CSK Kinase Assay

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Scientific Background:

CSK is a cytoplasmic tyrosine kinase that has been shown to downregulate the tyrosine kinase activity of the c-src through tyrosine phosphorylation of the c-src carboxy terminus[1]. A yeast 2-hybrid system has been used to identify proteins associated with CSK. The Src homology-3 (SH3) domain of CSK associates with a proline-rich region of PEP, a protein-tyrosine phosphatase expressed in hemopoietic cells[2]. This association is highly specific and it is speculated that PEP may be an effector and/or regulator of CSK in T cells and other hemopoietic cells.

1. Partanen, J. et al: Cyl encodes a putative cytoplasmic tyrosine kinase lacking the conserved tyrosine autophosphorylation site (Y416-src). Oncogene 6: 2013-2018.1991.
2. Cloutier, J.-F. et al: Association of inhibitory tyrosine protein kinase p50(csk) with protein tyrosine phosphatase PEP in T cells and other hemopoietic cells. EMBO J. 15: 4909-4918, 1996.

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.

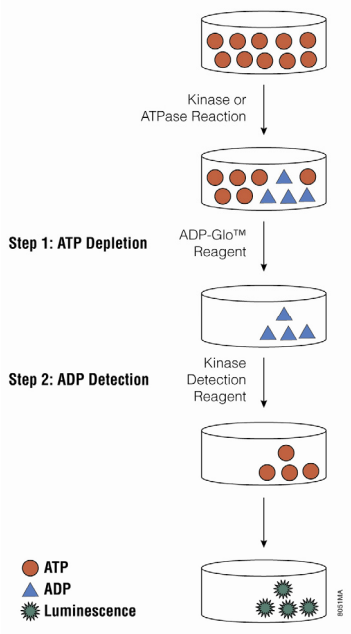

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

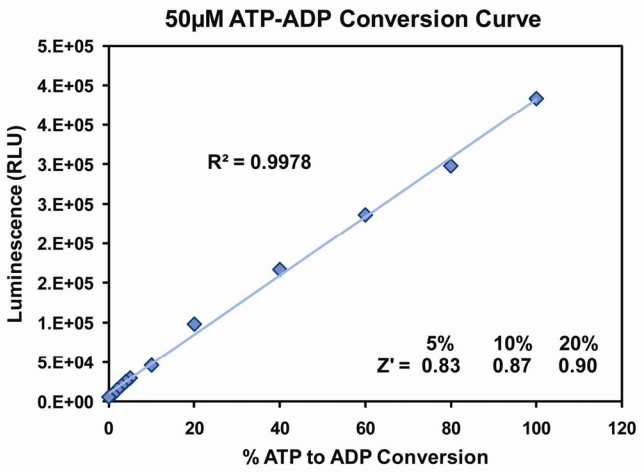

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.

For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Tyrosine Kinase 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 60 minutes.
- 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 0.5-1second).

Table 1. CSK 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.

CSK, ng	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.20	0
Luminescence	56330	45366	34112	25222	18055	12355	7716	4740	3063	984
S/B	57.2	46.1	34.7	25.6	18.3	12.6	7.8	4.8	3.1	1
% Conversion	66.4	53.0	39.3	28.5	19.7	12.8	7.1	3.5	1.5	0

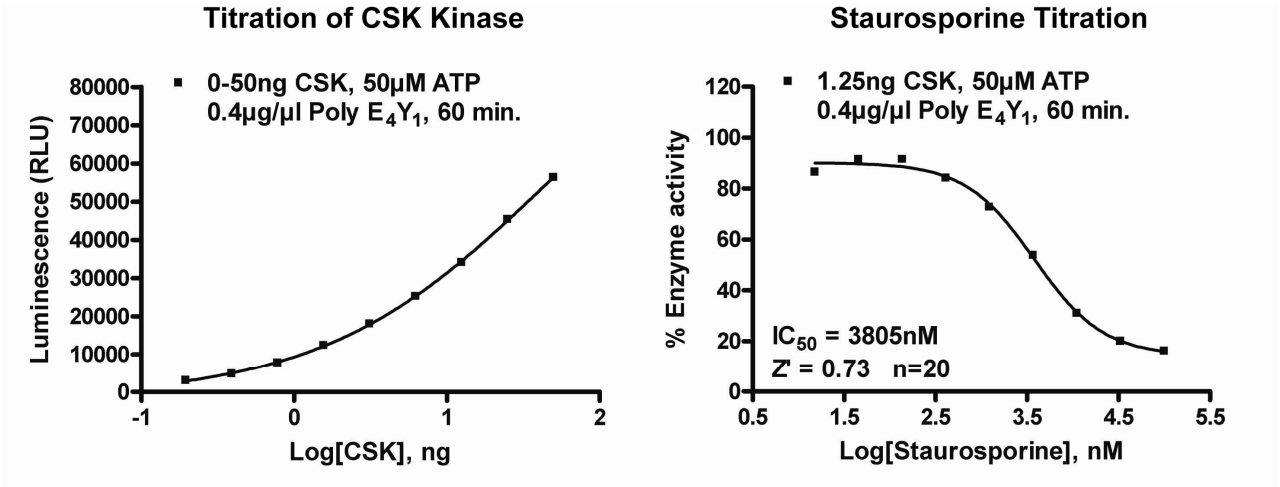

Figure 3. CSK Kinase Assay Development. (A) CSK enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 1.25ng of CSK to determine the potency of the inhibitor (IC_{50}). Z' factor was determined using 20 replicates of each of the minimum and maximum response (10 and 0µM staurosporine, respectively).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CSK Kinase Enzyme System	Promega	V2981
ADP-Glo + CSK Kinase Enzyme System	Promega	V9251

CSK Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 2mM $MnCl_2$; 50µM DTT.

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