ADP-Glo™ Kinase Assay Application Note

Ser-Thr Kinase Series

CK2α2 Kinase Assay

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Scientific Background:

CK2α2 or casein kinase II alpha 2 is a member of the CK2 family of Ser/Thr protein kinases. CK2α2 plays a fundamental role in cell function and is involved in DNA replication, regulation of basal and inducible transcription, translation and control of metabolism. CK2α2 prefers utilization of acidic proteins such as caseins as substrates. The CK2α2 holoenzyme is a tetramer composed of an alpha chain, an alpha' and two beta chains. The alpha and alpha' chains contain the catalytic site. CK2α2 is also a component of CK2-SPT16-SSRP1 complex comprised of SSRP1, SUPT16H, CSNK2A1, CSNK2A2 and CSNK2B (1). This complex associates following UV irradiation. CK2α2 act as a candidate gene for inherited abnormalities of sperm morphogenesis (2).

1. Keller, D. M. et.al: A DNA damage-induced p53 serine 392 kinase complex contains CK2, hSpt16, and SSRP1. Molec. Cell 7: 283-292, 2001.

2. Xu, X. et.al: Globozoospermia in mice lacking the casein kinase II alpha-prime catalytic subunit. Nature Genet. 23: 118-121, 1999.

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.

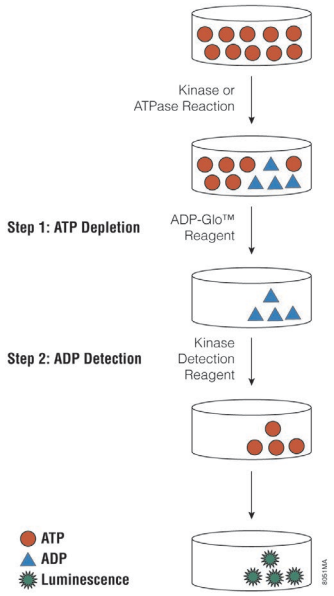

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

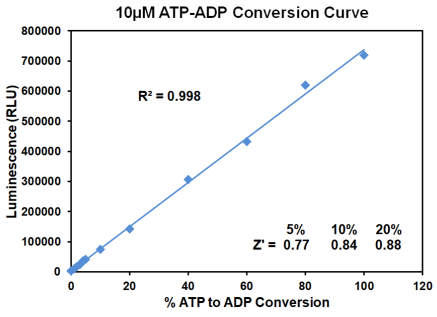

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	200	100	50	25	12.50	6.25	3.13	1.56	0.78	0.39	0.20	0
Luminescence	221,028	214,387	193,171	198,248	196,103	143,734	111,561	49,624	26,172	13,190	6,593	1,318
S/B	168	163	147	150	149	109	85	38	20	10	5	1
% Conversion	68	66	59	61	60	44	34	14	7	3	1	0

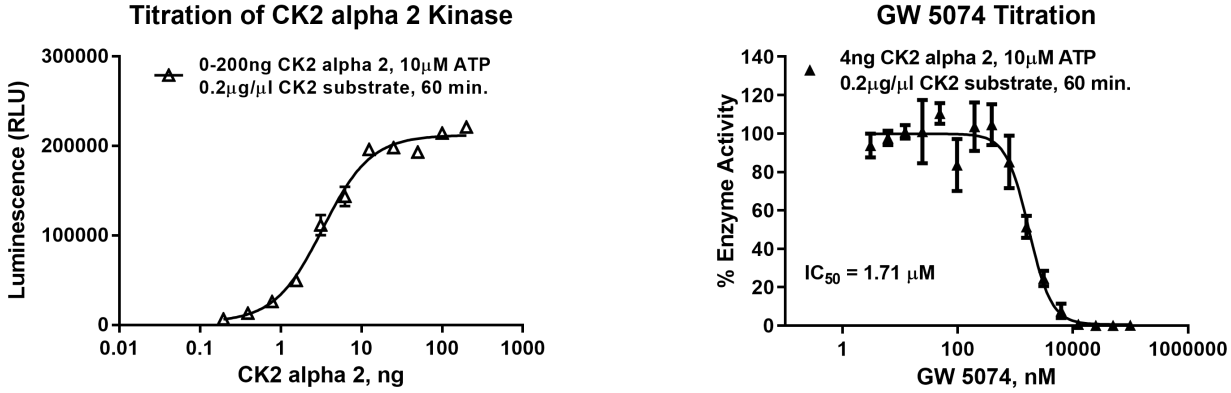

Figure 3. CK2α2 Kinase Assay Development. (A) CK2α2 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 4ng of CK2α2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
CK2α2 Kinase Enzyme System	10µg	VA7597
	1mg	VA7598
ADP-Glo™ + CK2α2 Kinase Enzyme System	1 Each	VA7599