

ADP-Glo™ Kinase Assay Application Notes

TYROSINE KINASE SERIES: c-MER



c-MER Kinase Assay

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Scientific Background:

c-MER is a tyrosine kinase and a member of the MER/AXL/TYRO3 receptor kinase family (1). The c-MER gene encodes a transmembrane protein with two fibronectin type-III domains, two Ig-like C2-type domains, and one tyrosine kinase domain. Functional knockout of c-MER in mice shows the mice to have macrophages deficient in the clearance of apoptotic thymocytes thereby demonstrating the critical role for c-MER in the engulfment and efficient clearance of apoptotic cells. Defects in c-MER have been associated with retinitis pigmentosa, a phenotype that can be reversed by replacement of the c-MER gene product (2).

1. Graham, D. K. et al: Cloning and mRNA expression analysis of a novel human protooncogene, c-mer. *Cell Growth Differ.* 5: 647-657, 1994.
2. Gal, A. et al: Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. *Nature Genet.* 26: 270-271, 2000.

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.

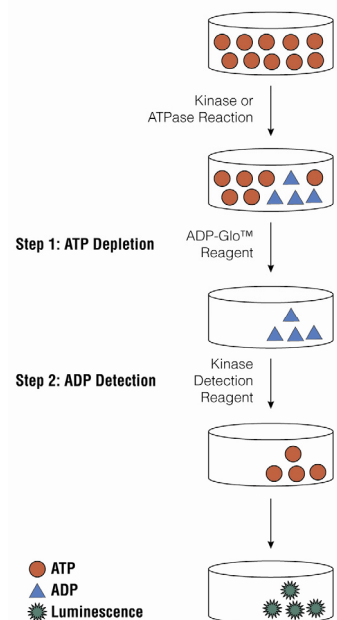


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

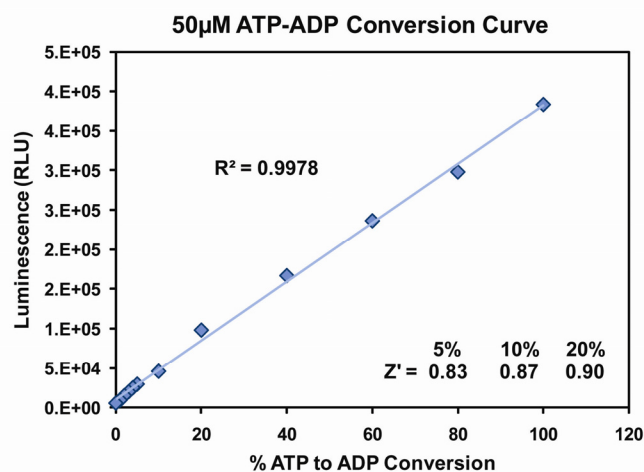


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.



For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay Technical Manual #TM313*, 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 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 0.5-1second).

Table 1. c-MER 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.

c-MER, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.39	0
Luminescence	115636	74102	55259	30035	24080	19480	14230	10272	7081	2651
S/B	43.6	28.0	20.8	11.3	9.1	7.3	5.4	3.9	2.7	1.0
% Conversion	53.5	34.2	25.4	13.6	10.9	8.7	6.3	4.4	2.9	0.0

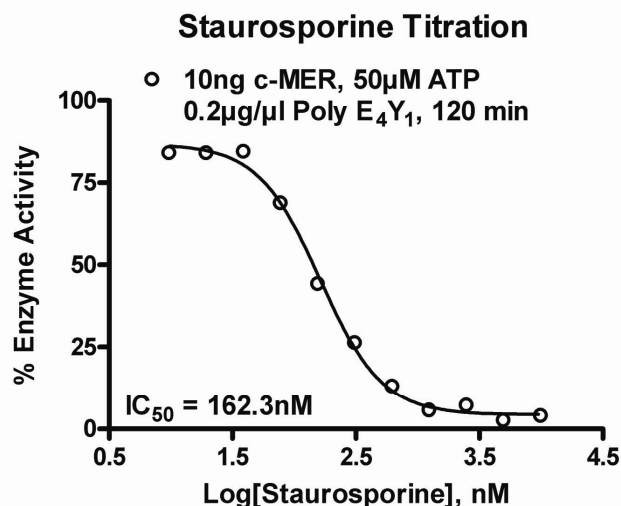
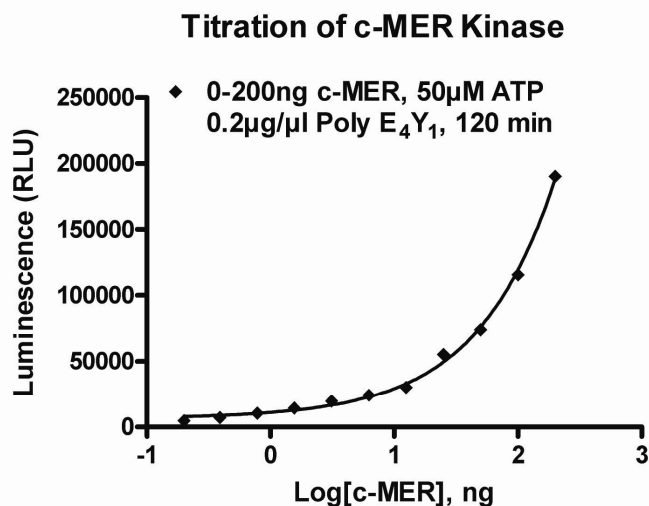


Figure 3. c-MER Kinase Assay Development: (A) c-MER enzyme was titrated using 50 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of c-MER to determine the potency of the inhibitor (IC₅₀).

Assay Components and Ordering Information:



Products

ADP-Glo™ Kinase Assay
 c-MER Kinase Enzyme System
 ADP-Glo + c-MER Kinase Enzyme System

Company

Promega
 Promega
 Promega

Cat.#

V9101
 V3541
 V9561

c-MER Kinase Buffer: 40mM Tris,7.5; 20mM MgCl₂; 0.1mg/ml BSA; 2mM MnCl₂, 50 μ M DTT.