ADP-Glo™ Kinase Assay Application Note

Tyrosine Kinase Series

TIE2 (R849W) Kinase Assay

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Scientific Background:

TIE2 or TEK is a receptor tyrosine kinase that is expressed principally on vascular endothelium. Disrupting TIE2 function in mice results in embryonic lethality with defects in embryonic vasculature, suggests a role in blood vessel maturation and maintenance. Angiopoietin-1 is a secreted growth factor that binds to and activates the TIE2 receptor tyrosine kinase (1). SHP2 and GRB2 are recruited to the activated TIE 2 kinase domain and are part of the cellular responses that mediate TIE2 function. TIE2 expression is upregulated in the endothelium of vascular "hot spots" in human breast cancer specimens. However, TIE2 is also overexpressed in areas of active angiogenesis in normal tissues (2).

1. Woolf, A S. et al: Angiopoietin growth factors and Tie receptor tyrosine kinases in renal vascular development. Pediatr Nephrol. 2001 Feb;16(2):177-84.

2. Peters, K G. et al: Functional significance of Tie2 signaling in the adult vasculature. Recent Prog Horm Res. 2004;59:51-71.

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.

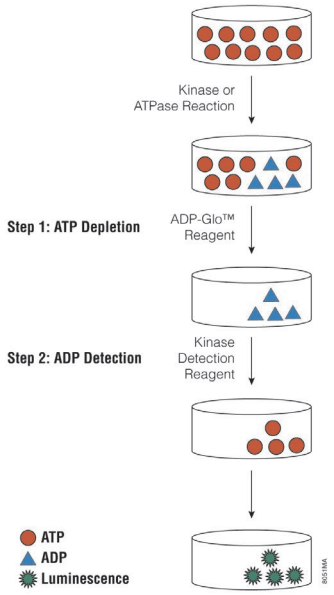

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

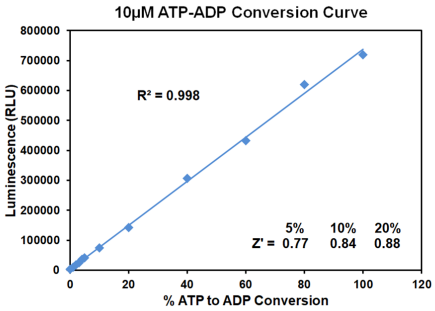

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	300	150	75	37.50	18.75	9.38	4.69	2.34	0
Luminescence	417,151	352,478	330,862	230,739	161,215	68,905	36,743	17,737	2,157
S/B	193	163	153	107	75	32	17	8	1
% Conversion	49	41	39	27	19	8	4	2	0

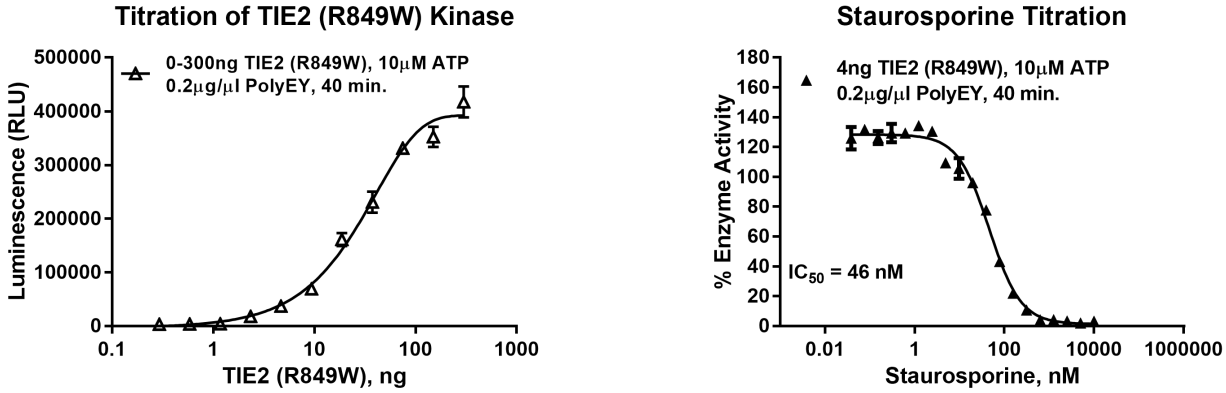

Figure 3. TIE2 (R849W) Kinase Assay Development. (A) TIE2 (R849W) enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 4ng of TIE2 (R849W) to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
TIE2 (R849W) Kinase Enzyme System	10µg	VA7309
	1mg	VA7310
ADP-Glo™ + TIE2 (R849W) Kinase Enzyme System	1 Each	VA7311