

PKAcβ Kinase Assay

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Scientific Background:

The catalytic subunit C-beta of PKA (PKAcβ) is a member of the Ser/Thr protein kinase family (the PKA catalytic subunit consist of three gene products: C-alpha, C-beta, and C-gamma) and has been assigned to human chromosome region 1p36.1 (1). PKAcβ is derived from a gene distinct from C-alpha and shows tissue-specific expression. At the amino acid level C-alpha and C-beta showed 93% homology.

1. Simard, J. et al: Assignment of the gene encoding the catalytic subunit C-beta of cAMP-dependent protein kinase to the p36 band on chromosome 1. Hum. Genet. 88: 653-657, 1992.

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.

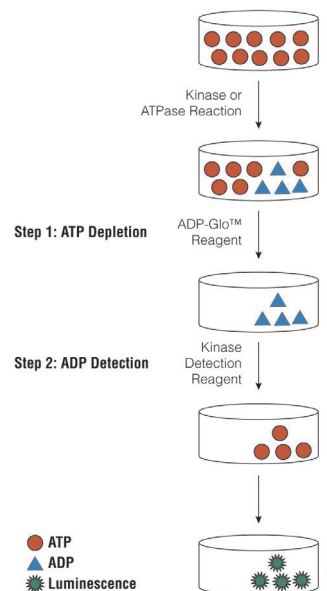


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

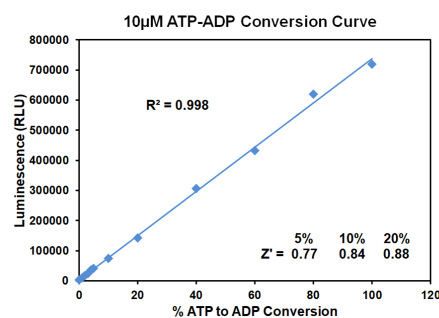


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10μM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 μ l of inhibitor or (5% DMSO)
 - ✓ 2 μ l of enzyme (defined from table 1)
 - ✓ 2 μ l of substrate/ATP mix
- Incubate at room temperature for indicated time (See Figure 3).
- Add 5 μ l of ADP-Glo™ Reagent.
- Incubate at room temperature for 40 minutes.
- Add 10 μ l 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	100	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0
Luminescence	230,606	206,005	171,017	110,731	66,229	32,529	18,299	10,662	5,869	1,100
S/B	210	187	155	101	60	30	17	10	5	1
% Conversion	57	51	42	27	16	7	4	2	0	0

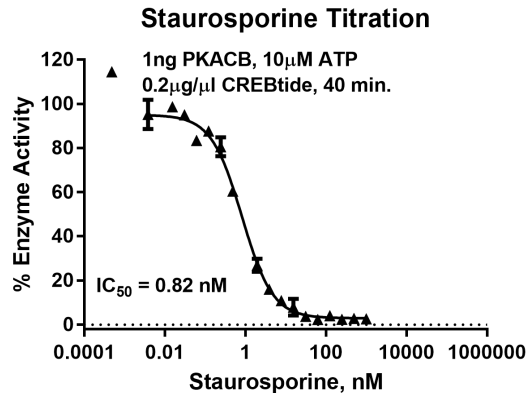
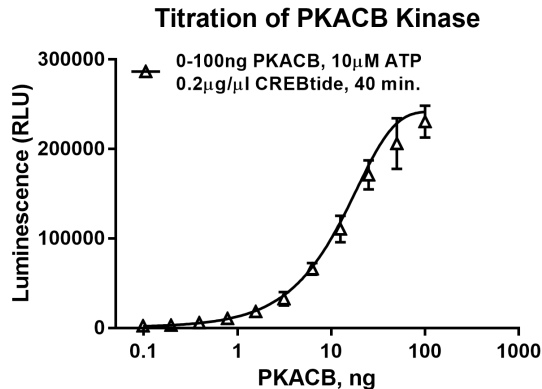


Figure 3. PKAC β Kinase Assay Development. (A) PKAC β enzyme was titrated using 10 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 1 ng of PKAC β to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
PKAC β Kinase Enzyme System	10 μ g	VA7255
	1mg	VA7256
ADP-Glo™ + PKAC β Kinase Enzyme System	1 Each	VA7257

