ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

RET (A883F) Kinase Assay

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Scientific Background:

RET or ret proto-oncogene is a member of the cadherin superfamily that encodes one of the receptor tyrosine kinases, which are cell-surface molecules that transduce signals for cell growth and differentiation. RET can undergo oncogenic activation in vivo and in vitro by cytogenetic rearrangement (1). Mutations in the RET gene are associated with the disorders multiple endocrine neoplasia, type IIA, multiple endocrine neoplasia, type IIB, Hirschsprung disease, and medullary thyroid carcinoma. RET signaling pathway, by regulating the development of both the nervous and lymphoid system in the gut, plays a key role in the molecular mechanisms that orchestrate intestine organogenesis (2).

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 60: 557-563, 1990.

2. Veiga-Fernandes, H. et.al: Tyrosine kinase receptors RET is a key regulator of Peyer's patch organogenesis. Nature 446: 547-551, 2007.

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.

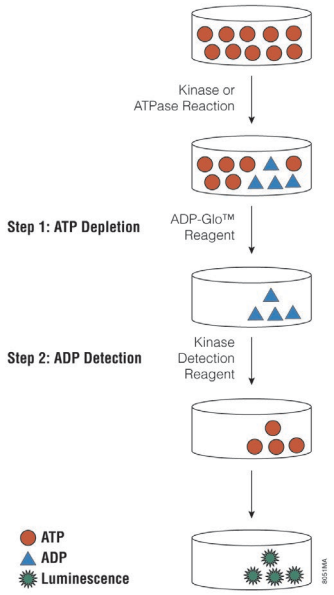

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

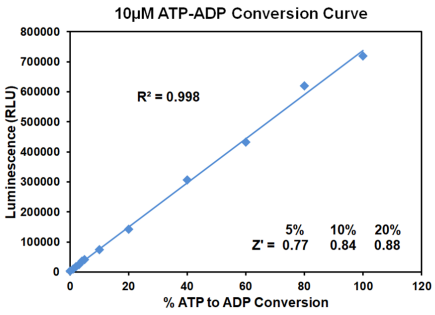

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	180	90	45	22.50	11.25	5.63	2.81	1.41	0.70	0.35	0
Luminescence	351,645	294,594	234,092	135,698	92,240	48,569	29,140	16,246	8,583	4,440	2,023
S/B	174	146	116	67	46	24	14	8	4	2	1
% Conversion	70	58	47	27	18	10	6	3	2	1	0

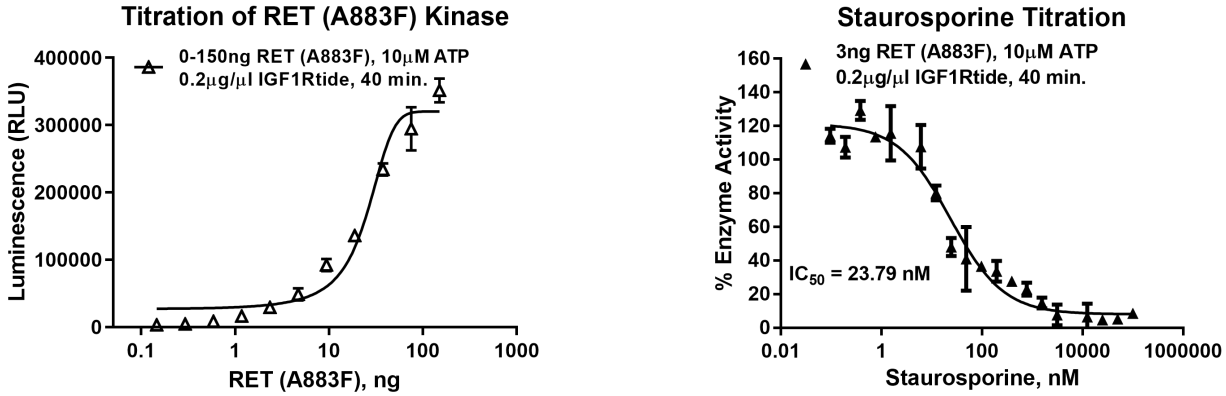

Figure 3. RET (A883F) Kinase Assay Development. (A) RET (A883F) enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 3ng of RET (A883F) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
RET (A883F) Kinase Enzyme System	10µg	VA7270
	1mg	VA7271
ADP-Glo™ + RET (A883F) Kinase Enzyme System	1 Each	VA7272