ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

TPR-TRKA (TRK-T1) Kinase Assay

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Scientific Background:

TRKA is a member of the neurotrophic tyrosine kinase receptor (NTKR) family which is a membrane-bound receptor that, upon neurotrophin binding, phosphorylates itself and members of the MAPK pathway. TRKA has a crucial role in the development and function of the nociceptive reception system as well as establishment of thermoregulation via sweating in humans (1). As one of the thyroid TRK oncogenes, TRK-T1 is created by an intrachromosomal rearrangement that juxtaposes the 5' end of the TPR gene to the TRK tyrosine kinase domain and shows frequent activation of the TRK in human papillary thyroid carcinoma (2).

1. Smeyne, R. J. et.al: Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. Nature 368: 246-249, 1994.

2. Greco A. et.al: TRK-T1 is a novel oncogene formed by the fusion of TPR and TRK genes in human papillary thyroid carcinomas. Oncogene. 1992 Feb;7(2):237-42.

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.

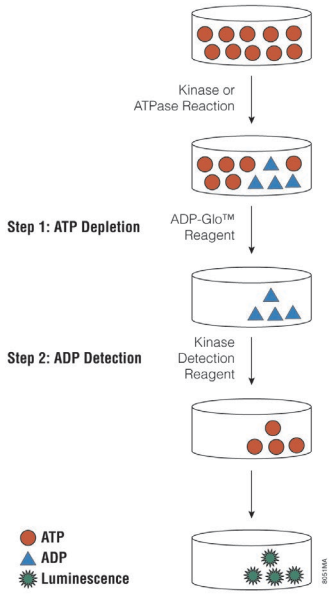

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

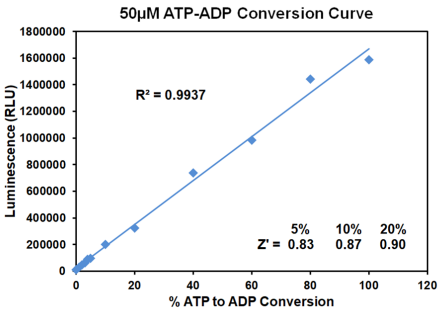

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0
Luminescence	624,532	318,481	158,883	81,422	41,287	22,815	17,562	8,466	4,562
S/B	137	70	35	18	9	5	4	2	1
% Conversion	47	24	12	6	3	1	1	0	0

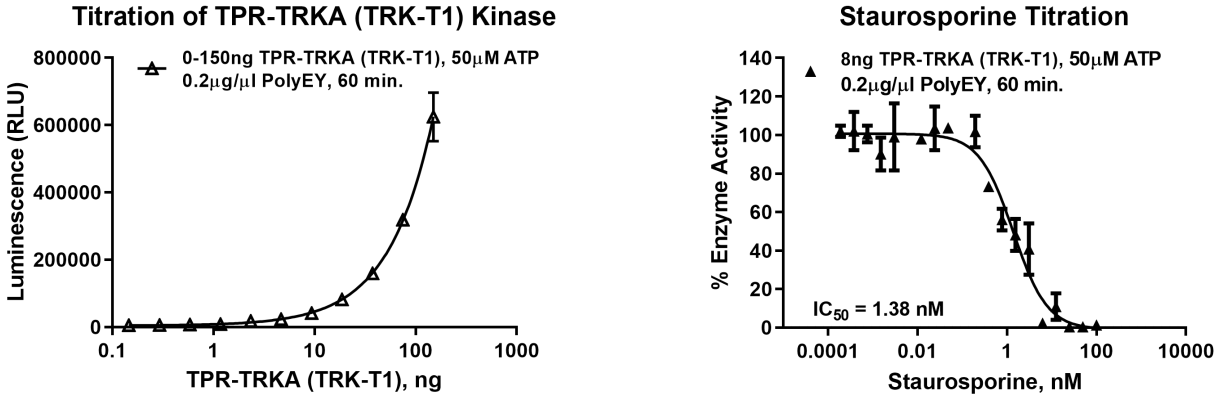

Figure 3. TPR-TRKA (TRK-T1) Kinase Assay Development. (A) TPR-TRKA (TRK-T1) enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 8ng of TPR-TRKA (TRK-T1) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
TPR-TRKA (TRK-T1) Kinase Enzyme System	10µg	VA7324
	1mg	VA7325
ADP-Glo™ + TPR-TRKA (TRK-T1) Kinase Enzyme System	1 Each	VA7326