CK1γ1 Kinase Assay

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Scientific Background:

CK1γ1 or casein kinase 1, gamma 1 is one of the most abundant serine/threonine kinases in eukaryotic cells and is mainly involved in growth and morphogenesis. CK1γ1 possesses the C-terminal sequence motif (MTM), which it shares with CSNK1G2 and CSNK1G3- this motif is associated with heterologous carboxy-terminal sequences (1). CK1γ1 couples Wnt receptor activation to the cytoplasmic signal transduction apparatus (2).

1. Kusuda, J. et.al: Cloning, expression analysis and chromosome mapping of human casein kinase 1 gamma-1 (CSNK1G1): identification of two types of cDNA encoding the kinase protein associated with heterologous carboxy-terminal sequences. Cytogenet. Cell Genet. 90: 298-302, 2000.

2. Davidson, G. et.al: Casein kinase 1-gamma couples Wnt receptor activation to cytoplasmic signal transduction. Nature 438: 867-872, 2005.

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.

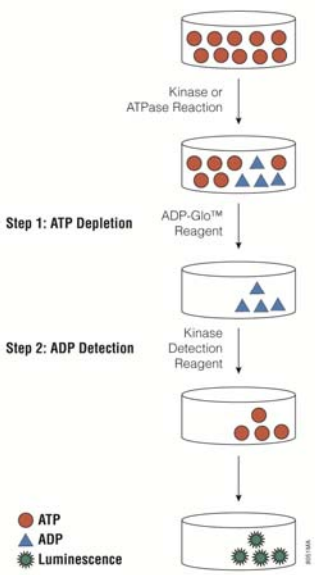


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

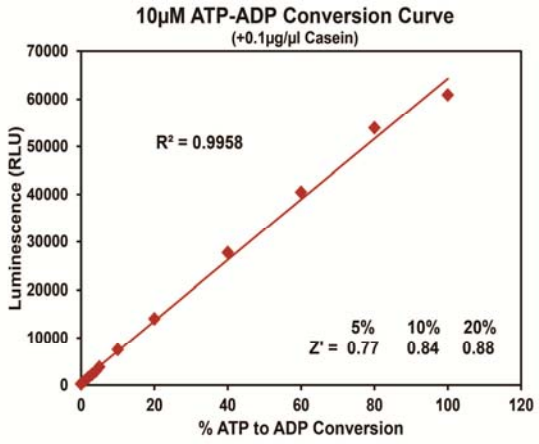


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

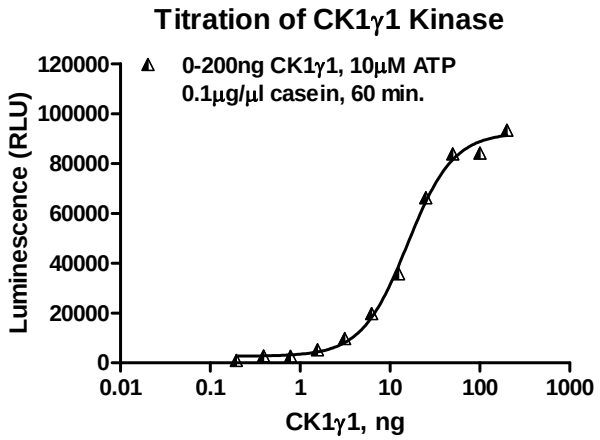
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, and the KES Protocol available at: http://www.promega.com/tbs/tm313/tm313.html, and http://www.promega.com/KESProtocol, respectively.

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 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 0.5-1sec).

Table 1. CK1γ1 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.

CK1γ1, ng	200	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0
Luminescence	93361	84188	83853	66261	35792	19739	9721	5271	2673	1308	946	401
S/B	233	210	209	165	89	49	24	13	7	3	2	1
% Conversion	94	85	84	67	36	20	9	5	2	1	0.6	0



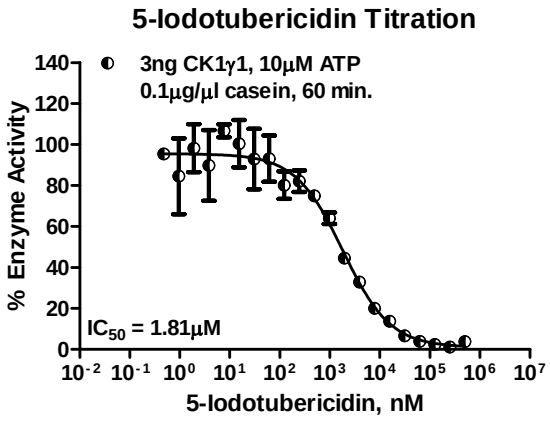


Figure 3. CK1γ1 Kinase Assay Development. **(A) CK1γ1 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) 5-iodotubericidin dose response was created using 3ng of CK1γ1 to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CK1γ1 Kinase Enzyme System	Promega	V4100
ADP-Glo™ + CK1γ1 Kinase Enzyme System	Promega	V4101

CK1γ1 Kinase Buffer: 40mM Tris, pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT