ADP-Glo™ Kinase Assay Application Note

Ser-Thr Kinase Series

TSSK2 Kinase Assay

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Scientific Background:

TSSK2 is an intronless serine-threonine kinase that was originally detected on mouse chromosome 16 (1). TSSK2 and TSSK1 share 72% overall amino acid identity and 83% identity in the kinase domain (2). Real-time PCR showed abundant TSSK2 expression in testis and lower levels in heart, brain, and placenta while Western blot analysis detected TSSK2 at the predicted molecular mass of 40kDa in human sperm and testis. Co-immunoprecipitation and yeast 2-hybrid analysis show that TSSK2 can interact with a protein TSKS, that also acts as a substrate for this protein kinase.

1. Nayak, S. et al: Immunohistochemical analysis of the expression of two serine-threonine kinases in the maturing mouse testis. Mech Dev. 1998 Jun;74(1-2):171-4.

2. Hao, Z. et al: Expression analysis of the human testis-specific serine/threonine kinase (TSSK) homologues: a TSSK member is present in the equatorial segment of human sperm. Molec. Hum. Reprod. 10: 433-444, 2004.

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.

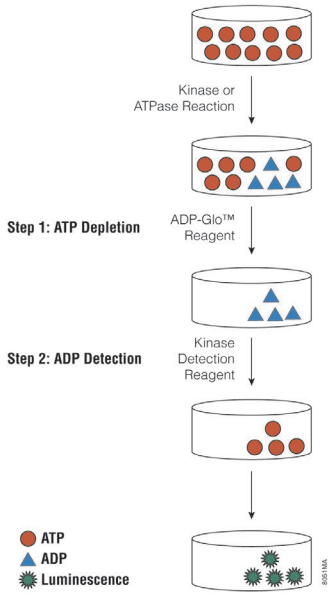

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

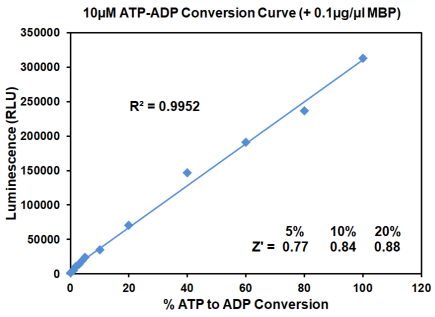

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	240	120	60	30	15	7.50	3.75	1.88	0
Luminescence	137,564	62,782	36,835	20,288	9,988	5,433	2,866	1,768	711
S/B	193	88	52	29	14	8	4	2	1
% Conversion	46	20	11	6	2	0	0	0	0

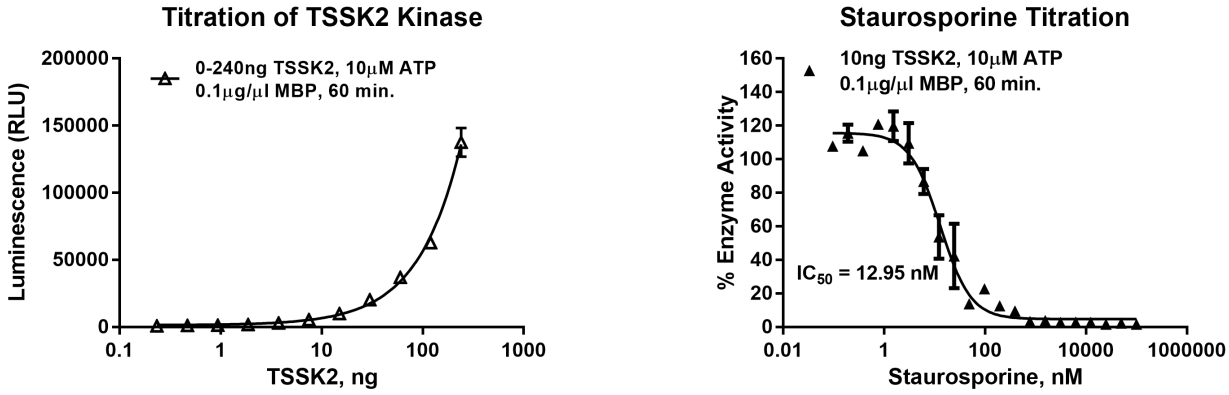

Figure 3. TSSK2 Kinase Assay Development. (A) TSSK2 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 10ng of TSSK2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
TSSK2 Kinase Enzyme System	10µg	VA7573
	1mg	VA7574
ADP-Glo™ + TSSK2 Kinase Enzyme System	1 Each	VA7575