ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

FGFR2 (N549H) Kinase Assay

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Scientific Background:

FGFR2 is a member of the fibroblast growth factor receptor family which play a role in mitogenesis and differentiation. FGFR2 is a high-affinity receptor for acidic, basic and/or keratinocyte growth factor, and mutations in FGFR2 are associated with Crouzon syndrome, Pfeiffer syndrome, Craniosynostosis, Apert syndrome, Jackson-Weiss syndrome, Saethre-Chotzen syndrome, and syndromic craniosynostosis (1). FGFR2 is required for early postimplantation development between implantation and the formation of the egg cylinder (2). FGFR2 contributes to the outgrowth, differentiation, and maintenance of the inner cell mass.

1. Arman, E.: Targeted disruption of fibroblast growth factor (FGF) receptor 2 suggests a role for FGF signaling in pregastrulation mammalian development. Proc. Nat. Acad. Sci. 95: 5082-5087, 1998.

2. Genomic screening of fibroblast growth-factor receptor 2 reveals a wide spectrum of mutations in patients with syndromic craniosynostosis. Am. J. Hum. Genet. 70: 472-486, 2002.

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.

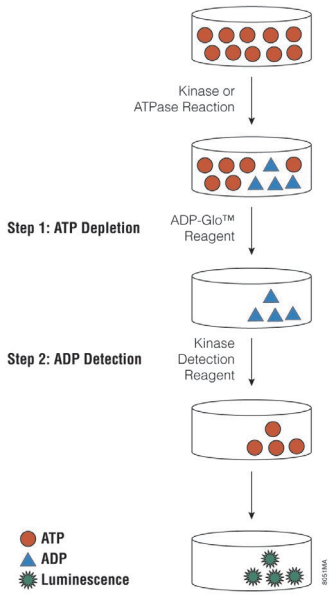

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

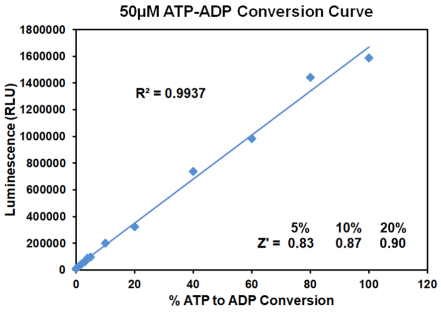

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 50µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction 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 indicated time (See Figure 3).
- 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 0.5-1 second).

Table 1. 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.

Enzyme, ng	200	100	50	25	12.50	6.25	3.13	1.56	0.78	0.20	0
Luminescence	1,370,915	785,298	606,472	325,073	228,074	117,635	70,583	33,327	19,132	9,033	3,970
S/B	345	198	153	82	57	30	18	8	5	2	1
% Conversion	91	52	40	21	14	7	4	1	0	0	0

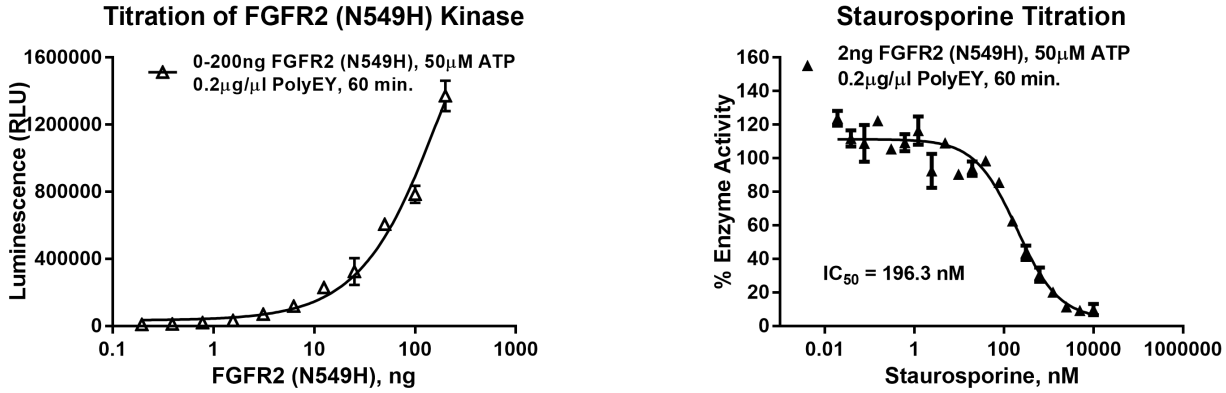

Figure 3. FGFR2 (N549H) Kinase Assay Development. (A) FGFR2 (N549H) enzyme was titrated using 50µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 2ng of FGFR2 (N549H) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
FGFR2 (N549H) Kinase Enzyme System	10µg	VA7150
	1mg	VA7151
ADP-Glo™ + FGFR2 (N549H) Kinase Enzyme System	1 Each	VA7152