MYO3β Kinase Assay

By Mary Sobol, M.S., Juliano Alves, Ph.D., Said A. Goueli, Ph.D., and Hicham Zegzouti, Ph.D., Promega Corporation

Scientific Background:

MYO3β is a member of the Class III myosins that are actin-dependent motor proteins containing an amino-terminal kinase domain. MYO3β contains a N-terminal kinase domain, followed by motor, neck, and tail domains. The MYO3β gene generates a variety of splice variants that contain 1 or 2 calmodulin-binding (IQ) motifs in the neck domain and 1 of 3 domains in the tail domain. Northern blot analysis shows expression of a 7-kb MYO3β transcript in the human retina but not in a RPE cell line (1). The MYO3β gene transcript is also detected in the kidney, intestine and testis. The MYO3β gene maps to chromosome 2q31.1-q31.2 by genomic sequence alignment.

1. Dose, A C. et al: A class III myosin expressed in the retina is a potential candidate for Bardet-Biedl syndrome. Genomics 79: 621-624, 2002.

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.

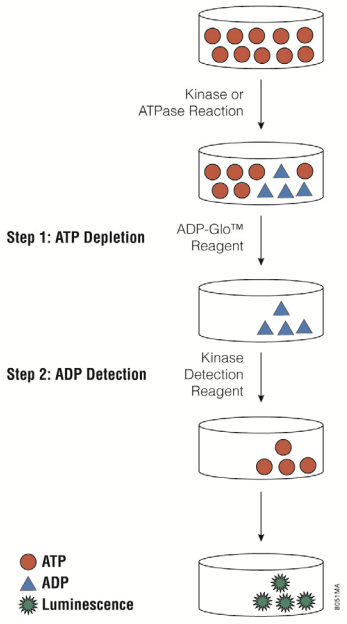

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

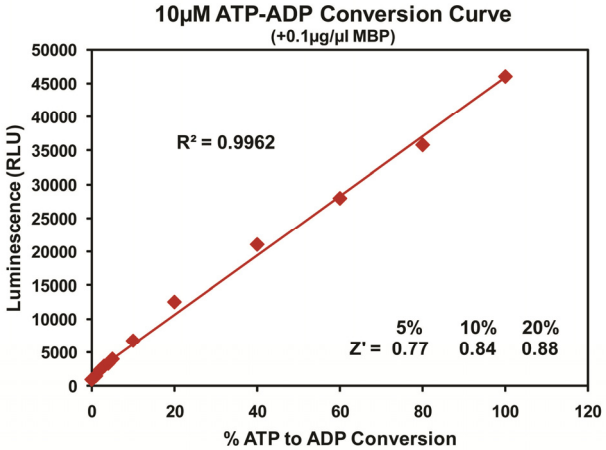

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 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 0.5-1second).

Table 1. MYO3β 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.

MYO3β, ng	100	50	25	13	6	3	2	1	0.5	0.2	0
RLU	78873	74237	71419	72331	61677	48164	34687	22409	11277	5797	791
S/B	100	94	90	91	78	61	44	28	14	7	1
% Conversion	100	97	93	94	80	62	45	28	14	6	0

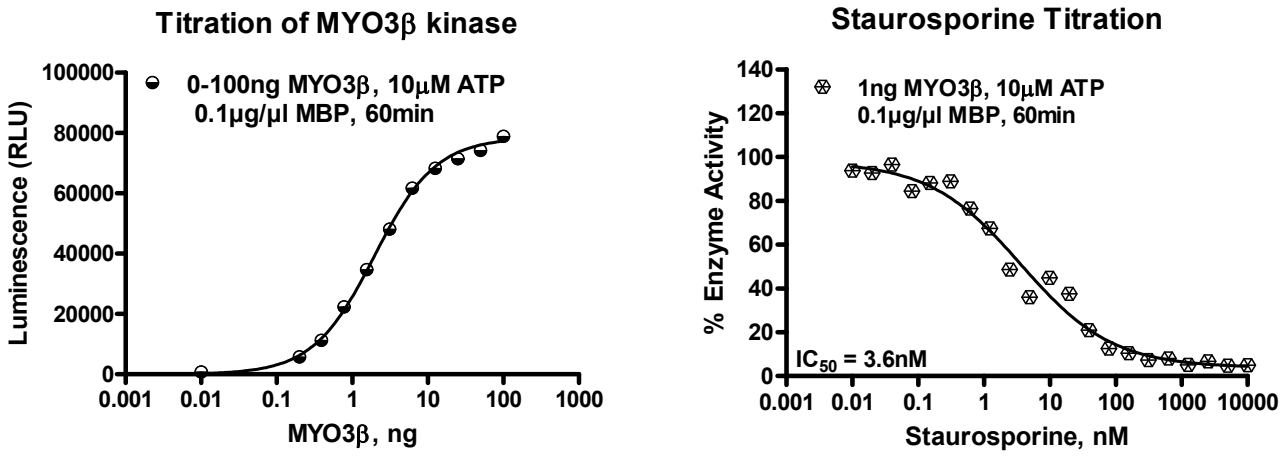

Figure 3. MYO3β Kinase Assay Development. (A) MYO3β enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1ng of MYO3β to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
MYO3β Kinase Enzyme System	Promega	V4074
ADP-Glo™ + MYO3β Kinase Enzyme System	Promega	V4075

MYO3β Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

Promega Corporation • 2800 Woods Hollow Road • Madison, WI 53711-5399 USA • Telephone 608-274-4330 • Fax 608-277-2601
promega.com