ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

BCR-RET Kinase Assay

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Scientific Background:

BCR-RET is a chimaeric fusion of BCR and RET genes generated by a balanced translocation of genes from t(10;22)(q11;q11) and this leads to aberrant activation of RET activity which can transform hematopoietic cells and skew the hematopoietic differentiation program towards the monocytic/macrophage lineage (1). The BCR-RET fusion is frequently found in chronic myelomonocytic leukemia (CMML) cases. The RET fusion genes seem to constitutively mimic the same signaling pathway as RAS mutations frequently involved in CMML.

1. Ballerini, P. et al: RET fusion genes are associated with chronic myelomonocytic leukemia and enhance monocytic differentiation. Leukemia. 2012 Nov;26(11):2384-9.

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.

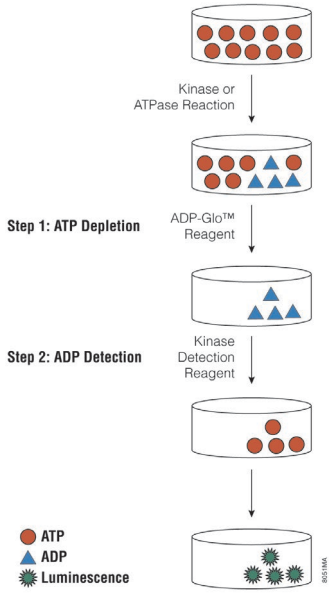

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

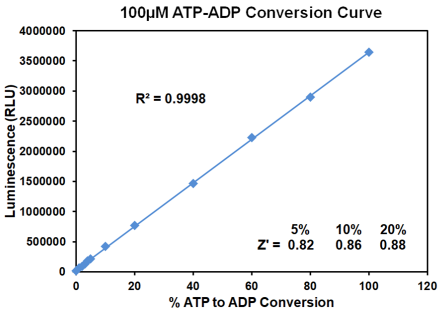

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	120	60	30	15	7.50	3.75	1.88	0.94	0
Luminescence	1,603,260	942,819	601,518	319,871	177,203	99,282	54,045	31,553	14,492
S/B	111	65	42	22	12	7	4	2	1
% Conversion	43	25	16	8	4	2	1	0	0

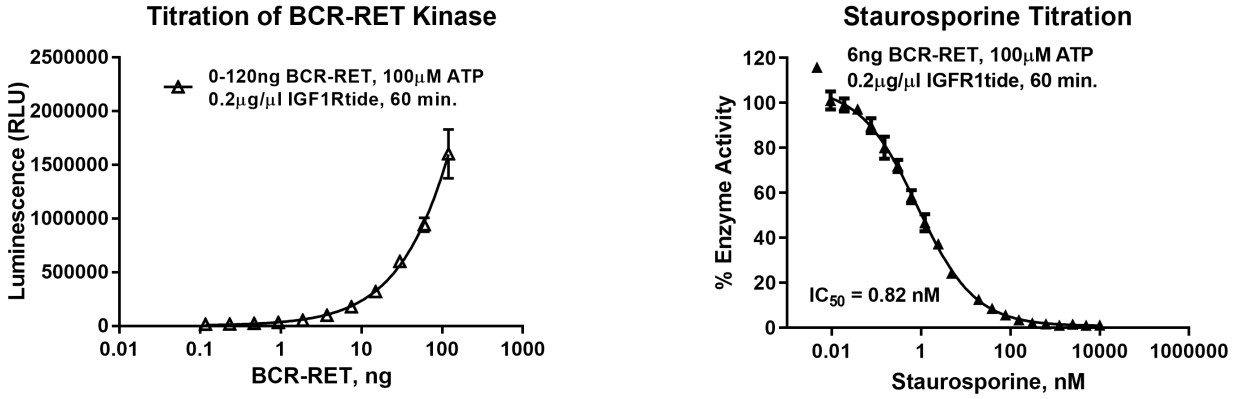

Figure 3. BCR-RET Kinase Assay Development. (A) BCR-RET enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 6ng of BCR-RET to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
BCR-RET Kinase Enzyme System	10µg	VA7030
	1mg	VA7031
ADP-Glo™ + BCR-RET Kinase Enzyme System	1 Each	VA7032