DNA-PK Kinase Assay

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Scientific Background:

DNA-Dependent Protein Kinase (DNA-PK) consists of an approximately 460kDa catalytic subunit and a heterodimeric DNA-binding subunit (Ku) containing an 85kDa and an 70kDa peptide (1). The human native DNA-PK is purified from HeLa cells, and the gene sequence can be found at accession number NM_006904.

1. **Gottlieb, T.M. and Jackson, S.P. (1993) The DNA-dependent protein kinase: Requirement for DNA ends and association with Ku antigen. Cell 72, 131–42.**

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.

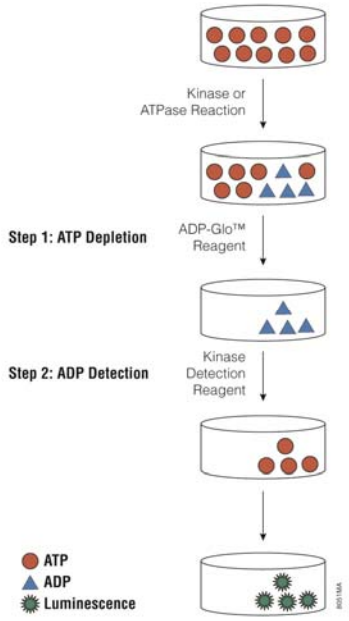

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

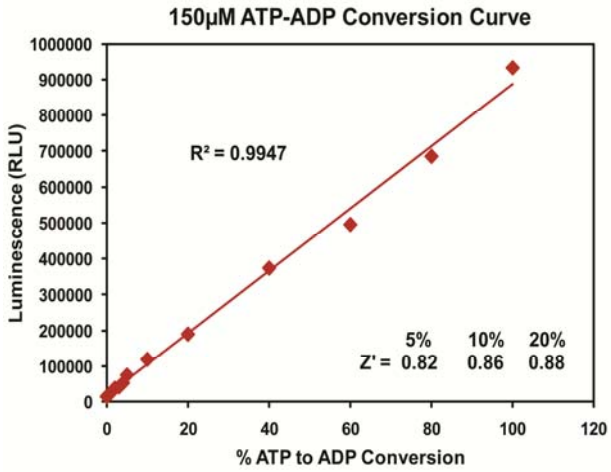

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 150µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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. DNA-PK 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.

DNA-PK, units	200	100	50	25	12.5	6.3	3.1	1.6	0.8	0
Luminescence	468642	434148	406918	224392	107039	40353	19507	8656	4691	2341
S/B	200	185	174	96	46	17	8	4	2	1
% Conversion	50	46	43	24	11	4	2	1	0.2	0

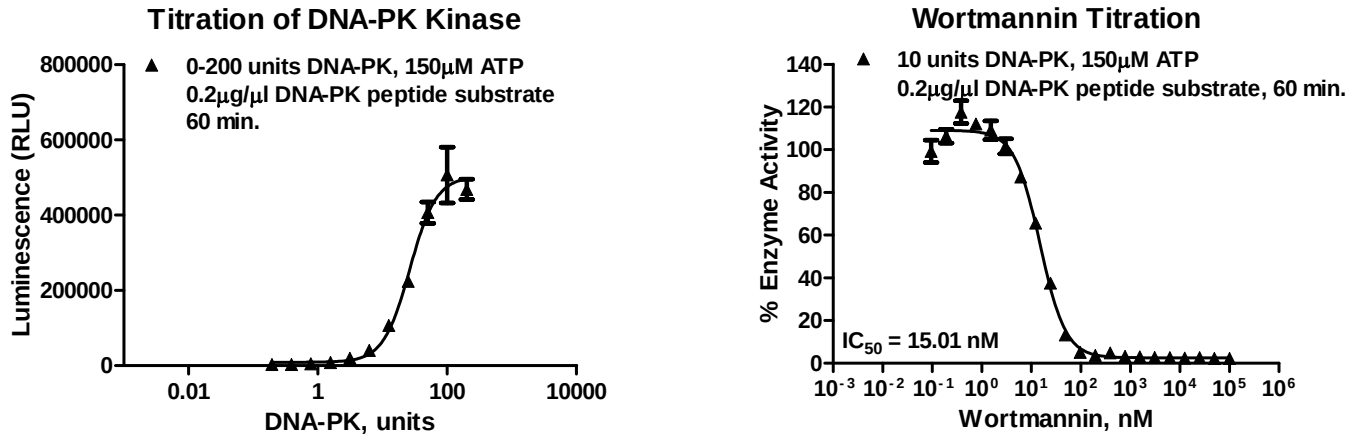

Figure 3. DNA-PK Kinase Assay Development. (A) DNA-PK enzyme was titrated using 150µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Wortmannin dose response was created using 10 units of DNA-PK to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
DNA-PK Kinase Enzyme System	Promega	V4106
ADP-Glo™ + DNA-PK Kinase Enzyme System	Promega	V4107

DNA-PK Kinase Buffer: 40mM Tris, pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 1X DNA-PK Activation Buffer; 50µM DTT.