PKA Kinase Assay

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Scientific Background:

The 40kDa cAMP-Dependent Protein Kinase (PKA), Catalytic Subunit, is a bovine recombinant enzyme expressed and purified from E. coli. Intracellular targets include ion channels (1), transcriptional activator proteins (2), and regulatory enzymes of glycogen metabolism (3). PKA does not require cAMP for activity, and the gene sequence can be found at accession number NM_174584.2.

1. Cohen, P. (1978) The role of cyclic-AMP-dependent protein kinase in the regulation of glycogen metabolism in mammalian skeletal muscle. Curr. Top. Cell. Regul. 14, 117–96.

2. Rossie, S. and Catterall, W.A. (1987) Cyclic-AMP-dependent phosphorylation of voltage-sensitive sodium channels in primary cultures of rat brain neurons. J. Biol. Chem. 262, 12735–44.

3. Montminy, M.R. and Bilezikjian, L.M. (1987) Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. Nature 328, 175–8.

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.

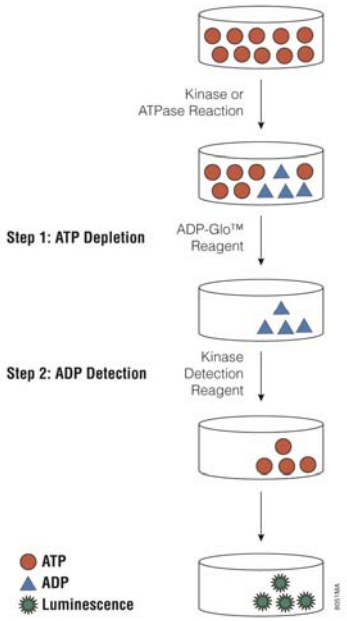

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

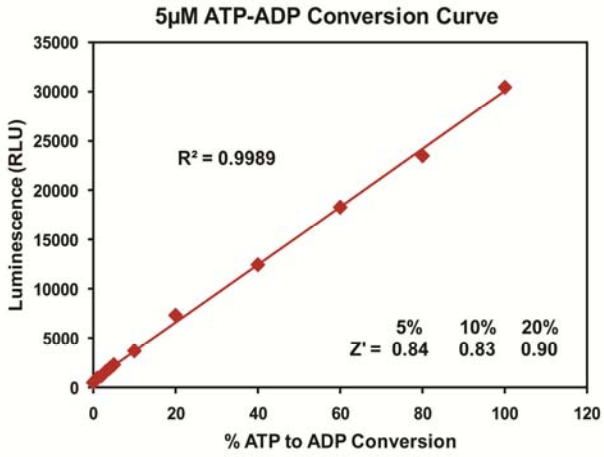

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 5µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 15 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. PKA 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.

PKA, units	0.2	0.1	0.05	0.025	0.0125	0.0062	0.0031	0.0015	0.0007	0.0003	0
RLU	49805	45270	39013	26834	17705	10271	6725	3631	2869	2487	291
S/B	171	155	134	92	61	35	23	13	10	9	1
% Conversion	90	82	71	49	32	19	12	7	5	4	0

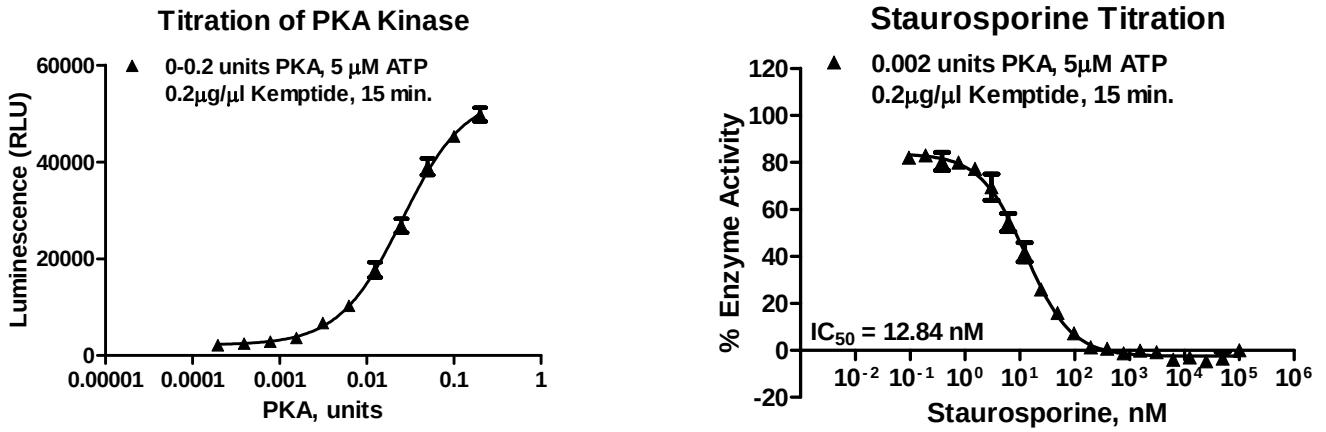

Figure 3. PKA Kinase Assay Development. (A) PKA enzyme was titrated using 5µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 0.002 units of PKA to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
PKA Kinase Enzyme System	Promega	V4246
ADP-Glo™ + PKA Kinase Enzyme System	Promega	V4247

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