ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

MET (D1228N) Kinase Assay

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Scientific Background:

MET is a proto-oncogene that encodes a transmembrane growth factor receptor which is a heterodimer of two di-sulphide linked chains of 50 kDa (alpha) and 145 kDa (beta). MET is widely expressed in the kidney, brain, lung, skin, and embryonic tissue (1). Hepatocyte growth factor (HGF) binds to MET and activates its tyrosine kinase activity. MET is overexpressed and activated in a variety of human cancers including pancreatic, colon, gastric, cervical and ovarian cancers and has been shown to be involved in tumor cell migration and invasion (2). MET(D1228N) is one of the native mutant forms of MET.

1. Giordano, S. et al: Biosynthesis of the protein encoded by the c-met proto-oncogene. Oncogene. 1989 Nov;4(11):1383-8.

2. Iyer, A. et al: Structure, tissue-specific expression, and transforming activity of the mouse met protooncogene. Cell Growth Differ. 1990 Feb;1(2):87-95.

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.

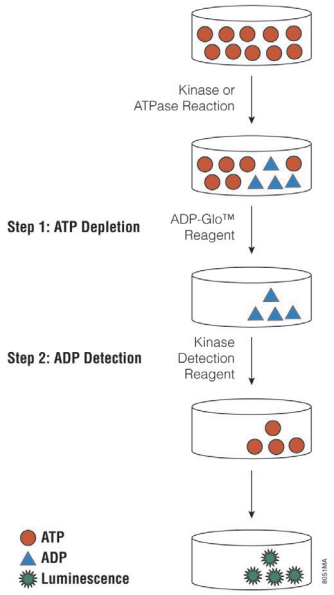

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

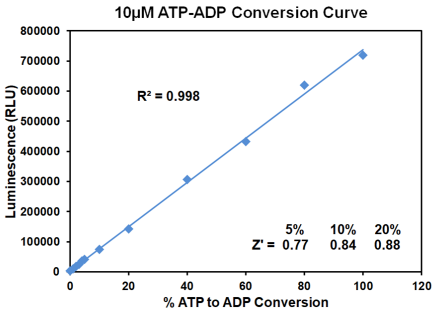

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	200	100	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0
Luminescence	235,807	185,454	193,931	152,425	108,437	54,378	31,743	14,249	7,830	3,652	2,314	830
S/B	284	223	234	184	131	66	38	17	9	4	3	1
% Conversion	75	59	62	48	34	17	9	4	2	0	0	0

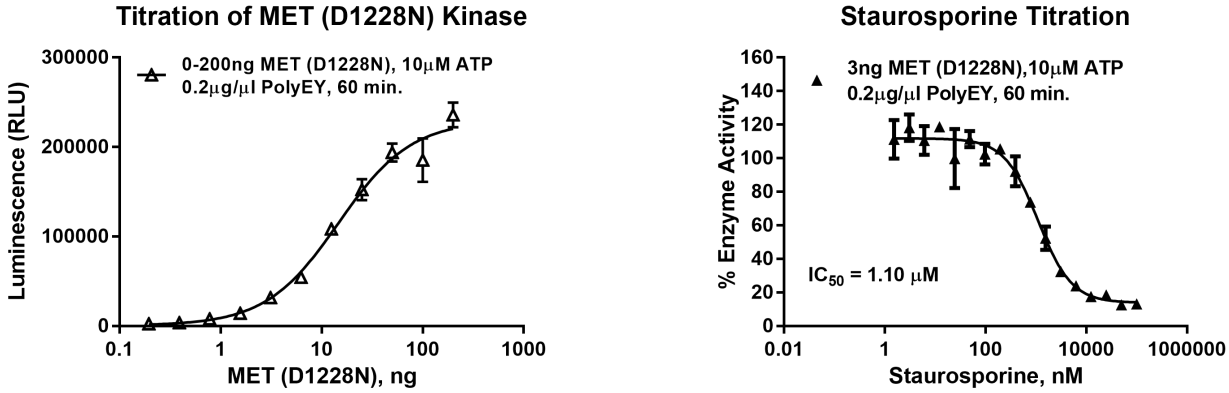

Figure 3. MET (D1228N) Kinase Assay Development. (A) MET (D1228N) enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 3ng of MET (D1228N) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
MET (D1228N) Kinase Enzyme System	10µg	VA7228
	1mg	VA7229
ADP-Glo™ + MET (D1228N) Kinase Enzyme System	1 Each	VA7230