ADP-Glo™ Kinase Assay Application Notes

TYROSINE KINASE SERIES: FAK

FAK Kinase Assay

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Scientific Background:

FAK (Focal Adhesion Kinase) is a non-receptor protein tyrosine kinase involved in signal transduction from integrin-enriched focal adhesion sites that mediate cell contact with the extracellular matrix. FAK-enhanced signals have been shown to mediate the survival of anchorage-dependent cells and are critical for efficient cell migration in response to growth factor receptor and integrin stimulation (1). Elevated expression of FAK in human tumors has been correlated with increased malignancy and invasiveness (2). Elevated FAK expression in anaplastic astrocytoma and glioblastoma tumor biopsy samples has been demonstrated.

1. Schaller, M D.: Biochemical signals and biological responses elicited by the focal adhesion kinase. Biochim Biophys Acta. 2001 Jul 25;1540(1):1-21.

2. Gabarra-Niecko, V. et al: FAK regulates biological processes important for the pathogenesis of cancer. Cancer Metastasis Rev. 2003 Dec;22(4):359-74.

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.

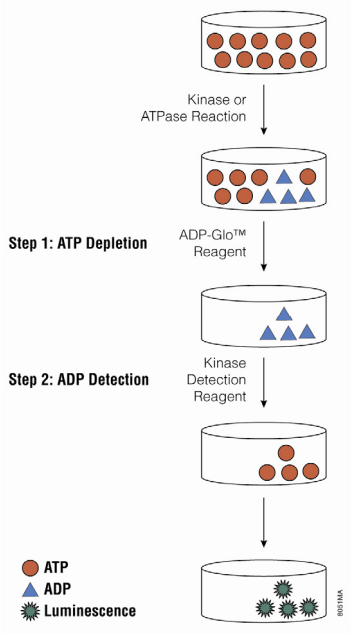

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

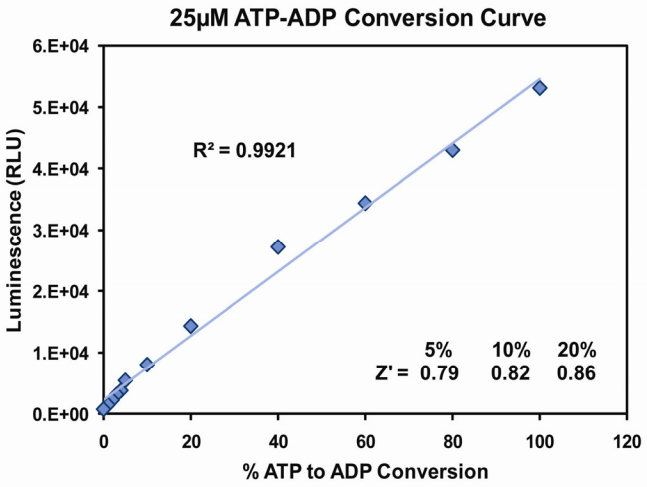

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.

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 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 0.5-1second).

Table 1. FAK 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.

FAK, ng	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.20	0
Luminescence	17859	19933	16778	12498	7107	4765	4157	2509	1770	680
S/B	26	29.5	24.5	18.38	10.5	7	6	3.5	2.5	1
% Conversion	24	27	22.5	16.5	8.5	5	4.5	2	1	0

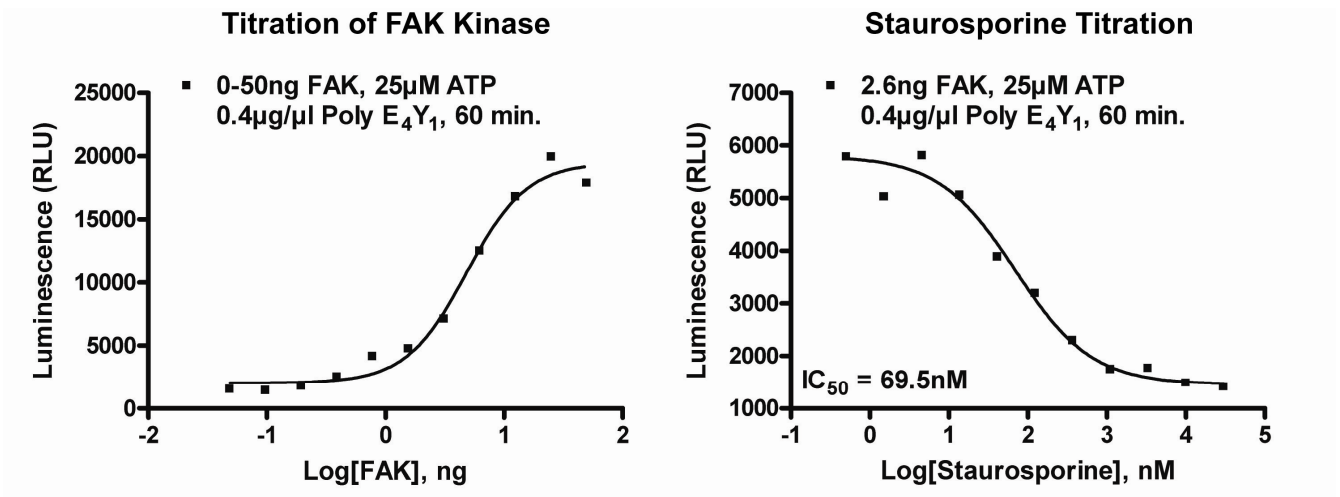

Figure 3. FAK Kinase Assay Development: (A) FAK enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 2.6ng of FAK to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
FAK Kinase Enzyme System	Promega	V1971
ADP-Glo + FAK Kinase Enzyme System	Promega	V9301

FAK Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 2mM $MnCl_2$; 50µM DTT.