ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: MRCKα

MRCKα Kinase Assay

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Scientific Background:

Myotonic Dystrophy Kinase-Related Cdc42-Binding Kinase α (MRCKα) is a Cdc42/Rac/Rho interactive/binding serine/threonine kinase with multiple functional domains (1). MRCK are effectors of RhoA and Cdc42, respectively, for actin reorganization. MRCKα is a critical regulator of signal transduction pathways in eukaryotic cells that are known principally for their role in regulating the cytoskeleton, and they do so by recruiting a variety of downstream effector proteins (2).

1. Ivan, T. et al: Genomic organization of human myotonic dystrophy kinase-related Cdc42-binding kinase α reveals multiple alternative splicing and functional diversity. Gene; 2003; 304:107-115.

2. Ivan, T. 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): 21209-21216.

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.

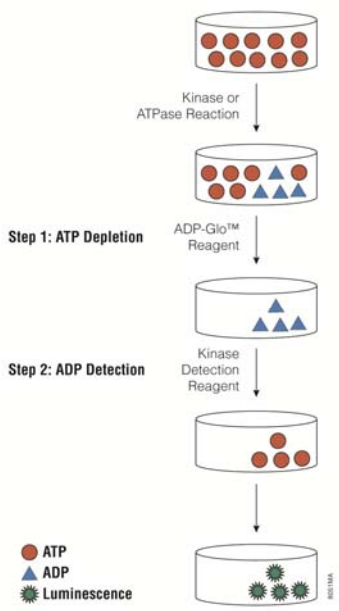


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

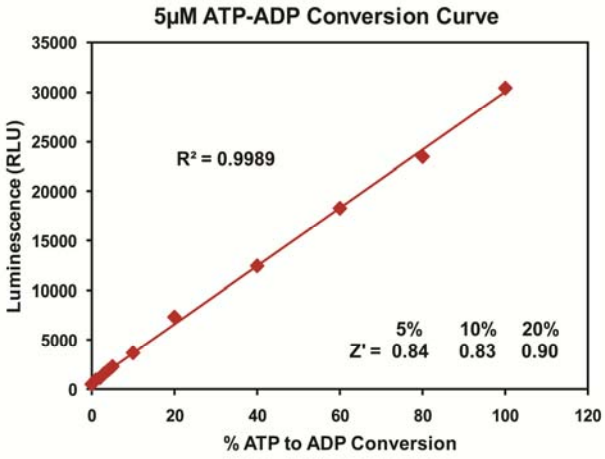


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 5µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

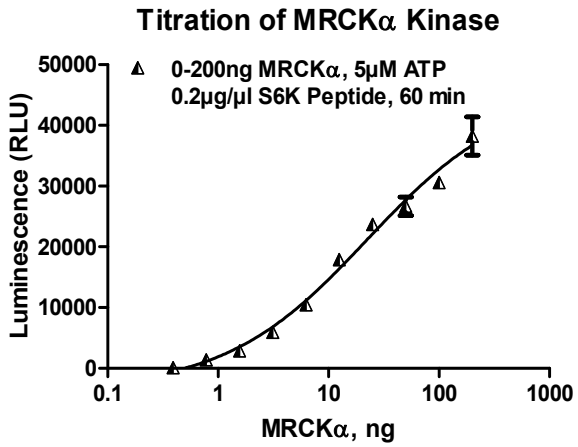
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, and the KES Protocol available at: http://www.promega.com/tbs/tm313/tm313.html, and http://www.promega.com/KESProtocol, respectively.

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 0.5-1sec).

Table 1. MRCKα 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.

MRCKα, ng	200	100	50	25	12.5	6.3	3.1	1.6	0.8	0
Luminescence	38239	30597	26676	23687	17902	10459	5920	2903	1400	93
S/B	411	329	287	255	192	112	64	31	15	1
% Conversion	108	85	74	65	48	27	15	7	3	0



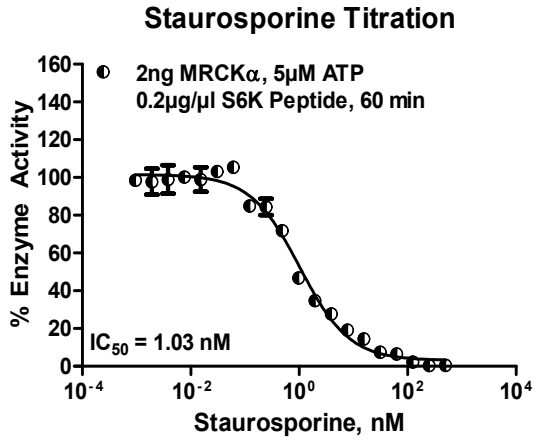


Figure 3. MRCKα Kinase Assay Development. **(A) MRCKα enzyme was titrated using 5µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 2ng of MRCKα to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
MRCKα Kinase Enzyme System	Promega	V5710
ADP-Glo™ + MRCKα Kinase Enzyme System	Promega	V5711

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