ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

MRCKβ Kinase Assay

By Juliano Alves, Laurie Engel, Said A. Goueli, and Hicham Zegzouti, Promega Corporation

Scientific Background:

Myotonic Dystrophy Kinase-Related cdc42-binding kinase beta (MRCKβ) belongs to the DMPK subfamily (1). The myotonic dystrophy kinase-related kinases and myotonic dystrophy kinase-related Cdc42 binding kinase (MRCK) are effectors of RhoA and Cdc42, respectively, where they are involved in actin cytoskeletal reorganization and neurite outgrowth (2). Effects of the repeat expansion on the DMPK gene may be responsible for muscle and heart features of Myotonic Dystrophy.

1. Lam. L T. et al: Characterization of a monoclonal antibody panel shows that the myotonic dystrophy protein kinase, DMPK, is expressed almost exclusively in muscle and heart. Hum. Mol. Genet. 2000; 9(14): 2167-73.

2. Tan, I. et al: Phosphorylation of a novel myosin binding subunit of protein phosphatase 1 reveals a conserved mechanism in the regulation of actin cytoskeleton. J. Biol Chem. 2001; 276(24):21209-16.

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.

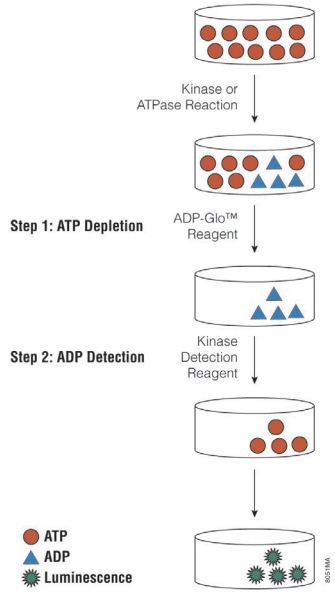

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

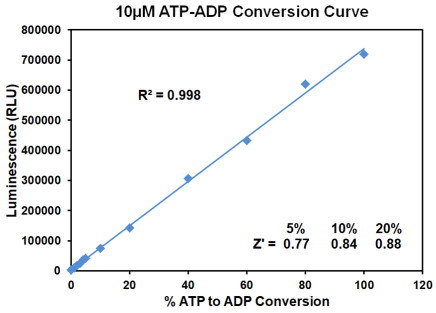

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	180	90	45	22.50	11.25	5.63	2.81	1.41	0.70	0.35	0.18	0
Luminescence	344,541	279,378	217,029	204,120	121,235	71,369	37,230	23,892	12,873	7,862	5,208	2,410
S/B	143	116	90	85	50	30	15	10	5	3	2	1
% Conversion	81	66	51	48	28	17	9	5	3	2	1	0

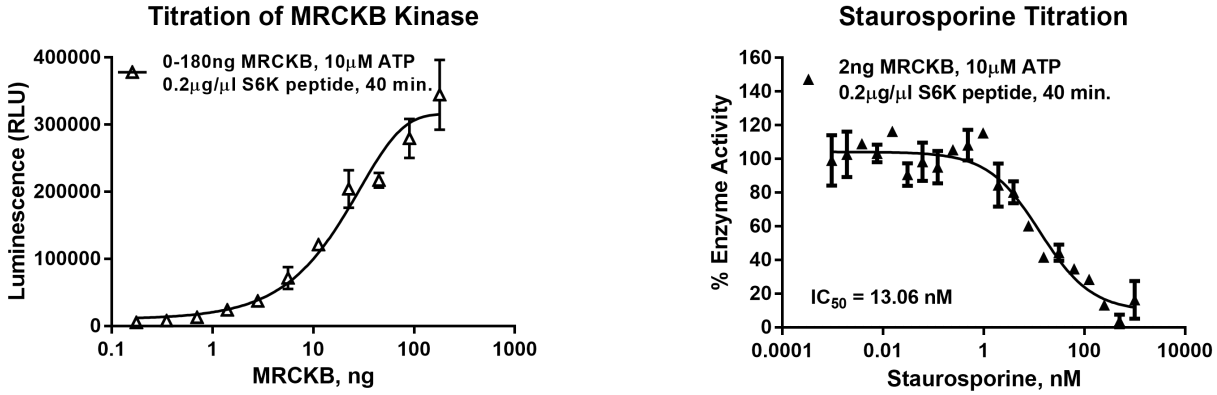

Figure 3. MRCKβ Kinase Assay Development. (A) MRCKβ enzyme was titrated using 10 µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2 ng of MRCKβ to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
MRCKβ Kinase Enzyme System	10µg	VA7243
	1mg	VA7244
ADP-Glo™ + MRCKβ Kinase Enzyme System	1 Each	VA7245