ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

QIK Kinase Assay

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Scientific Background:

QIK is a serine/threonine protein kinase that contains an N-terminal kinase domain, a central domain with ubiquitin-associate motif, and a C-terminal PKA phosphorylation site. QIK can phosphorylate IRS1 and overexpression of QIK in adipocyte elevates the phosphorylation of IRS1 (1). The QIK-mediated phosphorylation of IRS1 may modulate the efficiency of insulin signal transduction and could be responsible for insulin resistance associated with diabetes (1). Insulin disrupts TORC2 activity by induction of QIK which then stimulates the phosphorylation and cytoplasmic translocation of TORC2. Phosphorylated TORC2 is subsequently degraded by the 26S proteasome (2).

1. Horike N, et al: Adipose-specific expression, phosphorylation of ser794 in insulin receptor substrate-1, and activation in diabetic animals of salt-inducible kinase-2. J. Biol. Chem. 278: 18440-18447, 2003.

2. Dentin R, et al: Insulin modulates gluconeogenesis by inhibition of the coactivator TORC2. Nature 449: 366-369, 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.

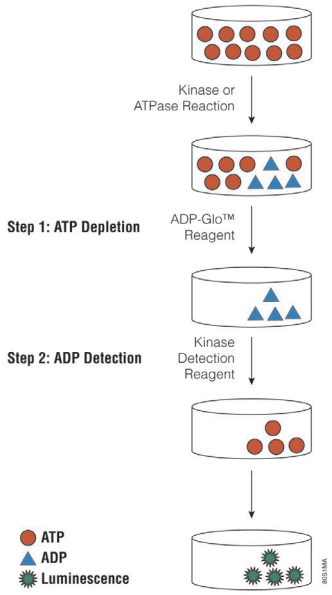

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

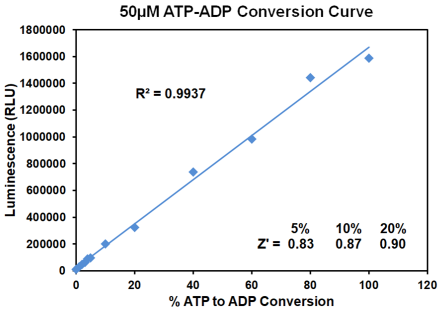

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	160	80	40	20	10	5	2.50	1.25	0.63	0
Luminescence	1,543,300	984,151	771,383	533,451	295,458	141,044	82,861	39,625	24,465	8,768
S/B	176	112	88	61	34	16	9	5	3	1
% Conversion	90	57	44	30	16	7	4	1	0	0

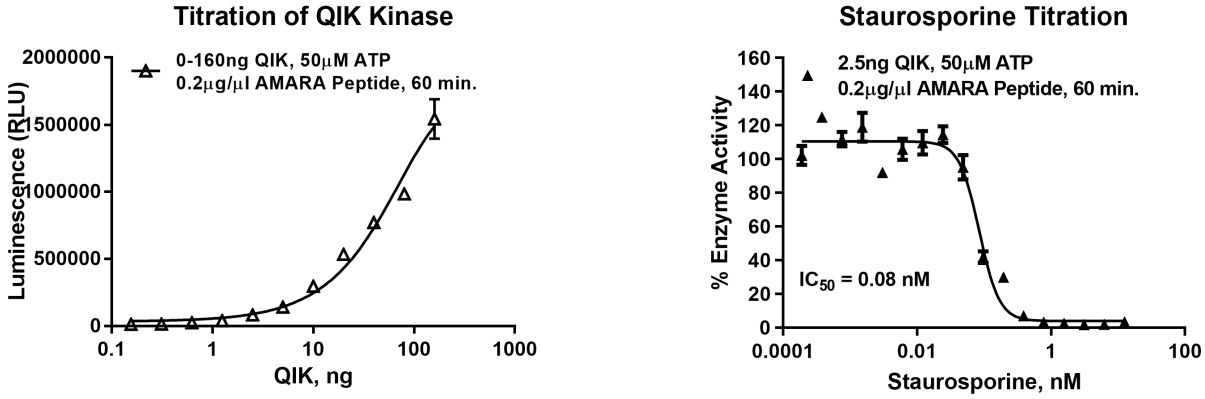

Figure 3. QIK Kinase Assay Development. (A) QIK enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2.5ng of QIK to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
QIK Kinase Enzyme System	10µg	VA7267
	1mg	VA7268
ADP-Glo™ + QIK Kinase Enzyme System	1 Each	VA7269