ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

LTK Kinase Assay

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Scientific Background:

LTK is a member of the ros/insulin receptor family of tyrosine kinases. LTK express a gene encoding a putative transmembrane protein-kinase devoid of an extracellular domain and it may encode a signal transduction subunit for one or more of the hematopoietic receptors (1). LTK gene produces not only the putative receptor tyrosine kinase for an unknown ligand but also multiple protein products that may have different functions (2). LTK causes upregulation of the PI3K pathway and possibly form a genetic component of susceptibility to abnormal proliferation of self-reactive B cells in SLE.

1. Ben-Neriah, Y. et.al: Leukocytes express a novel gene encoding a putative transmembrane protein-kinase devoid of an extracellular domain. (Letter) Nature 333: 672-676, 1988.

2. Toyoshima, H. et.al: Differently spliced cDNAs of human leukocyte tyrosine kinase receptor tyrosine kinase predict receptor proteins with and without a tyrosine kinase domain and a soluble receptor protein. Proc. Nat. Acad. Sci. 90: 5404-5408, 1993.

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.

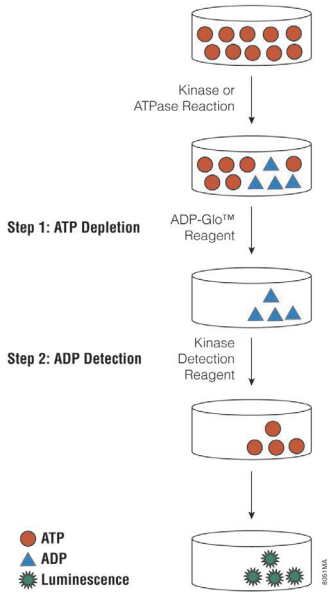

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

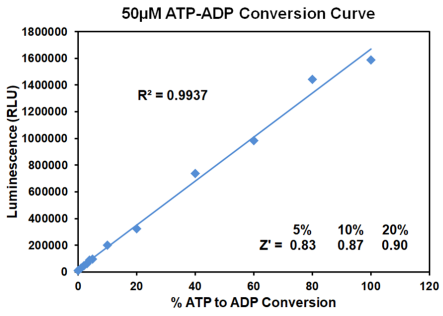

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	300	150	75	37.50	18.75	9.38	2.34	0
Luminescence	870,618	564,252	393,302	196,496	105,770	38,389	11,706	5,056
S/B	172	112	78	39	21	8	2	1
% Conversion	62	40	27	13	6	1	0	0

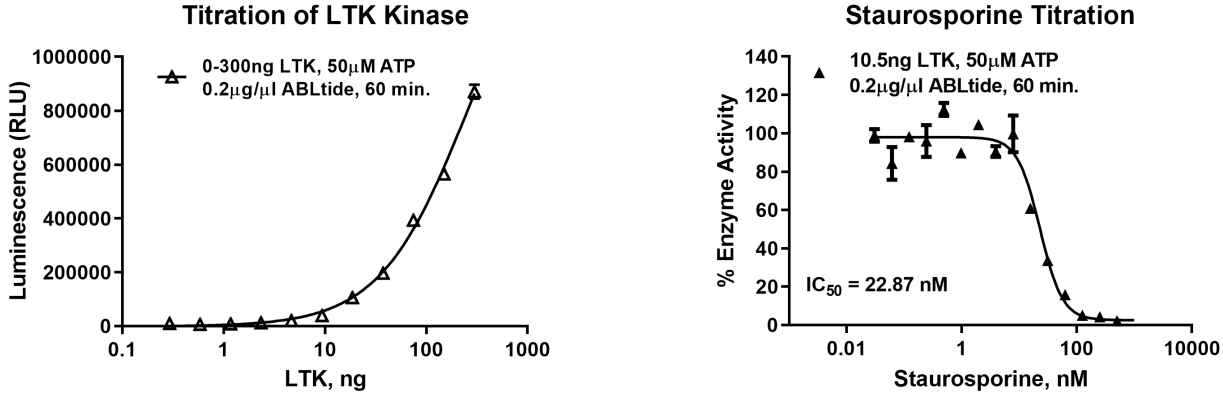

Figure 3. LTK Kinase Assay Development. (A) LTK enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 10.5ng of LTK to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
LTK Kinase Enzyme System	10µg	VA7483
	1mg	VA7484
ADP-Glo™ + LTK Kinase Enzyme System	1 Each	VA7485