ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

TXK Kinase Assay

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Scientific Background:

TXK or RLK is a member of the TEC family of non-receptor tyrosine kinases. TXK is expressed in T-cells and is an important component of signaling pathways downstream of lymphocyte antigen receptor. TXK is phosphorylated in response to T-cell receptor stimulation and can be activated by phosphorylation by Src family kinases. However, TXK is phosphorylated independent of PI-3K activity (1). Excessive TXK protein expression is seen in patients with Behcet's disease. Over production of TXK leads to increased Th1 cell function that is involved in the pathogenesis of Behcet's disease (2).

1. Czar, M J. et al: Biochemical and genetic analyses of the Tec kinases Itk and Rlk/Txk. Biochem Soc Trans. 2001 Nov;29(Pt 6):863-7.

2. Suzuki, N. et al: Skewed Th1 responses caused by excessive expression of Txk, a member of the Tec family of tyrosine kinases, in patients with Behcet's disease. Clin Med Res. 2006 Jun;4(2):147-51.

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.

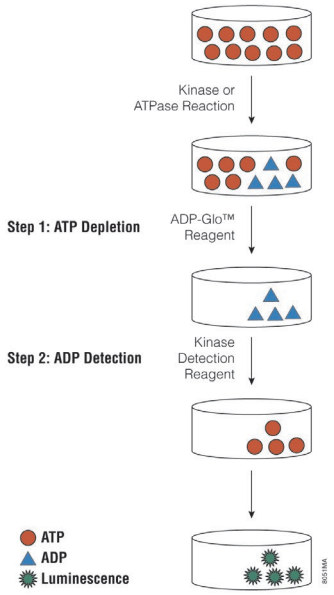

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

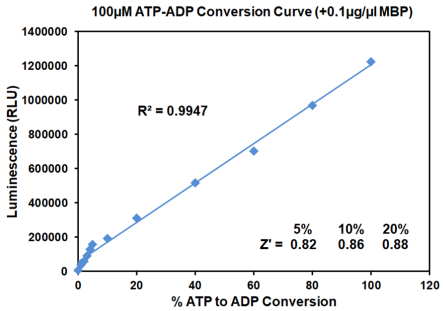

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 100µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	300	150	75	37.50	18.75	9.38	4.69	0
Luminescence	886,602	328,377	187,799	81,263	38,265	19,352	12,036	7,294
S/B	122	45	26	11	5	3	2	1
% Conversion	91	29	13	1	0	0	0	0

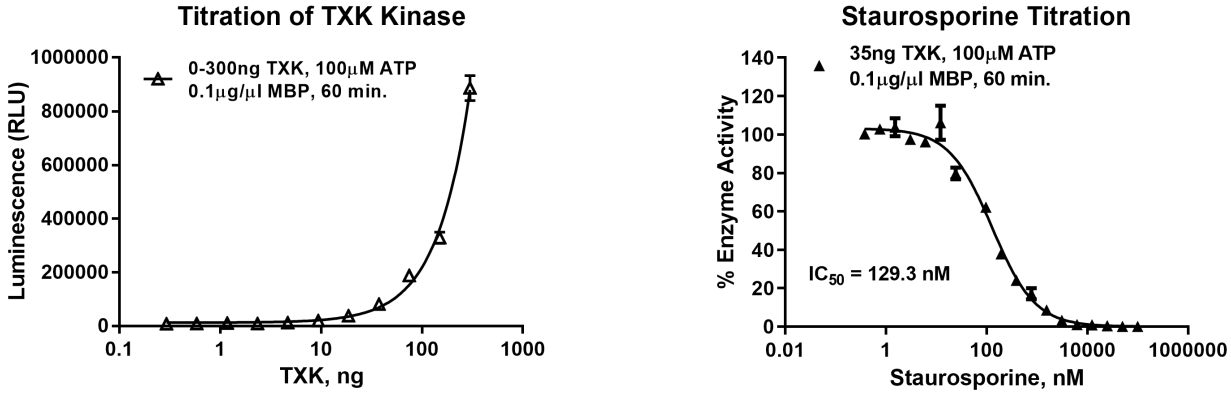

Figure 3. TXK Kinase Assay Development. (A) TXK enzyme was titrated using 100µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 35ng of TXK to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
TXK Kinase Enzyme System	10µg	VA7576
	1mg	VA7577
ADP-Glo™ + TXK Kinase Enzyme System	1 Each	VA7578