ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

PTC3 (NCOA4-RET) Kinase Assay

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Scientific Background:

RET/PTC3 is fused of RET and the activating NCOA4 gene by intrachromosomal paracentric inversions in chromo-some 10 (1). Likes RET/PTC1, it is the most frequent RET rearrangements in papillary thyroid carcinoma (PTC) (2), especially in radiation-induced tumours. The RET/PTC3 rearrangements may be typical for radiation-induced childhood PTC with a short latency period. The RET/PTC rearrangements also have been shown in benign thyroid lesions, including Hashimoto's thyroiditis (HT).

1. Santoro M, et al: Molecular characterization of RET/PTC3; a novel rearranged version of the RET proto-oncogene in a human thyroid papillary carcinoma. Oncogene 1994, 9:509-516.

2. Nikiforov YE: RET/PTC rearrangement in thyroid tumors. Endocr Pathol 2002, 13:3-16.

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.

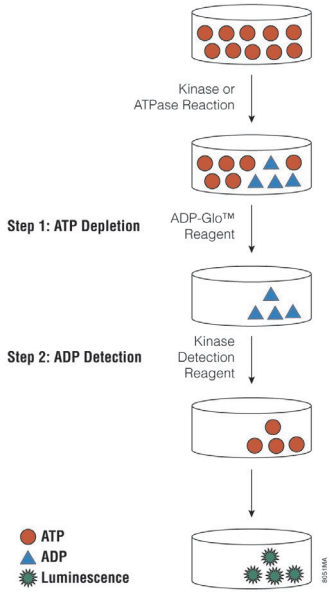

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

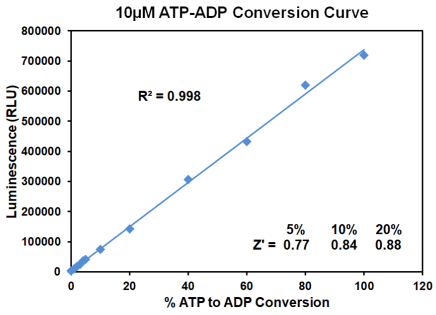

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	80	40	20	10	5	2.50	1.25	0.63	0.31	0.16	0
Luminescence	389,337	281,901	166,109	107,503	59,208	38,457	20,272	11,601	6,823	4,434	2,137
S/B	182	132	78	50	28	18	9	5	3	2	1
% Conversion	53	38	22	14	8	5	2	1	0	0	0

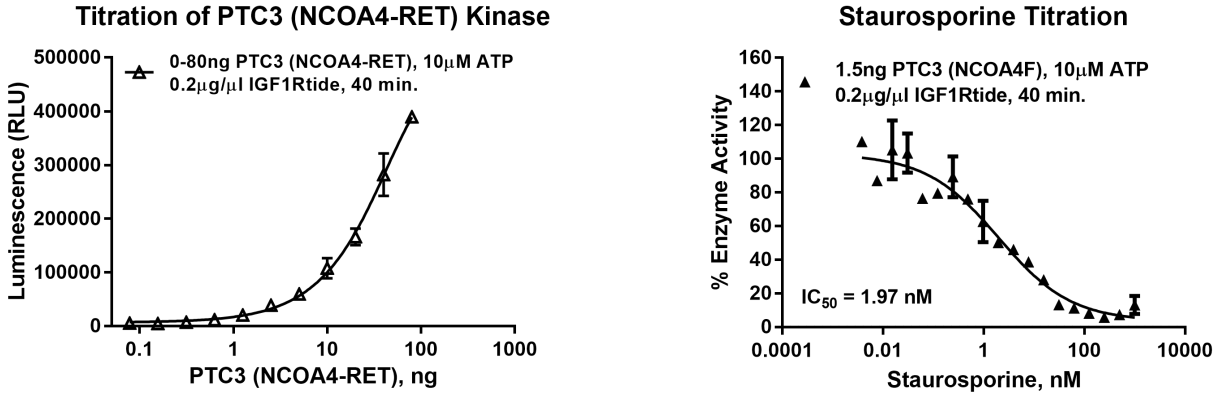

Figure 3. PTC3 (NCOA4-RET) Kinase Assay Development. (A) PTC3 (NCOA4-RET) enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1.5ng of PTC3 (NCOA4-RET) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
PTC3 (NCOA4-RET) Kinase Enzyme System	10µg	VA7264
	1mg	VA7265
ADP-Glo™ + PTC3 (NCOA4-RET) Kinase Enzyme System	1 Each	VA7266