ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

# TSSK1B Kinase Assay

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## Scientific Background:

TSSK1B (testis-specific serine kinase 1B) is a member of a unique subfamily of protein kinases belonging to the AMPK branch on the human kinome tree. TSSK1B is a serine/threonine kinases that is highly expressed in testis and lower express in pancreas, and weak expression in a few other tissues. TSSK members are present in the equatorial segment of human sperm. TSSK1B share 72% amino acid identity and 83% identity in the kinase domain with other family TSSK members and the TSSK1 gene is intronless. In vitro kinase assay shows phosphorylation of TSKS by TSSK1 and TSSK2.

1. 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.

2. Xu, B. et al: Validation of a testis specific serine/threonine kinase [TSSK] family and the substrate of TSSK1 & 2, TSKS, as contraceptive targets. Soc Reprod Fertil Suppl. 2007;63:87-101.

## 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.

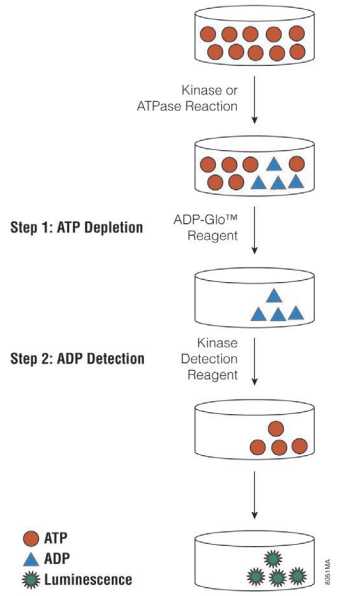


**Figure 1. Principle of the ADP-Glo™ Kinase Assay.** The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

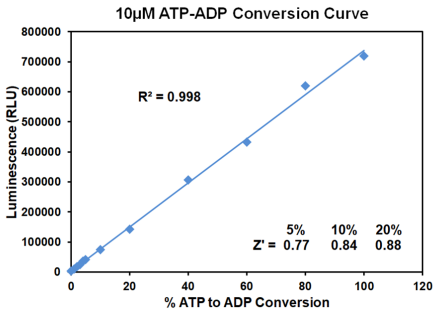


**Figure 2. Linearity of the ADP-Glo Kinase Assay.** ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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 | 200 | 100 | 50 | 25 | 12.50 | 6.25 | 3.13 | 1.56 | 0.78 | 0.39 | 0.20 | 0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Luminescence | 398,160 | 379,445 | 296,809 | 206,106 | 118,985 | 59,537 | 34,147 | 18,880 | 10,529 | 5,658 | 4,138 | 1,771 |
| S/B | 225 | 214 | 168 | 116 | 67 | 34 | 19 | 11 | 6 | 3 | 2 | 1 |
| % Conversion | 84 | 80 | 63 | 43 | 25 | 12 | 7 | 3 | 2 | 1 | 0 | 0 |

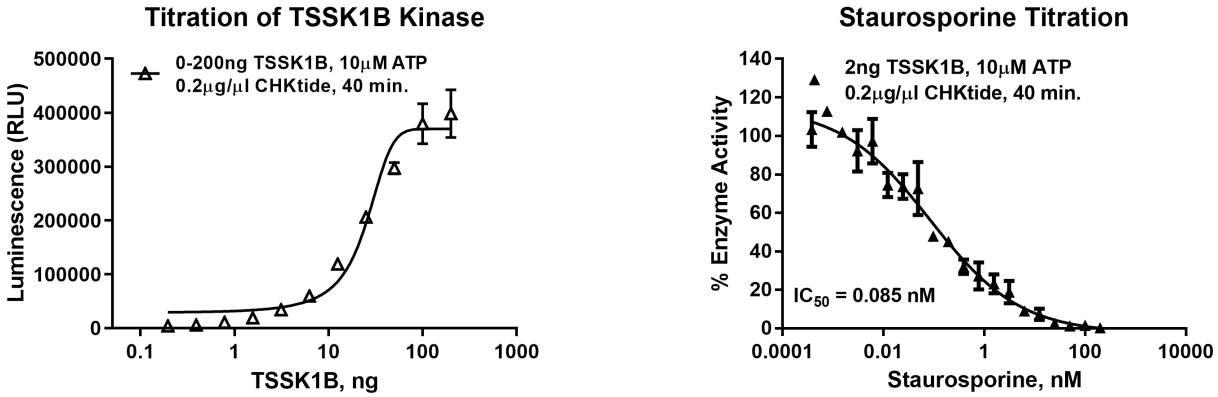


**Figure 3. TSSK1B Kinase Assay Development.** (A) TSSK1B enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2ng of TSSK1B to determine the potency of the inhibitor ($IC_{50}$).

## Ordering Information:

| Products | Size | Cat. # |
|---|---|---|
| TSSK1B Kinase Enzyme System | 10µg | VA7345 |
| | 1mg | VA7346 |
| ADP-Glo™ + TSSK1B Kinase Enzyme System | 1 Each | VA7347 |