RET Kinase Assay

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Scientific Background:

RET gene codes for a transmembrane tyrosine kinase which is a subunit of a multimeric complex that acts as a receptor for four structurally related molecules: GDNF, neurturin, artemin and persephin (1). Germline mutations of RET cause a dominantly inherited dysgenesis of the enteric nervous system known as Hirschsprung's disease. RET is constitutively activated by point mutations in hereditary medullary thyroid carcinomas (MTCs). Several single nucleotide polymorphisms of the RET gene have been described. Multiple endocrine neoplasia type 2A (MEN 2A) have been reported to be associated with two mutations of the protooncogene RET (2).

1. Geneste, O. et al: Two distinct mutations of the RET receptor causing Hirschsprung's disease impair the binding of signalling effectors to a multifunctional docking site. Hum Mol. Genet. 1999; 8(11):1989-99.

2. Kahn, Tessitore, A. et al: A novel case of multiple endocrine neoplasia type 2A associated with two de novo mutations of the RET protooncogene. J. Clin. Endocrinol. Metab. 1999; 84(10):3522-7.

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.

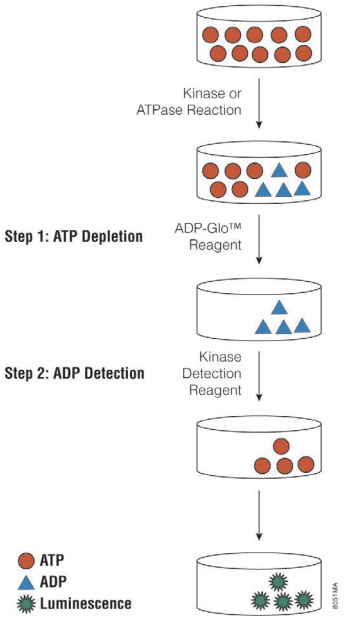
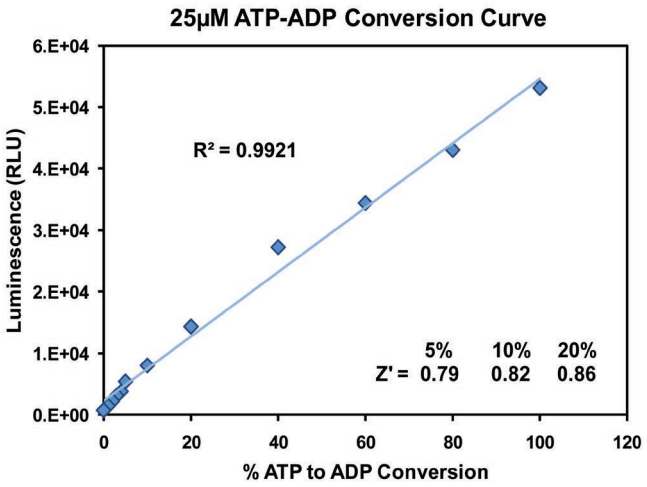


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.



For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase 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 60 minutes.
- 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 0.5-1second).

Table 1. RET 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, ng	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.00
Luminescence	101095	93775	83298	66260	51833	37355	23601	14506	8679	1977
S/B	51	47	42	34	26	19	12	7	4	1
% Conversion	86	80	70	55	42	29	17	9	4	0

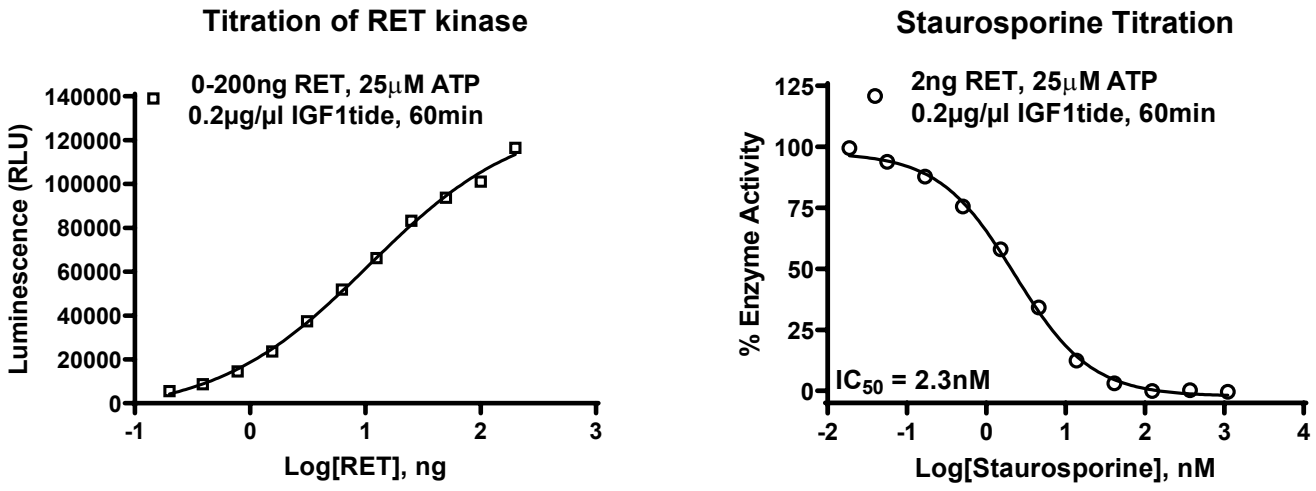


Figure 3. RET Kinase Assay Development: (A) RET enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 2ng of RET to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
RET Kinase Enzyme System	Promega	V3761
ADP-Glo + RET Kinase Enzyme System	Promega	V8061

RET Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.