ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

TRKA (G595R) Kinase Assay

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Scientific Background:

TRKA is a member of the trk proto-oncogene family and encodes a 140kDa, 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.

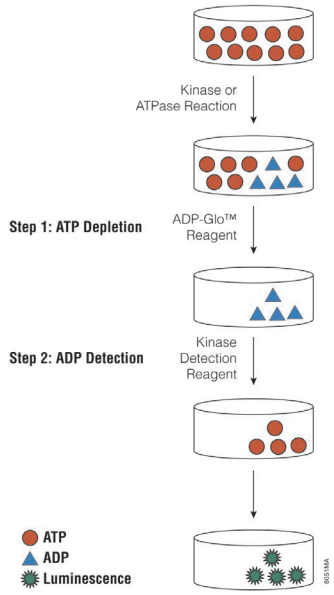

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

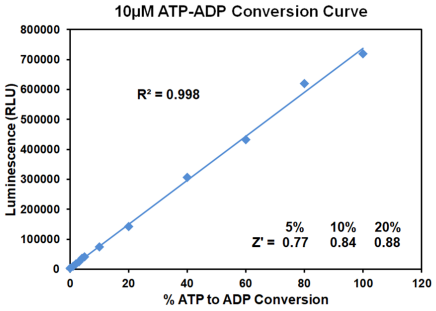

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0
Luminescence	416,391	276,452	190,235	92,081	43,535	19,238	9,217	5,061	2,248
S/B	185	123	85	41	19	9	4	2	1
% Conversion	49	32	22	10	5	2	1	0	0

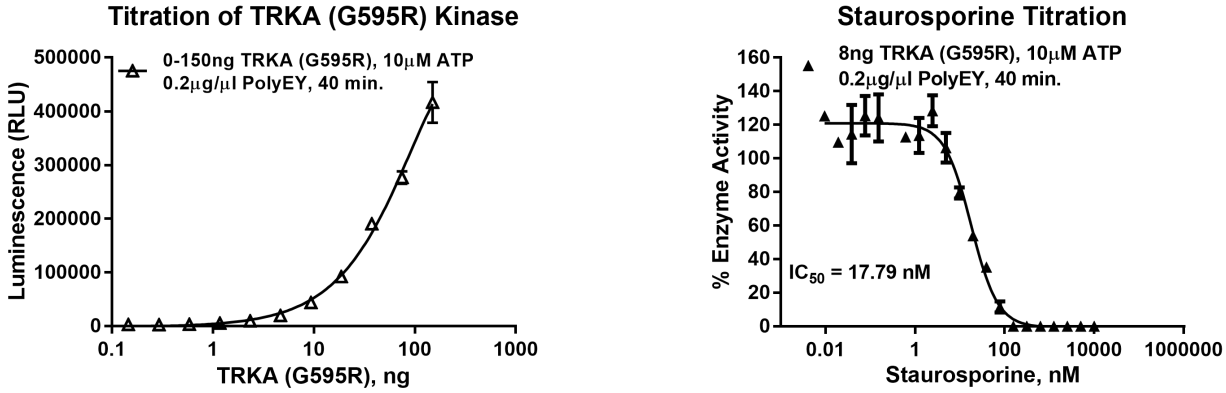

Figure 3. TRKA (G595R) Kinase Assay Development. (A) TRKA (G595R) enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 8ng of TRKA (G595R) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
TRKA (G595R) Kinase Enzyme System	10µg	VA7336
	1mg	VA7337
ADP-Glo™ + TRKA (G595R) Kinase Enzyme System	1 Each	VA7338