FES Kinase Assay

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Scientific Background:

FES is a protooncogene that encodes a protein-tyrosine kinase distinct from c-Src, c-Abl and other nonreceptor tyrosine kinases. FES was originally identified as the cellular homolog of several transforming retroviral oncoproteins (1). FES plays a role in regulating cytoskeletal rearrangements and inside out signalling that accompany receptor ligand, cell matrix and cell-cell interaction. Genetic analysis using transgenic mouse model implicate FES in the regulation of inflammation and innate immunity (2). FES modulates the innate immune response of macrophages to LPS challenge, in part, by regulating the internalization and down-regulation of the TLR4 receptor complex.

1. Smithgall T E. et al: The c-Fes family of protein-tyrosine kinases. Crit Rev Oncog. 1998;9(1):43-62.

2. Greer, P.: Closing in on the biological functions of Fps/Fes and Fer. Nat Rev Mol Cell Biol. 2002 Apr;3(4):278-89.

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.

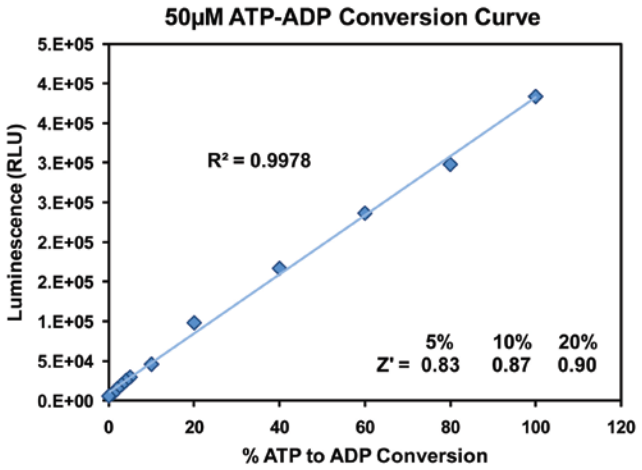

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Tyrosine 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 10-20 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. FES 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.

FES, ng	100	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0
Luminescence	210862	210342	195201	174922	140135	101442	69212	43700	23601	13797	2644
S/B	80	80	74	66	53	38	26	17	8.9	5.2	1
% Conversion	84	84	78	69	55	39	26	15	6.9	2.8	0

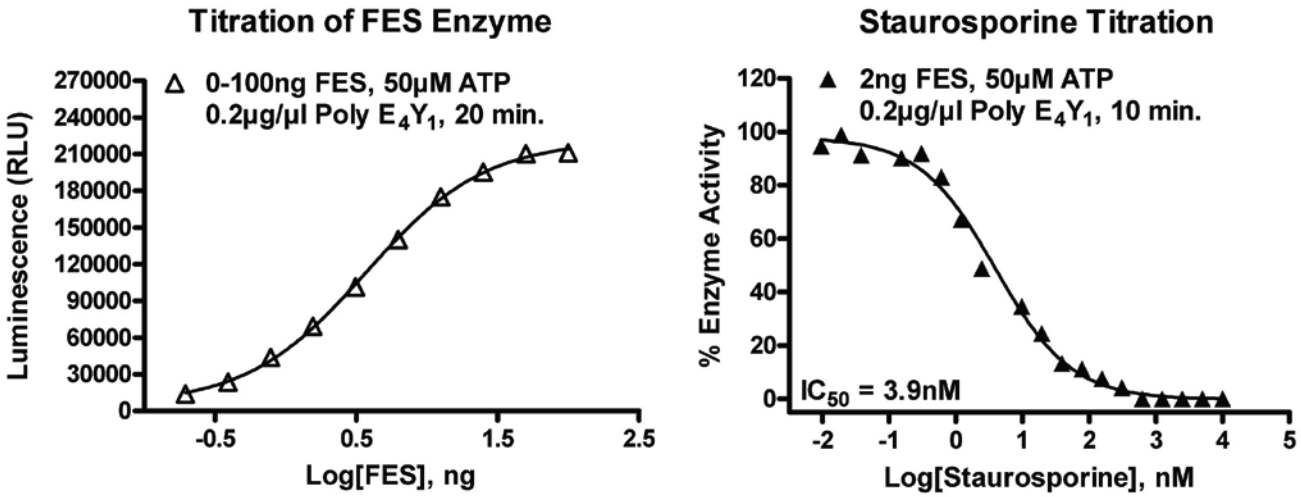

Figure 3. FES Kinase Assay Development. (A) FES enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 2ng of FES to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
FES Kinase Enzyme System	Promega	V1981
ADP-Glo™ + FES Kinase Enzyme System	Promega	V9311

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