ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

TEC Kinase Assay

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Scientific Background:

TEC is a member of the Tec family of non-receptor protein-tyrosine kinases that are involved in the intracellular signaling mechanisms of cytokine receptors, lymphocyte surface antigens, heterotrimeric G-protein coupled receptors, and integrin molecules. TEC is an integral component of T cell signaling and has a distinct role in T cell activation. Defects in TEC may be associated with myelodysplastic syndrome. TEC plays a crucial role in regulating FGF2 secretion under various physiological conditions (1) and it inhibits CD25 expression in human T-lymphocyte (2).

1. Ebert,A.D.et.al: Tec-kinase-mediated phosphorylation of fibroblast growth factor 2 is essential for unconventional secretion. Traffic 11 (6), 813-826 (2010).

2. Susaki,K.et.al: TEC protein tyrosine kinase inhibits CD25 expression in human T-lymphocytes. Immunol. Lett. 127 (2), 135-142 (2010)

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.

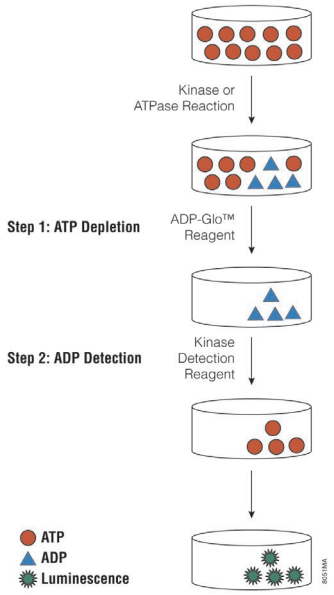

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

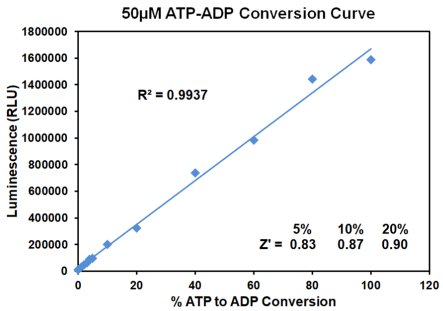

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	0
Luminescence	938,428	347,291	143,942	61,594	22,277	11,973	6,715	4,458
S/B	211	78	32	14	5	3	2	1
% Conversion	71	26	11	4	1	0	0	0

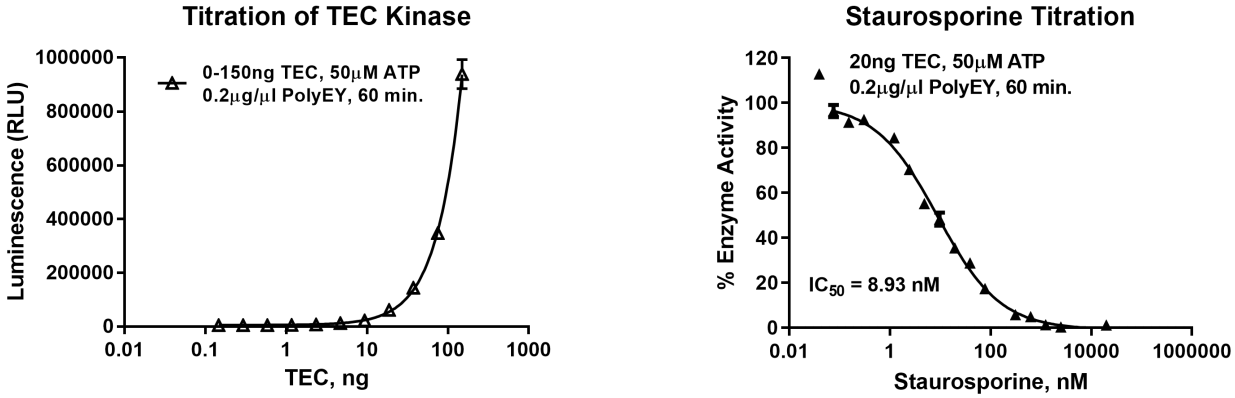

Figure 3. TEC Kinase Assay Development. (A) TEC enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 20ng of TEC to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
TEC Kinase Enzyme System	10µg	VA7306
	1mg	VA7307
ADP-Glo™ + TEC Kinase Enzyme System	1 Each	VA7308