ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

PTC1 (CCDC6-RET) Kinase Assay

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Scientific Background:

RET/PTC1 is fused of RET and the activating CCDC6 gene by intrachromosomal paracentric inversions in chromo-some 10 (1). Likes RET/PTC3, it is the most frequent RET rearrangements in papillary thyroid carcinoma (PTC) (2), especially in radiation-induced tumours. The RET/PTC1 rearrangements may be a marker for later-occurring PTC of radiation-exposed children and adults. The RET/PTC rearrangements also have been shown in benign thyroid lesions, including Hashimoto's thyroiditis (HT).

1. Grieco M. et al: PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. Cell 1990, 60:557-563.

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.

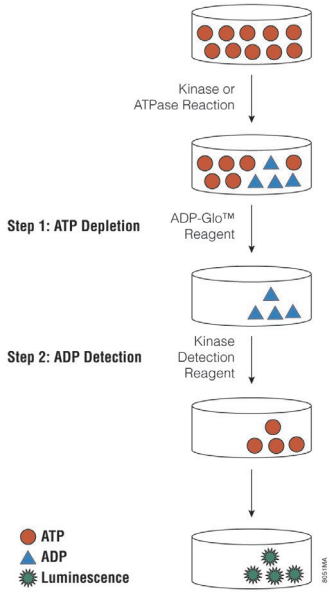

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

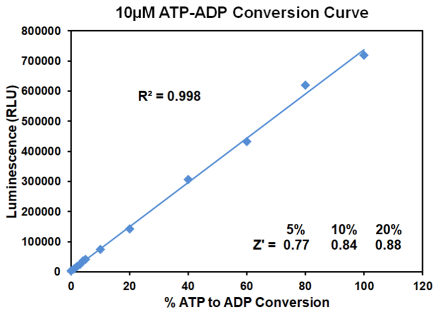

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	0.08	0
Luminescence	483,448	447,544	345,835	251,194	175,667	109,015	65,288	38,304	23,083	12,451	8,722	3,038
S/B	159	147	114	83	58	36	21	13	8	4	3	1
% Conversion	65	60	47	34	23	14	8	5	3	1	1	0

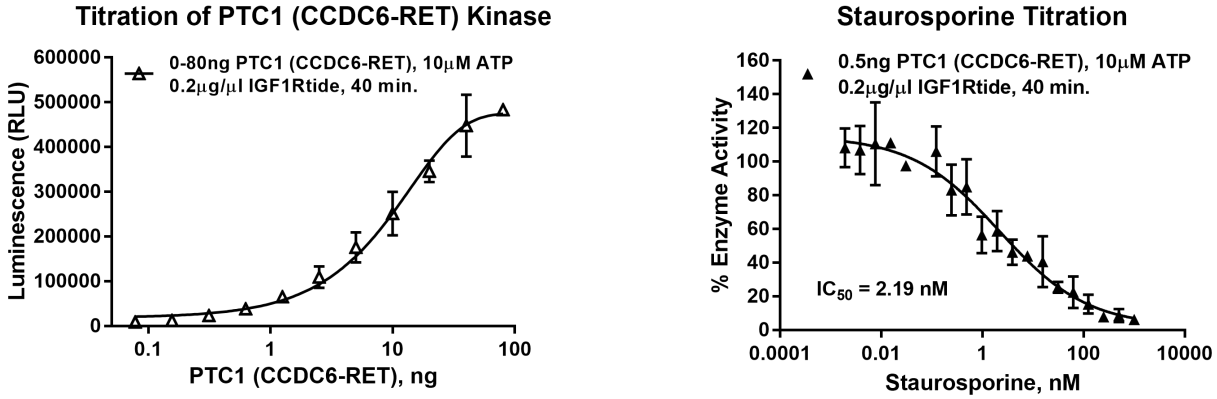

Figure 3. PTC1 (CCDC6-RET) Kinase Assay Development. (A) PTC1 (CCDC6-RET) enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 0.5ng of PTC1 (CCDC6-RET) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
PTC1 (CCDC6-RET) Kinase Enzyme System	10µg	VA7261
	1mg	VA7262
ADP-Glo™ + PTC1 (CCDC6-RET) Kinase Enzyme System	1 Each	VA7263