ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

BRSK1 Kinase Assay

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Scientific Background:

BRSK1 is serine/threonine kinases 1 which required for presynaptic differentiation in Caenorhabditis elegans that are needed for neuronal polarization (1). BRSK1 is highly expressed in all specific adult brain regions followed by fetal brain and adult spinal cord. It is also expressed in adult heart, pancreas, testis, ovary, lung, and kidney, and in fetal liver (2).

1. Kishi, M. et.al: Mammalian SAD kinases are required for neuronal polarization. Science 307: 929-932, 2005.

2. Nagase, T. et.al: Prediction of the coding sequences of unidentified human genes. XX. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. DNA Res. 8: 85-95, 2001.

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.

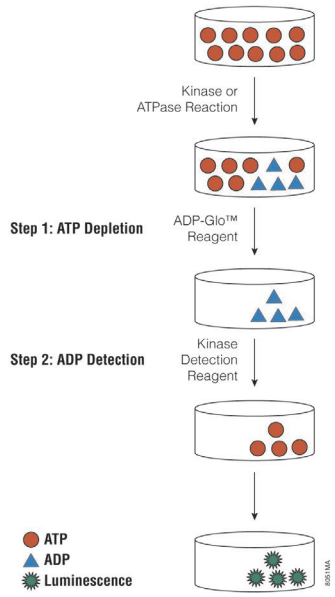

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

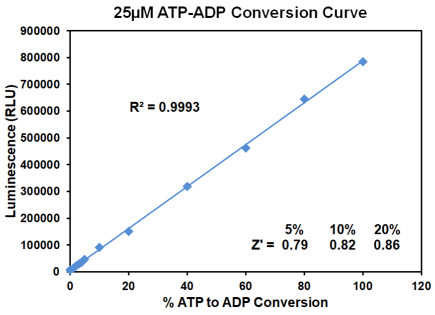

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	0.63	0
Luminescence	481,944	387,479	228,162	132,604	53,487	29,922	14,437	5,078	3,085
S/B	156	126	74	43	17	10	5	2	1
% Conversion	56	45	26	14	5	2	0	0	0

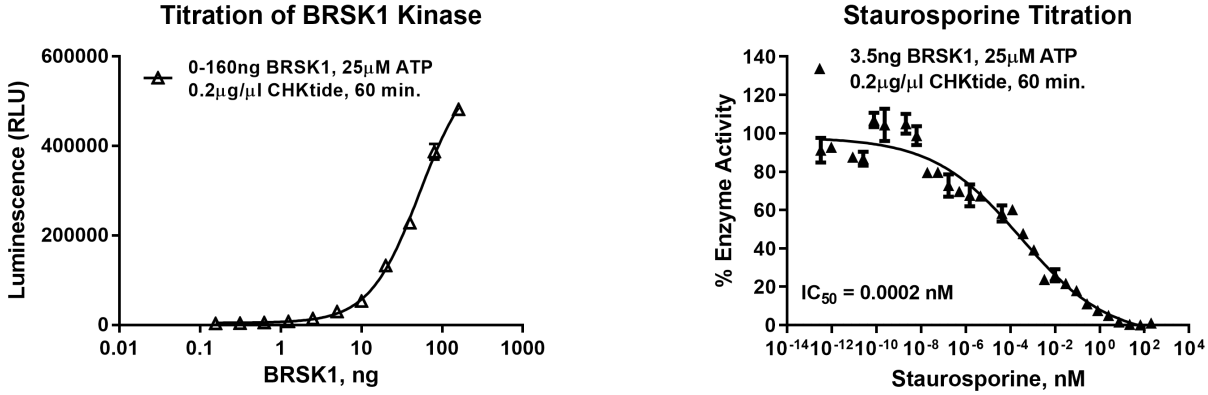

Figure 3. BRSK1 Kinase Assay Development. (A) BRSK1 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 3.5ng of BRSK1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
BRSK1 Kinase Enzyme System	10µg	VA7390
	1mg	VA7391
ADP-Glo™ + BRSK1 Kinase Enzyme System	1 Each	VA7392