TRKA Kinase Assay

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Scientific Background:

TRKA is a member of the trk proto-oncogene family and encodes a 140-kilodalton, membrane-spanning protein tyrosine kinase that is the functional receptor for nerve growth factor (NGF). NGF elicits the rapid phosphorylation of gp140trk on tyrosine residues leading to increased c-Fos expression, DNA synthesis and morphologic transformation (1). A decreased expression of TRKA on the striatal cholinergic neurons has been observed which may contribute, when it reaches a crucial threshold, to the death of cholinergic neurons observed in Alzheimer disease (2).

1. Kaplan, D R. et al: The trk proto-oncogene product: a signal transducing receptor for nerve growth factor. Science. 1991 Apr 26;252(5005):554-8.

2. Boissiere, F. et al: Neurotrophin receptors and selective loss of cholinergic neurons in Alzheimer disease. Mol Chem Neuropathol. 1996 May-Aug;28(1-3):219-23.

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.

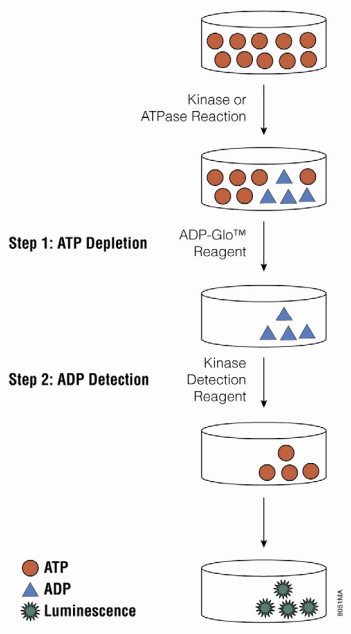

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

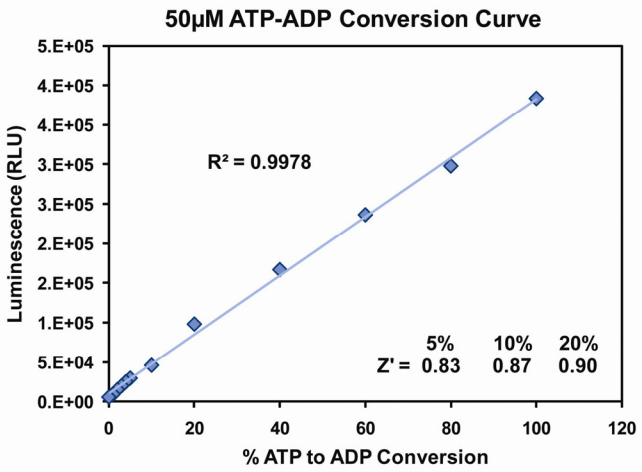

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.

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 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. KDR 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.

TRKA, ng	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0
Luminescence	149023	131117	110443	88731	67374	48713	32933	21115	13670	2592
S/B	57	51	43	34	26	19	12.7	8.1	5.3	1
% Conversion	73	64	54	42	32	22	13.9	7.8	4.0	0

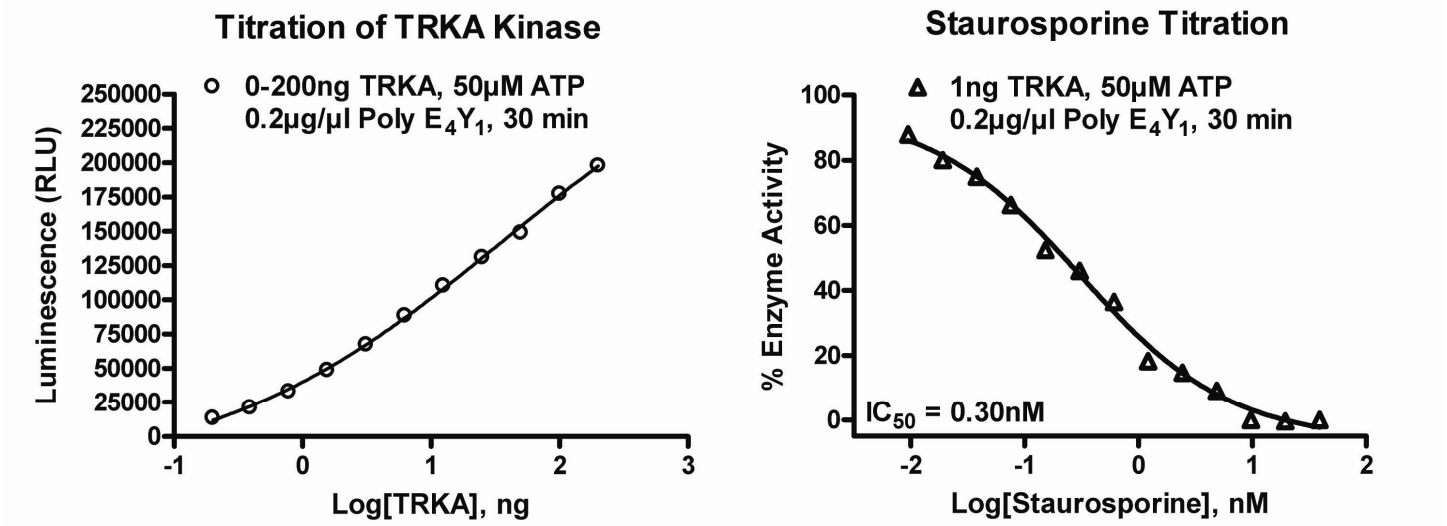

Figure 3. TRKA Kinase Assay Development: (A) TRKA enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1ng of TRKA to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
TRKA Kinase Enzyme System	Promega	V2931
ADP-Glo + TRKA Kinase Enzyme System	Promega	V9761

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

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