ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: CDC7/DBF4

Promega

CDC7/DBF4 Kinase Assay

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Scientific Background:

CDC7 is a cell division cycle 7 homolog protein that is critical for the G1/S transition and is also essential for initiation of DNA replication as cell division occurs. CDC7 is expressed in many normal tissues, but the overexpression of CDC7 may be associated with neoplastic transformation for some tumors and transformed cell lines (1). CDC7/DBF4 kinase promotes S phase by alleviating an inhibitory activity in Mcm4 that evolved to integrate several protein kinases (2).

1. Hess, G. F. et.al: A human homolog of the yeast CDC7 gene is overexpressed in some tumors and transformed cell lines. Gene 211: 133-140, 1998.

2. Sheu, Y.-J. et.al: The Dbf4-Cdc7 kinase promotes S phase by alleviating an inhibitory activity in Mcm4. Nature 463: 113-117, 2010.

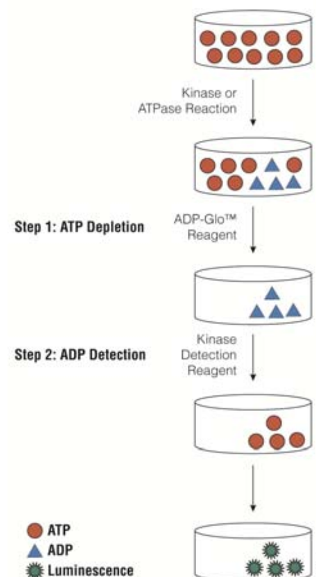


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

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.

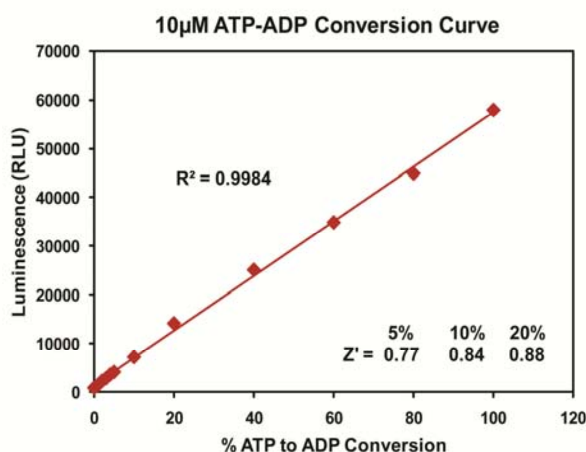


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

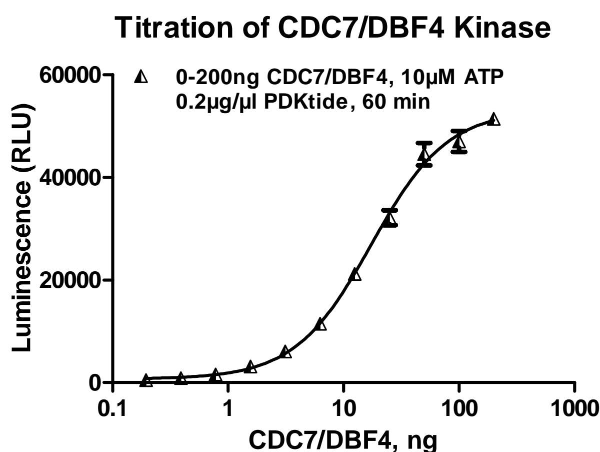
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, and the KES Protocol available at: http://www.promega.com/tbs/tm313/tm313.html, and http://www.promega.com/KESProtocol, respectively.

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. CDC7/DBF4 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.

CDC7/DBF4, ng	200	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0
Luminescence	51385	46978	44506	32128	21183	11498	6054	3121	1564	861	486	173
S/B	297	272	257	186	122	66	35	18	9	5	3	1
% Conversion	59	55	52	39	25	13	7	3	2	1	0.3	0



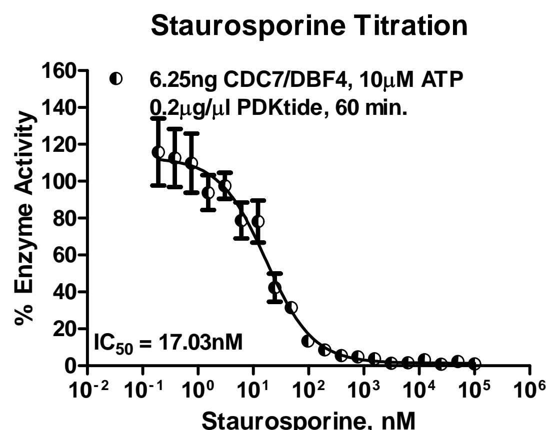


Figure 3. CDC7/DBF4 Kinase Assay Development. (A) CDC7/DBF4 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 6.25ng of CDC7/DBF4 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Promega   SignalChem Specialists in Signaling Proteins

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
CDC7/DBF4 Kinase Enzyme System	Promega	V5088
ADP-Glo™ + CDC7/DBF4 Kinase Enzyme System	Promega	V5089

CDC7/DBF4 Kinase Buffer: 40mM Tris, pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT