ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

BRSK2 Kinase Assay

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Scientific Background:

BRSK2 is a brain-selective serine/threonine kinase 2 that is mainly expressed in the brain, with weaker expression in testis and pancreas. BRSK2 expressed in insect cells specifically phosphorylates WEE1A, CDC25C and CDC25B in an in vitro assay. DNA damage induced by ultraviolet (UV) irradiation or methyl methane sulfonate, but not by ionizing radiation, enhanced endogenous BRSK2 kinase activity in a caffeine-sensitive manner and caused translocation of BRSK2 from the cytoplasm to the nucleus (1). Overexpression of BRSK2 induces G2/M arrest in HeLa cells while small interfering RNA against BRSK2 partly abrogated UV-induced G2/M arrest. Mammalian BRSK2 kinases are required for neuronal polarization (2).

1. Lu, R. et.al: Human SAD1 kinase is involved in UV-induced DNA damage checkpoint function. J. Biol. Chem. 279: 31164-31170, 2004.

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

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.

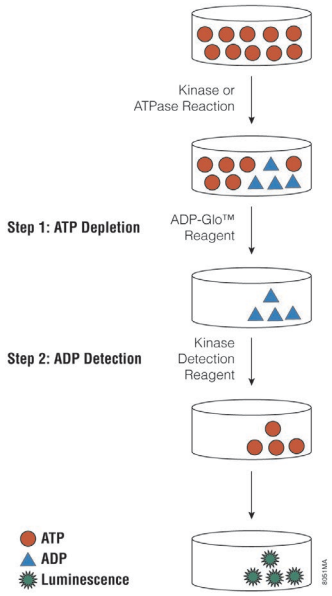

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

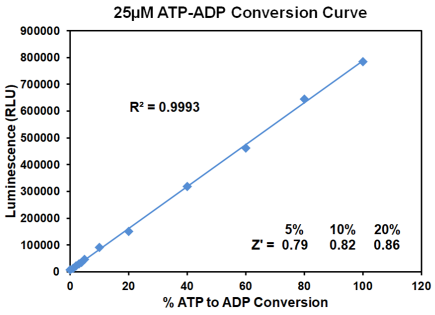

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	1.25	0.63	0
Luminescence	403,342	249,647	168,755	86,119	42,957	19,375	6,281	4,652	3,050
S/B	132	82	55	28	14	6	2	2	1
% Conversion	47	28	19	9	4	1	0	0	0

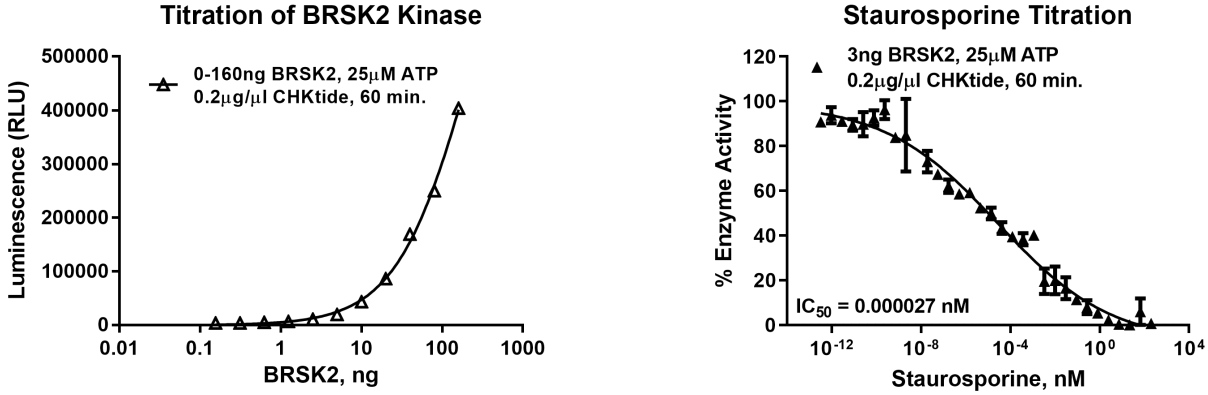

Figure 3. BRSK2 Kinase Assay Development. (A) BRSK2 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 3ng of BRSK2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
BRSK2 Kinase Enzyme System	10µg	VA7393
	1mg	VA7394
ADP-Glo™ + BRSK2 Kinase Enzyme System	1 Each	VA7395