EGFR Kinase Assay

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Scientific Background:

EGFR is the receptor for members of the EGF family and is a transmembrane glycoprotein that has tyrosine kinase activity. Binding of epidermal growth factor to EGFR induces receptor dimerization and tyrosine autophosphorylation and leads to cell proliferation, differentiation, motility, and cell survival. Activation of EGFR triggers mitogenic signaling in gastrointestinal mucosa, and its expression is upregulated in colon cancers and most neoplasms. Activation of EGFR triggers activation of the ERK-signaling pathway in normal gastric epithelial and colon cancer cell lines. Inactivation of EGFR with selective inhibitors significantly reduces ERK2 activation, c-fos mRNA expression and cell proliferation.

1. Wang K, et al: Epidermal growth factor receptor-deficient mice have delayed primary endochondral ossification because of defective osteoclast recruitment. J. Biol. Chem. 279: 53848-53856, 2004.

2. Kobayashi S, et al: EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. New Eng. J. Med. 352: 786-792, 2005.

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.

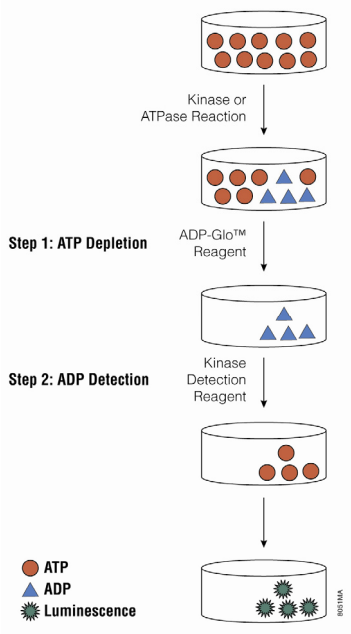

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

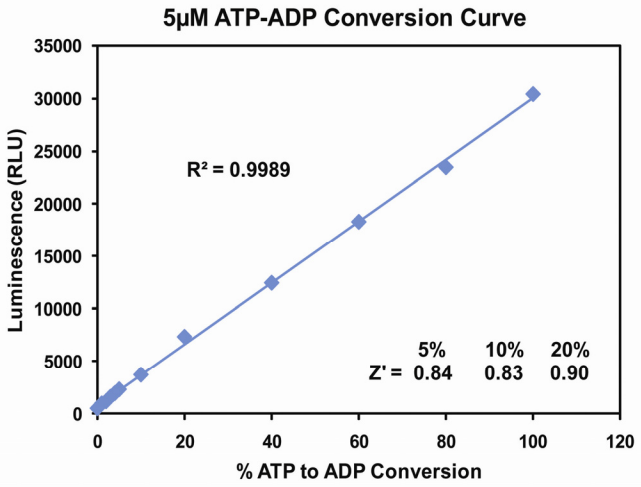

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 5µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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 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. EGFR 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.

EGFR, ng	100	50	25	12.5	6.3	3.1	1.56	0.78	0
Luminescence	20792	16758	15593	9910	7561	3620	2408	1653	429
S/B	48	39	36	23	18	8.4	5.6	3.9	1
% Conversion	97	78	72	45	34	14.9	9.1	5.5	0

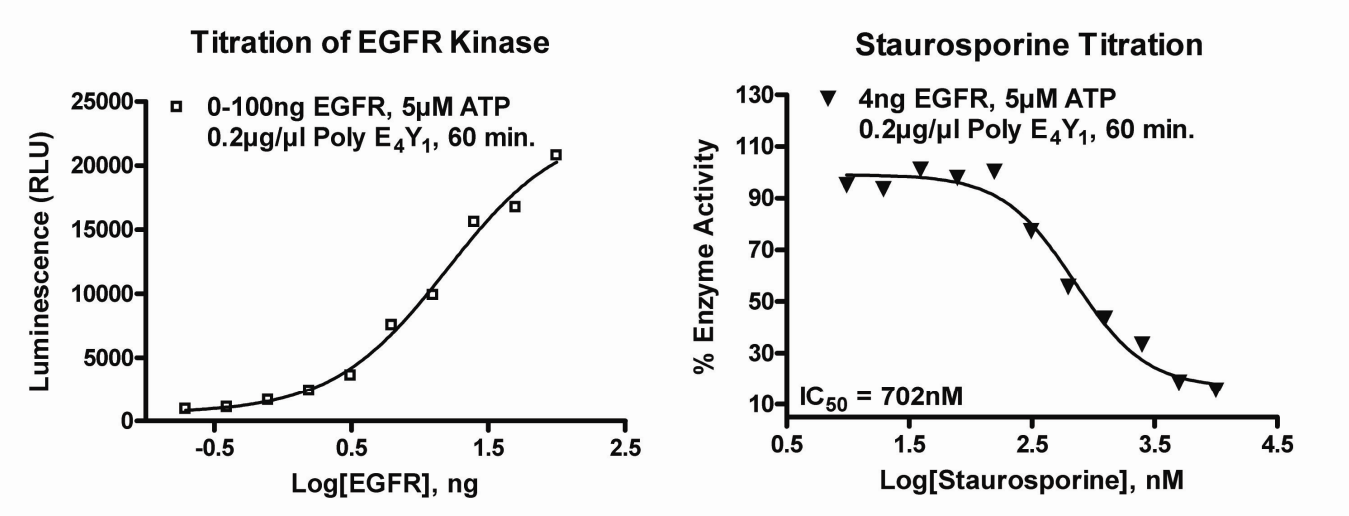

Figure 3. EGFR Kinase Assay Development. (A) EGFR enzyme was titrated using 5µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 4ng of EGFR to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
EGFR Kinase Enzyme System	Promega	V3831
ADP-Glo + EGFR Kinase Enzyme System	Promega	V9261

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

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