BRK Kinase Assay

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Scientific Background:

BRK is a member of the non-receptor tyrosine kinases (PTKs) that contains an amino terminal SH3 and SH2 domain as well as the catalytic domain (1). BRK expression is low or undetectable in normal mammary tissue and benign lesions. However, approximately two-thirds of breast tumors express appreciable levels, and 27% of tumors over express BRK by fivefold or more (2).

1. Mitchell, PJ. et al: Cloning and characterisation of cDNAs encoding a novel non-receptor tyrosine kinase, brk, expressed in human breast tumours. Oncogene. 1994 Aug;9(8):2383-90.

2. Mitchell, PJ. et al: Characterisation and chromosome mapping of the human non receptor tyrosine kinase gene, brk. Oncogene. 1997 Sep 18;15(12):1497-502.Erratum in: Oncogene 1998 Jul 9;17(1):129.

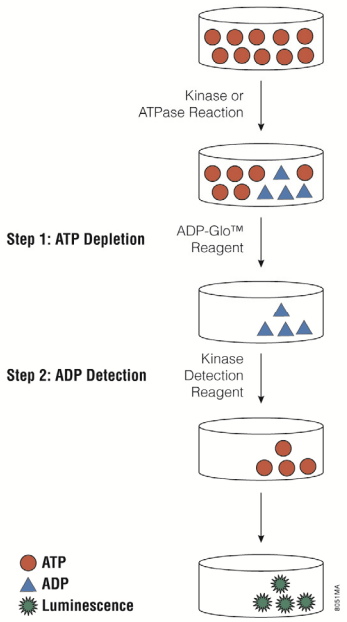

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

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.

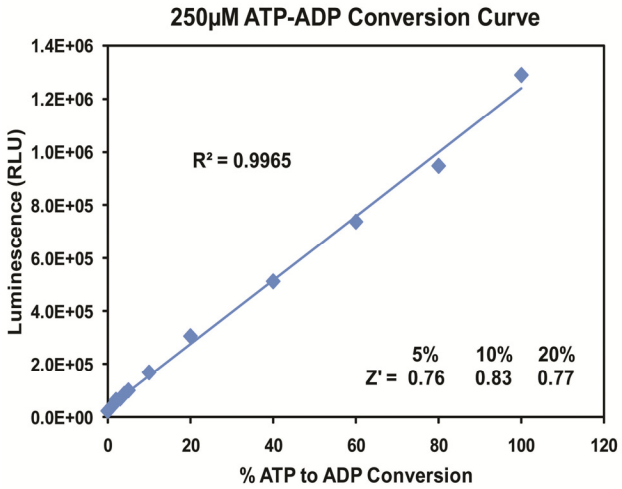

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 250µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Tyrosine Kinase 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 60 minutes.
- 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 0.5-1second).

Table 1. BRK 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.

BRK, ng	200	100	50	25	12.5	6.3	3.1	0
Luminescence	304011	209944	165520	101993	53564	26187	17643	6209
S/B	49	34	27	16	9	4	2.8	1
% Conversion	22	15	11	6	3	1	0.1	0

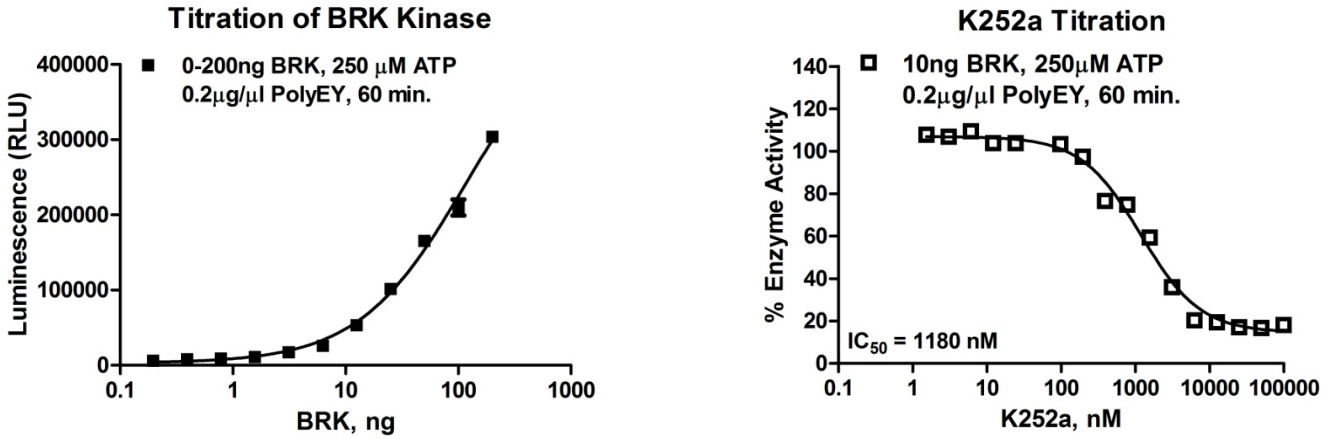

Figure 3. BRK Kinase Assay Development. **(A) BRK enzyme was titrated using 250µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) K252a dose response was created using 10ng of BRK to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
BRK Kinase Enzyme System	Promega	V4054
ADP-Glo™ + BRK Kinase Enzyme System	Promega	V4055

BRK Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 2.5mM MnCl2, 50µM DTT.