ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

TRKC Kinase Assay

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Scientific Background:

TRKC is a member of the TRK family of tyrosine kinase receptors and is the high affinity catalytic receptor for the neurotrophin NT-3 (neurotrophin-3). TRKC mediates the multiple cellular effects of the NT-3 neurotrophic factor, which includes neuronal differentiation and survival (1). TRKC has been implicated in insulin signaling pathway through interactions with the MUSK protein receptor and the VEGF receptor. Mutations in the TRKC gene have been associated with medulloblastomas, secretory breast carcinomas and other cancers (2).

1. Lamballe, F. et al: trkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3". Cell, 1991; 66 (5): 967–79.

2. Marchetti, A. et al: Frequent mutations in the neurotrophic tyrosine receptor kinase gene family in large cell neuroendocrine carcinoma of the lung. Hum Mutat. 2008 May;29(5):609-16.

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.

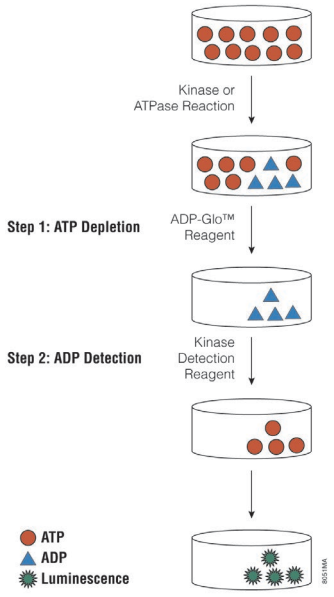

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

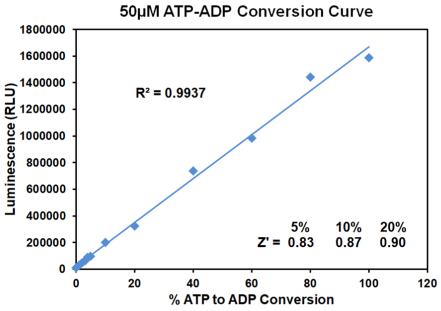

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	240	120	60	30	15	7.50	3.75	0
Luminescence	1,741,710	915,464	306,956	174,413	153,950	60,490	38,040	12,599
S/B	138	73	24	14	12	5	3	1
% Conversion	98	51	16	8	7	2	0	0

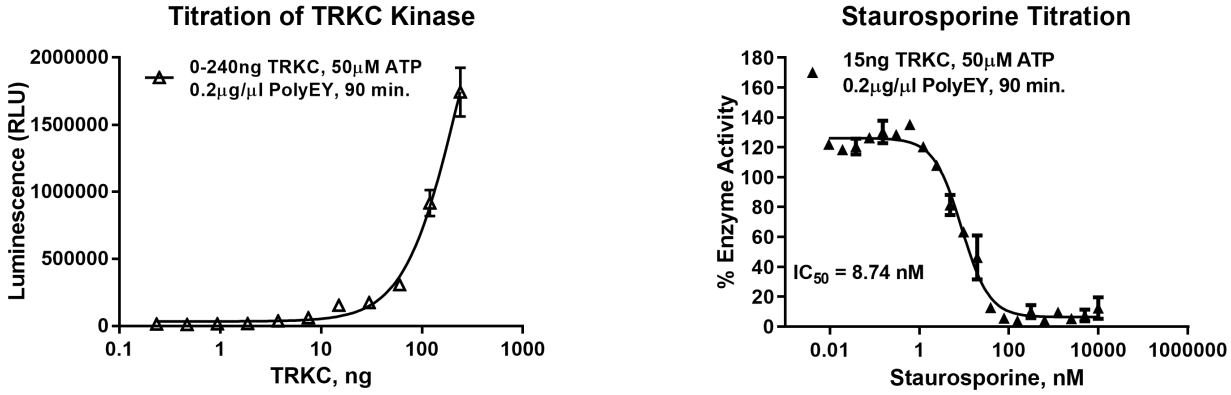

Figure 3. TRKC Kinase Assay Development. (A) TRKC enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 15ng of TRKC to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
TRKC Kinase Enzyme System	10µg	VA7342
	1mg	VA7343
ADP-Glo™ + TRKC Kinase Enzyme System	1 Each	VA7344