ADP-Glo™ Kinase Assay Application Notes

TYROSINE KINASE SERIES: TRKB

TRKB Kinase Assay

By Juliano Alves, Ph.D., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

TRKB is a member of the neurotrophic tyrosine receptor kinase (NTRK) family. TRKB is the high affinity catalytic receptor for several "neurotrophins", which are small protein growth factors that induce the survival and differentiation of distinct cell populations (1). TRKB is a membrane-bound receptor that, upon neurotrophin binding, phosphorylates itself and members of the MAPK pathway (2). Signalling through TRKB leads to cell differentiation. Mutations in the TRKB gene have been associated with obesity and mood disorders.

1. Gregory, T. et al: Signal Transduction Mediated by the Truncated trkB Receptor Isoforms, trkB.T1 and trkB.T2. J. Neurosci. 1997; 17(8); 2683-2690.

2. Pearse, R N. et al: A neurotrophin axis in myeloma: TrkB and BDNF promote tumor-cell survival. Blood. 2005;105(11):4429-36.

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.

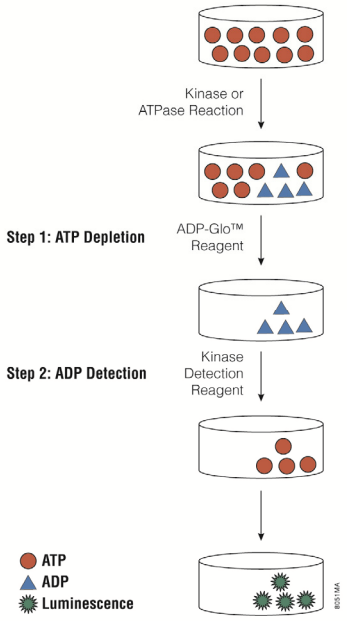

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

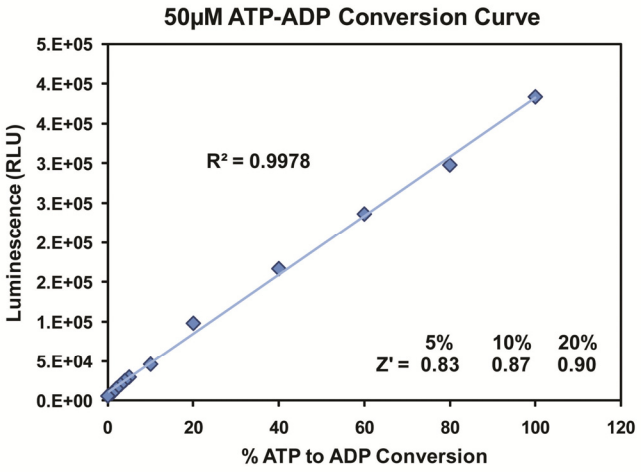

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. TRKB 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.

TRKB, ng	200	100	50	25	13	6.3	0
Luminescence	184955	97369	43183	13446	3967	1827	1002
S/B	185	97	43	13	4	2	1
% Conversion	64	32	12	1.8	0.8	0.3	0

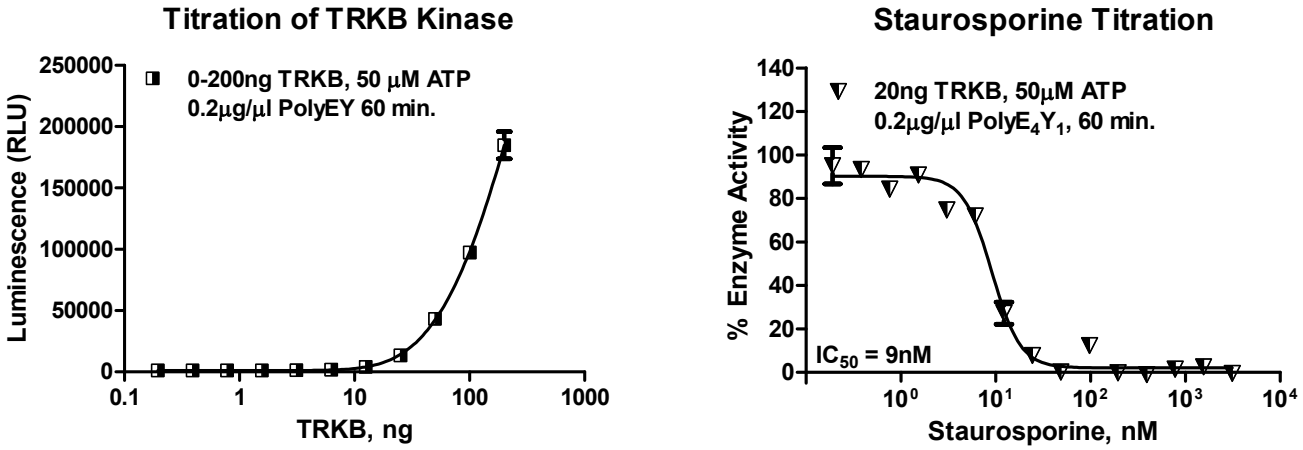

Figure 3. TRKB Kinase Assay Development. **(A) TRKB enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 20ng of TRKB to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
TRKB Kinase Enzyme System	Promega	V4048
ADP-Glo™ + TRKB Kinase Enzyme System	Promega	V4049

TRKB Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 2.5mM $MnCl_2$, 50µM DTT.