

RET (V804L) 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.

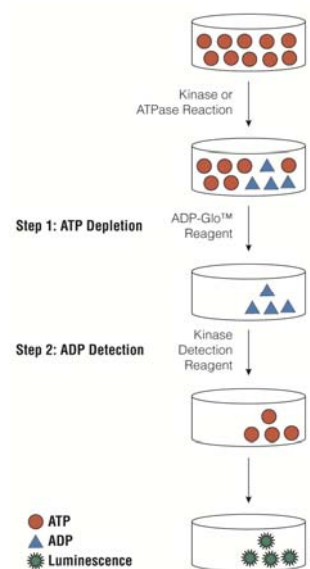


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

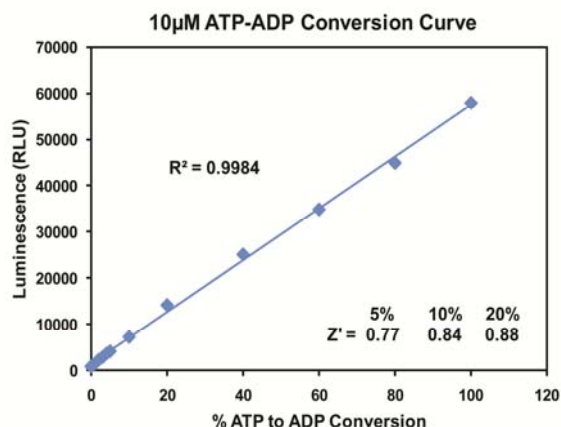
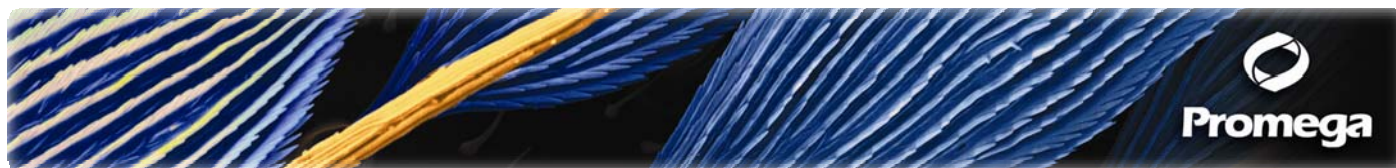


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10μM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.



For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay Technical Manual #TM313*, and the KES Protocol available at: <http://www.promega.com/tbs/tm313/tm313.html>, and <http://www.promega.com/KESProtocol>, respectively.

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Tyrosine Kinase Buffer.
- Add to the wells of 384 low volume plate:
1 μ l of inhibitor or (5% DMSO)
2 μ l of enzyme (defined from table 1)
2 μ l of substrate/ATP mix
- Incubate at room temperature for 60 minutes.
- Add 5 μ l of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10 μ l of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1sec).

Table 1. RET (V804L) 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.

RET (V804L), ng	200	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0
Luminescence	94936	79535	61133	51083	30094	16701	12985	5761	3678	1436	328
S/B	289	242	186	156	92	51	40	18	11	4	1
% Conversion	112	94	72	60	36	20	15	7	4	2	0

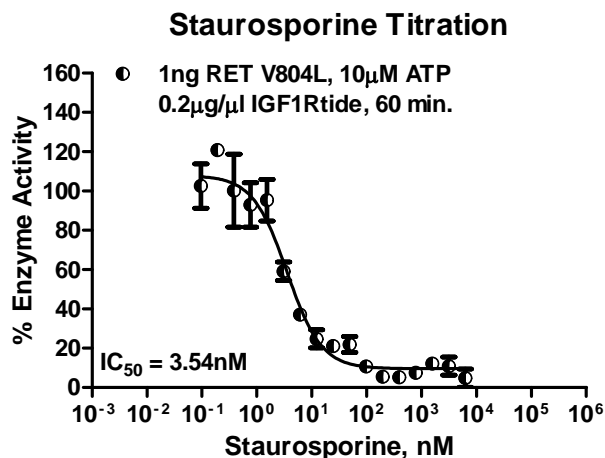
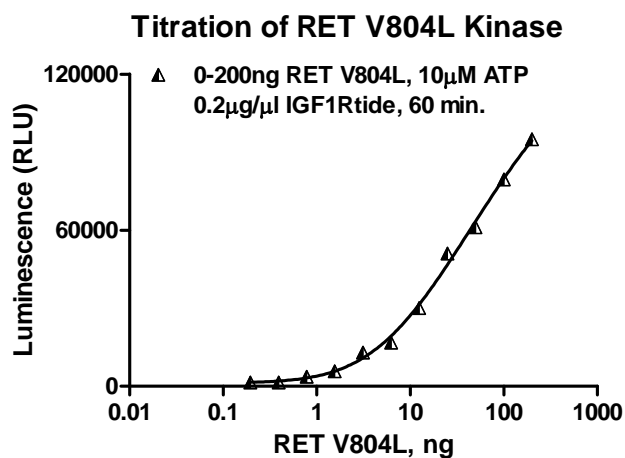


Figure 3. RET (V804L) Kinase Assay Development. (A) RET (V804L) enzyme was titrated using 10 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1ng of RET (V804L) to determine the potency of the inhibitor (IC₅₀).

Assay Components and Ordering Information:



Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
RET (V804L) Kinase Enzyme System	Promega	V4472
ADP-Glo™ + RET (V804L) Kinase Enzyme System	Promega	V4473

RET (V804L) Kinase Buffer: 40mM Tris, pH 7.5; 20mM MgCl₂; 0.1mg/ml BSA; 50 μ M DTT.