ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

TMP3-ROS1 Kinase Assay

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

Scientific Background:

TPM3 is a member of the tropomyosin family of actin-binding proteins which are dimers of coiled-coil proteins that provide stability to actin filaments and regulate access of other actin-binding proteins. Mutations in TPM3 gene result in autosomal dominant nemaline myopathy and other muscle disorders (1). TPM3 gene locus is involved in translocations with other loci including c-ros oncogene 1 (ROS1) which result in the formation of the TPM3-ROS1 fusion protein that act as an oncogene. TPM3-ROS1 fusion gene product has been detected in lung adenocarcinoma is higher in lung adenocarcinoma with wild-type EGFR (2).

1. Lawlor, M W. et al: Mutations of tropomyosin 3 (TPM3) are common and associated with type 1 myofiber hypotrophy in ongenital fiber type disproportion. Hum Mutat. 2010 Feb;31(2):176-83.

2. Zhao C. et al: Detecting ALK, ROS1 and RET Fusion Genes in Cell Block Samples. Transl Oncol. 2014 Jun 17;7 (3):363-7.

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.

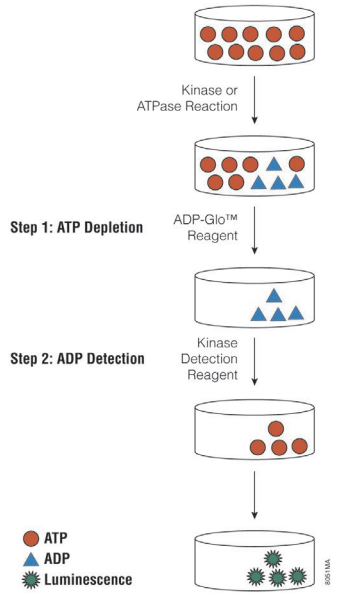

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

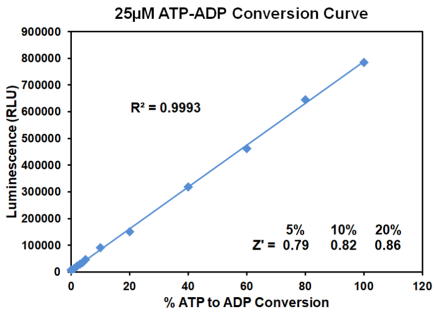

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0.59	0.29	0
Luminescence	1,157,170	827,511	663,528	511,772	351,200	177,284	75,979	37,740	19,966	13,181	5,339
S/B	217	155	124	96	66	33	14	7	4	2	1
% Conversion	128	91	73	56	38	19	8	3	1	1	0

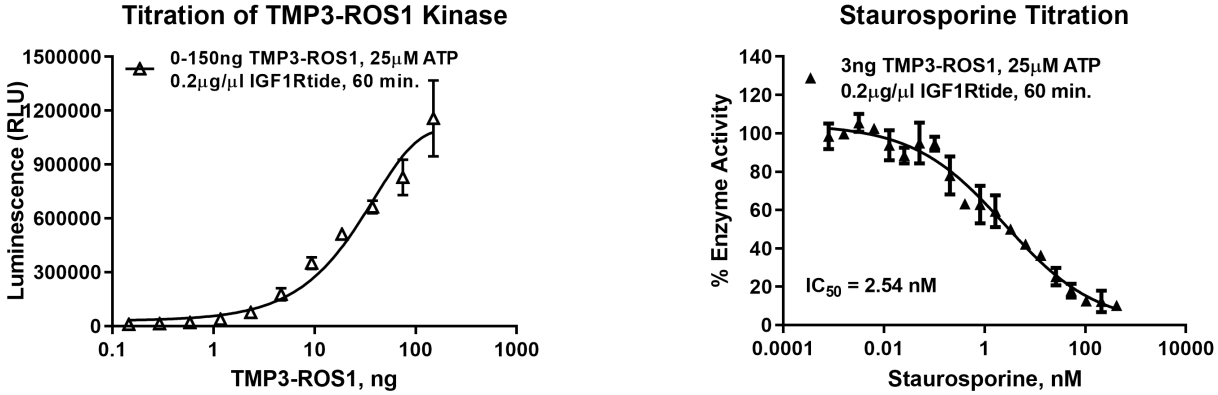

Figure 3. TMP3-ROS1 Kinase Assay Development. (A) TMP3-ROS1 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 3ng of TMP3-ROS1 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
TMP3-ROS1 Kinase Enzyme System	10µg	VA7318
	1mg	VA7319
ADP-Glo™ + TMP3-ROS1 Kinase Enzyme System	1 Each	VA7320