

ADP-Glo™ Kinase Assay Application Notes

TYROSINE KINASE SERIES: SRC



SRC Kinase Assay

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Scientific Background:

SRC family belongs to non-receptor tyrosine kinases. SRC was originally identified as a transforming protein of the Rous sarcoma virus (RSV) that had enzymatic ability to phosphorylate tyrosine in protein substrates (1). SRC is overexpressed and activated in a large number of human malignancies and has been linked to the development of cancer and progression to distant metastases (2). In addition to increasing cell proliferation, a key role of SRC in cancer seems to be the ability to promote invasion and motility, functions that might contribute to tumour progression.

1. Collett, M S. et al: Protein kinase activity associated with the avian sarcoma virus src gene product. Proc Natl Acad Sci U S A. 1978 Apr;75(4):2021-4.
2. Jacobs, C. et al: Expression of pp60c-src protein kinase in adult and fetal human tissue: high activities in some sarcomas and mammary carcinomas. Cancer Res. 1983 Apr;43(4):1696-702.

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.

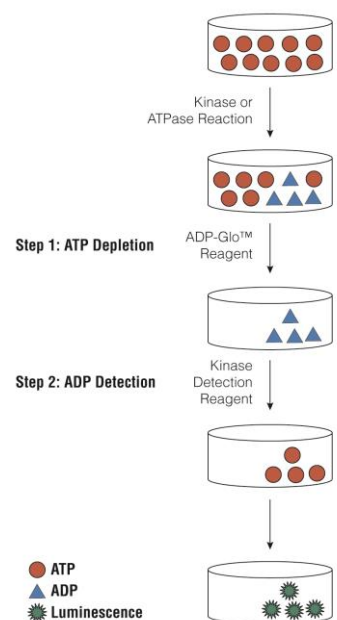


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

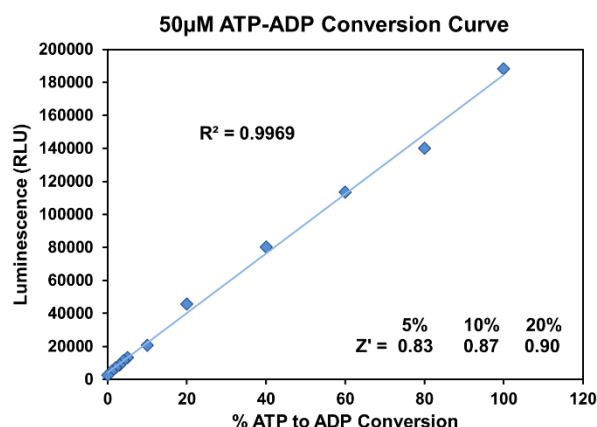


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.



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

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase 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 60 minutes.
- 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 0.5-1second).

Table 1. SRC 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.

SRC, ng	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0
Luminescence	178214	149823	97196	63378	37218	21433	13010	8092	5151	2335
S/B	76.3	64.2	41.6	27.1	15.9	9.2	5.6	3.5	2.2	1
% Conversion	96.5	80.8	51.6	32.8	18.3	9.6	4.9	2.2	0.5	0

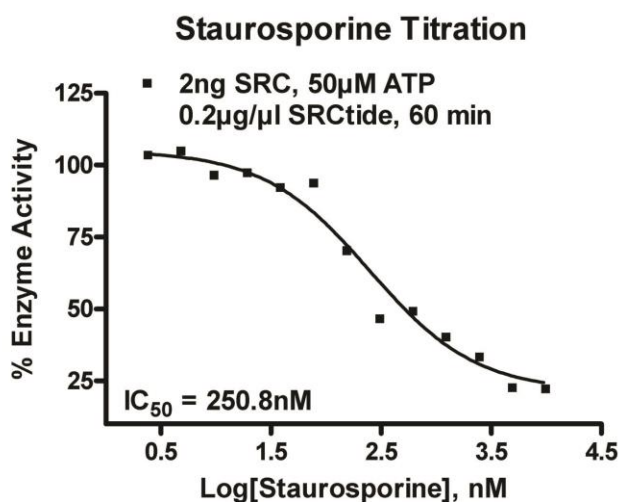
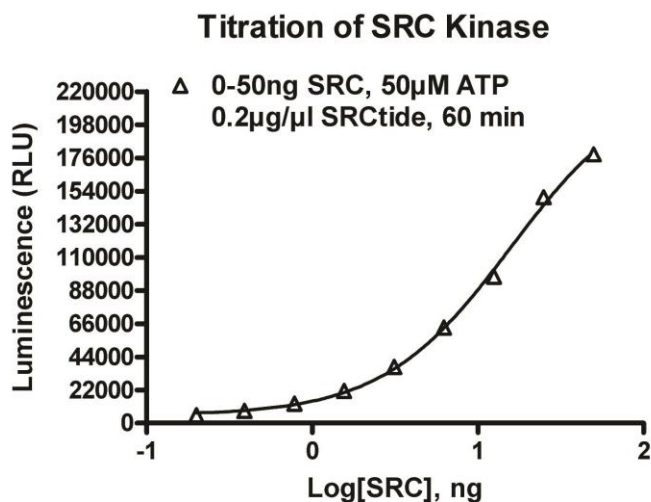


Figure 3. SRC Kinase Assay Development: (A) SRC enzyme was titrated using 50 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 2ng of SRC to determine the potency of the inhibitor (IC₅₀).

Assay Components and Ordering Information:



Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
SRC Kinase Enzyme System	Promega	V2921
ADP-Glo + SRC Kinase Enzyme System	Promega	V9741

SRC Kinase Buffer: 40mM Tris, pH 7.5; 20mM MgCl₂; 0.1mg/ml BSA; 2mM MnCl₂; 50 μ M DTT.

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