ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

YSK4 (MAP3K19) Kinase Assay

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Scientific Background:

YSK4 (MAP3K19) belongs to STE Ser/Thr protein kinase family and STE20 subfamily. The YSK4 gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, and rat (1). The important paralog is MAP3K1 gene. The RCK ligand-binding domains form an octameric gating ring (2) in the large-conductance Ca2+-activated K+ channels. The YSK4 kinase function has not been made clearly yet.

1. Hillier LW, et al: Generation and annotation of the DNA sequences of human chromosomes 2 and 4. Nature, 2005 Apr 7.

2. Ye S. et al: Crystal Structures of a Ligand-free MthK Gating Ring: Insights into the Ligand Gating Mechanism of K(+) Channels. Cell (2006).

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.

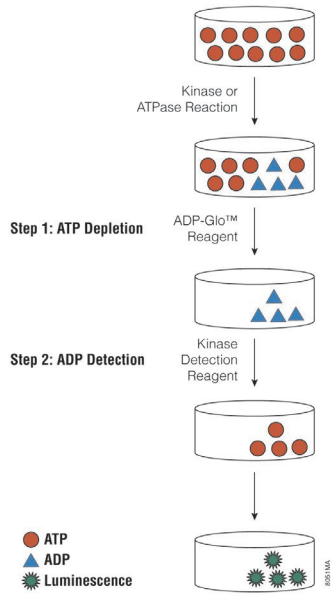

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

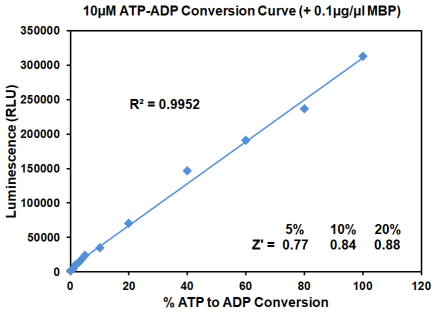

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	150	75	37.50	18.75	9.38	4.69	2.34	1.17	0.59	0.29	0.15	0
Luminescence	189,318	142,996	149,090	116,077	80,616	43,180	21,281	8,355	3,457	1,531	987	591
S/B	320	242	252	196	136	73	36	14	6	3	2	1
% Conversion	108	81	85	66	45	23	10	3	0	0	0	0

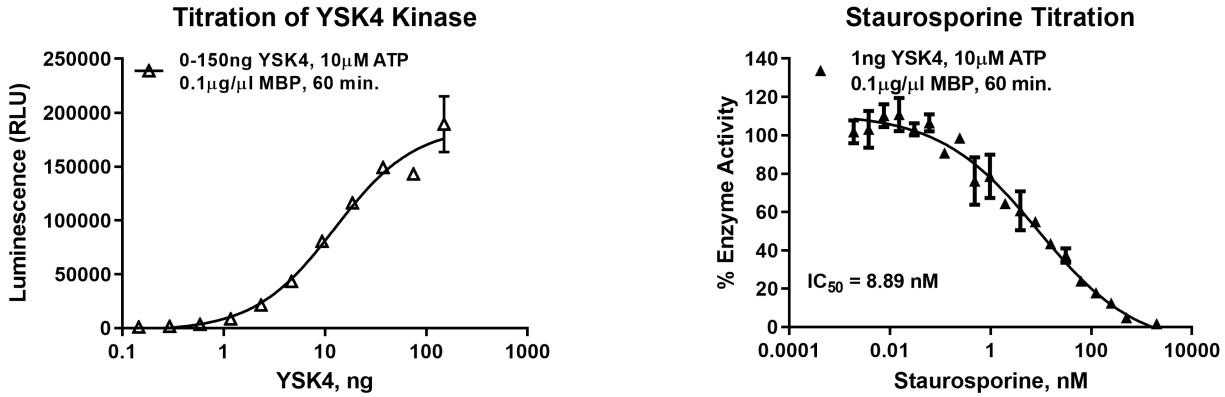

Figure 3. YSK4 (MAP3K19) Kinase Assay Development. (A) YSK4 (MAP3K19) enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1ng of YSK4 (MAP3K19) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
YSK4 (MAP3K19) Kinase Enzyme System	10µg	VA7354
	1mg	VA7355
ADP-Glo™ + YSK4 (MAP3K19) Kinase Enzyme System	1 Each	VA7356