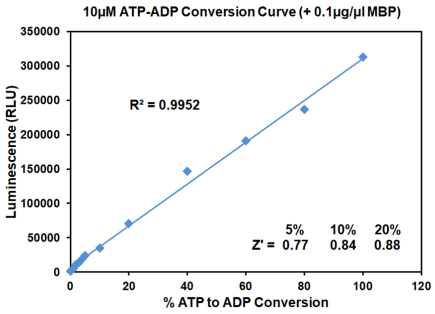

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	300	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0.59	0
Luminescence	212,396	174,785	147,925	120,753	74,329	44,221	20,436	10,300	4,843	2,482	1,465
S/B	145	119	101	82	51	30	14	7	3	2	1
% Conversion	68	55	46	38	22	12	5	1	0	0	0

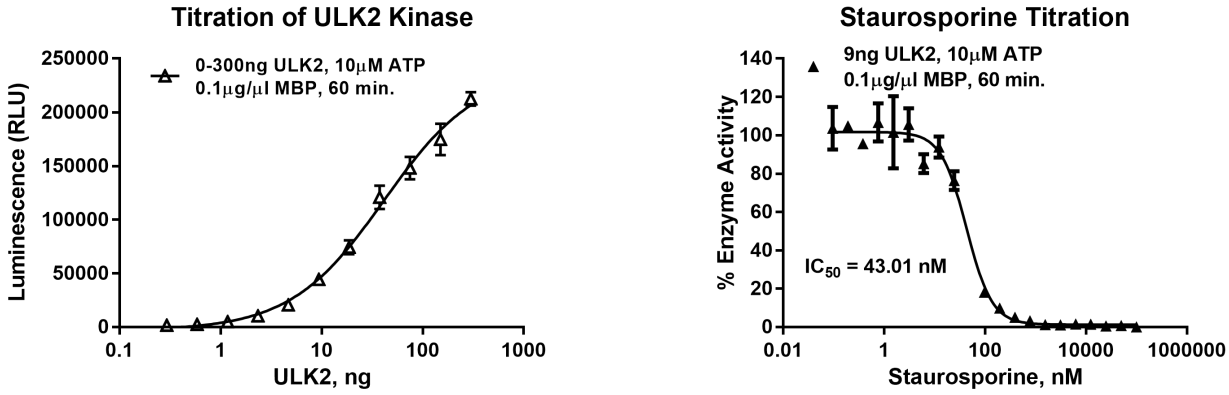

Figure 3. ULK2 Kinase Assay Development. (A) ULK2 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 9ng of ULK2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
ULK2 Kinase Enzyme System	10µg	VA7582
	1mg	VA7583
ADP-Glo™ + ULK2 Kinase Enzyme System	1 Each	VA7584