

# CAMK4 Kinase Assay

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## Scientific Background:

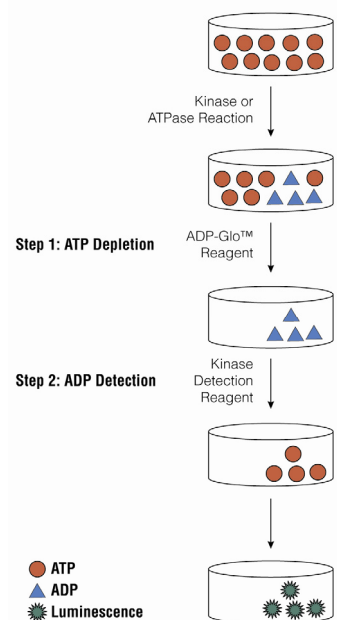
CAMK4 is a multifunctional serine/threonine protein kinase and a member of Ca(2+)/calmodulin-dependent protein kinase family. CAMK4 is localized in neurons in the hippocampus, amygdala, anterior cingulate cortex, somatosensory cortex, and insular cortex (1). CAMK4 is involved in neural activity-dependent signaling in the neuronal nucleus and thought to play an important role in the consolidation/retention of hippocampus-dependent long-term memory (2).

1. Sikela, J. M. et al: Chromosomal localization of the human gene for brain Ca(2+)/calmodulin-dependent protein kinase type IV. *Genomics* 4: 21-27, 1989.
2. Kang, H. et al: An important role of neural activity-dependent CaMKIV signaling in the consolidation of long-term memory. *Cell* 106: 771-783, 2001.

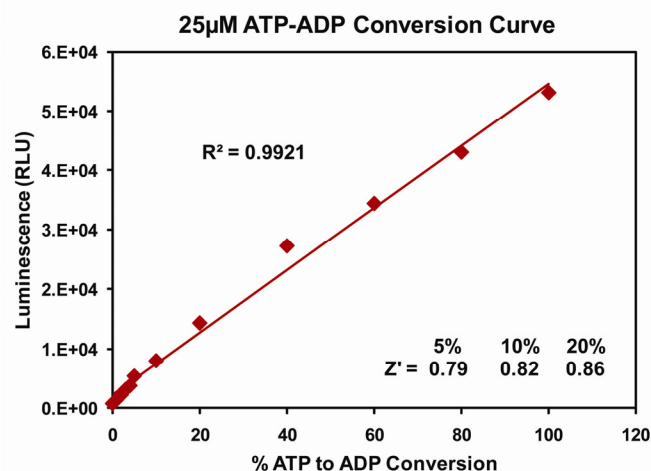
## ADP-Glo™ Kinase Assay

### 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 25μM 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.



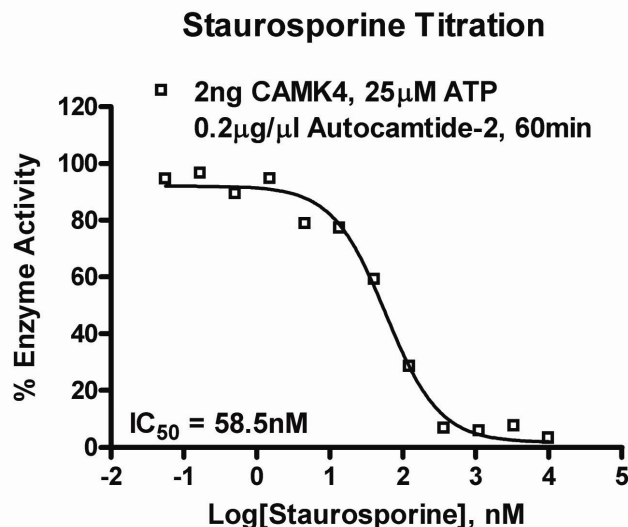
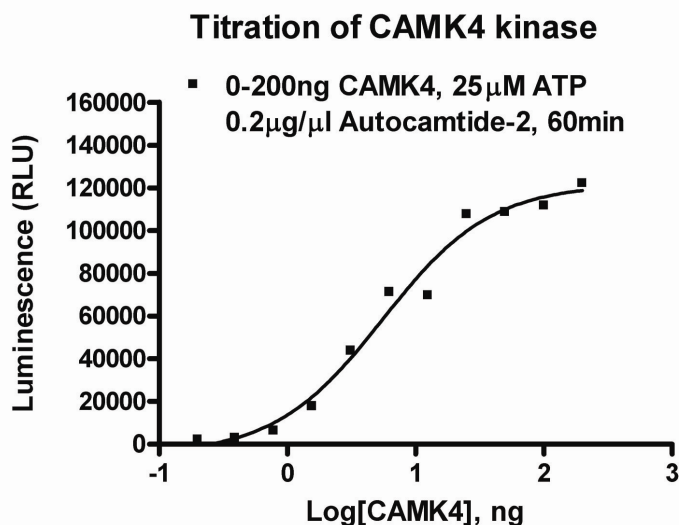
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay Technical Manual #TM313*, available at [www.promega.com/tbs/tm313/tm313.html](http://www.promega.com/tbs/tm313/tm313.html)

## Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase Buffer.
- Add to the wells of 384 low volume plate:
  - 1  $\mu$ l of inhibitor or (5% DMSO)
  - 2  $\mu$ l of enzyme (defined from table 1)
  - 2  $\mu$ l of substrate/ATP mix
- Incubate at room temperature for 15 minutes.
- Add 5  $\mu$ l of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10  $\mu$ l of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1second).

**Table 1. CAMK4 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.

CAMK4, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0
RLU	97317	106545	76496	69677	58740	43658	17714	6337	1601
S/B	61	67	48	44	37	27	11	4	1
% Conversion	90	99	70	63	53	38	13	2	0



**Figure 3. CAMK4 Kinase Assay Development:** (A) CAMK4 enzyme was titrated using 25  $\mu$ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 2ng of CAMK4 to determine the potency of the inhibitor ( $IC_{50}$ ).

### Assay Components and Ordering Information:



#### Products

ADP-Glo™ Kinase Assay  
CAMK4 Kinase Enzyme System  
ADP-Glo + CAMK4 Kinase Enzyme System

#### Company

Promega  
Promega  
Promega

#### Cat.#

V9101  
V2951  
V9091

CAMK4 Kinase Buffer: 40mM Tris, 7.5; 20mM MgCl<sub>2</sub>; 0.1mg/ml BSA; 50  $\mu$ M DTT; Ca<sup>2+</sup>/Calmodulin solution (0.03  $\mu$ g/ $\mu$ l Calmodulin, 1mM Tris, pH 7.3, 0.5mM CaCl<sub>2</sub>).