ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

MYLK2 Kinase Assay

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Scientific Background:

MYLK2 is a member of the myosin light chain kinase family and is a calcium/calmodulin dependent enzyme that is exclusively expressed in adult skeletal muscle (1). MYLK2 has been proposed to participate in signaling pathways (calcium signaling pathway, focal adhesion, regulation of actin cytoskeleton) and cellular processes (neuromuscular synaptic transmission, protein/amino acid phosphorylation). MYLK2 is involved in multiple molecular functions as a result of various subdomains that participate in ATP binding, calmodulin binding, nucleotide binding, protein serine/threonine kinase activity and transferase activity) (2).

1. Soung, Y.H. et al. Mutational analysis of the kinase domain of MYLK2 gene in common human cancers.Pathol Res Pract. 2006;202(3):137-40.

2. Toth-Zsamboki, E. et al. P2X1-mediated ERK2 activation amplifies the collagen-induced platelet secretion by enhancing myosin light chain kinase activation.J. Biol Chem. 2003; 21;278(47):46661-7.

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.

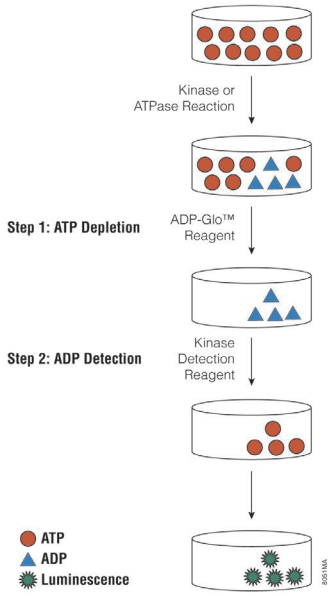

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

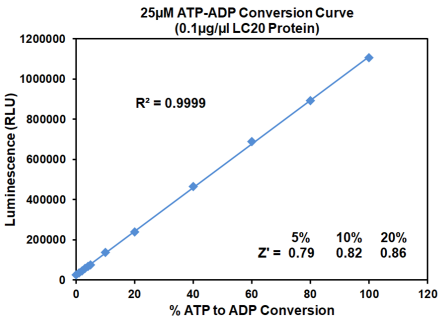

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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
Luminescence	256,328	125,543	74,338	58,875	40,764	27,698	15,480	9,936	5,105
S/B	50	25	15	12	8	5	3	2	1
% Conversion	21	9	4	3	1	0	0	0	0

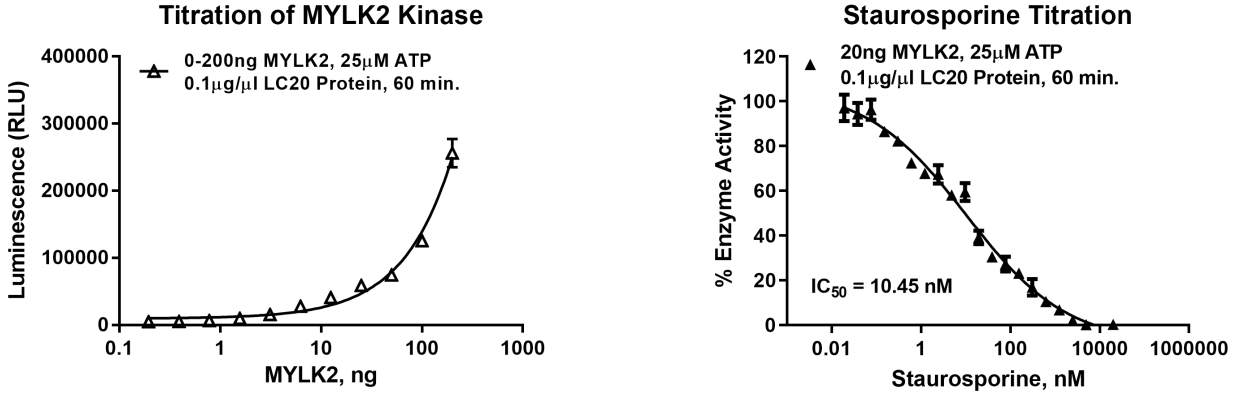

Figure 3. MYLK2 Kinase Assay Development. (A) MYLK2 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 20ng of MYLK2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
MYLK2 Kinase Enzyme System	10µg	VA7510
	1mg	VA7511
ADP-Glo™ + MYLK2 Kinase Enzyme System	1 Each	VA7512