ADP-Glo™ Kinase Assay Application Notes

TYROSINE KINASE SERIES: HER4

HER4 Kinase Assay

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Scientific Background:

HER4 or ERBB4 is a transmembrane receptor tyrosine kinase that belongs to epidermal growth factor receptor family (1). Through its interaction with its activating ligand heregulin, HER4 can regulate proliferation and differentiation of cells (2). HER4 ectodomain is cleaved by a metalloprotease, gamma-secretase that releases the HER4 intracellular domain from the membrane and facilitates its translocation to the nucleus. The kinase activity of HER4 is both necessary and sufficient to trigger an antiproliferative response in human breast cancer cells. Increased expression of HER4 has been detected in various cancers and this usually correlates to better survival.

1. Carolyn, I. et al: HER4 Mediates Ligand-Dependent Antiproliferative and Differentiation Responses in Human Breast Cancer Cells. Mol.Cellular Biol., 2001, 21: 4265-4275.

2. Jorma, A. et al: Proteolytic Cleavage and Phosphorylation of a Tumor-associated ErbB4 Isoform Promote Ligand-independent Survival and Cancer Cell Growth. MolBiolCell, 2006; 17, 1: 67-79.

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.

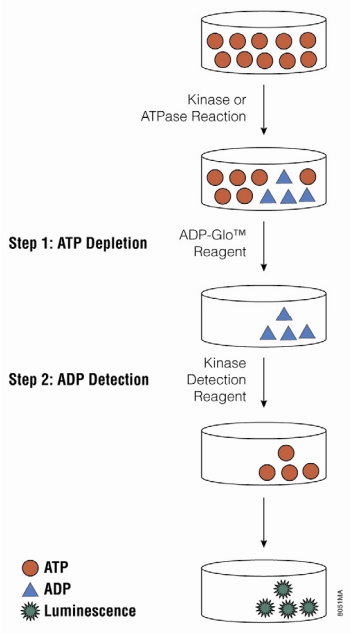

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

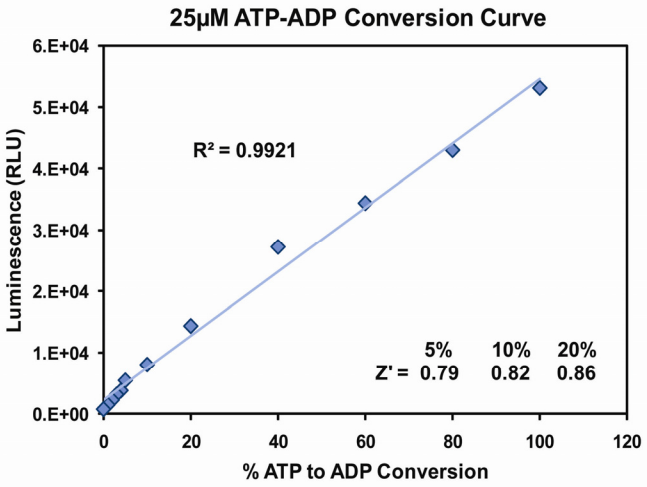

Figure 2. Linearity of the ADP-Glo™ Kinase Assay. ATP-to-ADP conversion curve was prepared at 25µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 192 replicates of each of the % conversions shown.

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 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. HER4 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.

HER4, ng	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.20	0.10	0
Luminescence	30990	31339	29660	26971	21917	18126	12300	7371	5305	3093	555
S/B	55.84	56.47	53.44	48.60	39.49	32.66	22.16	13.28	9.56	5.57	1
% Conversion	51.27	51.89	48.89	44.09	35.07	28.30	17.89	9.09	5.40	1.45	0

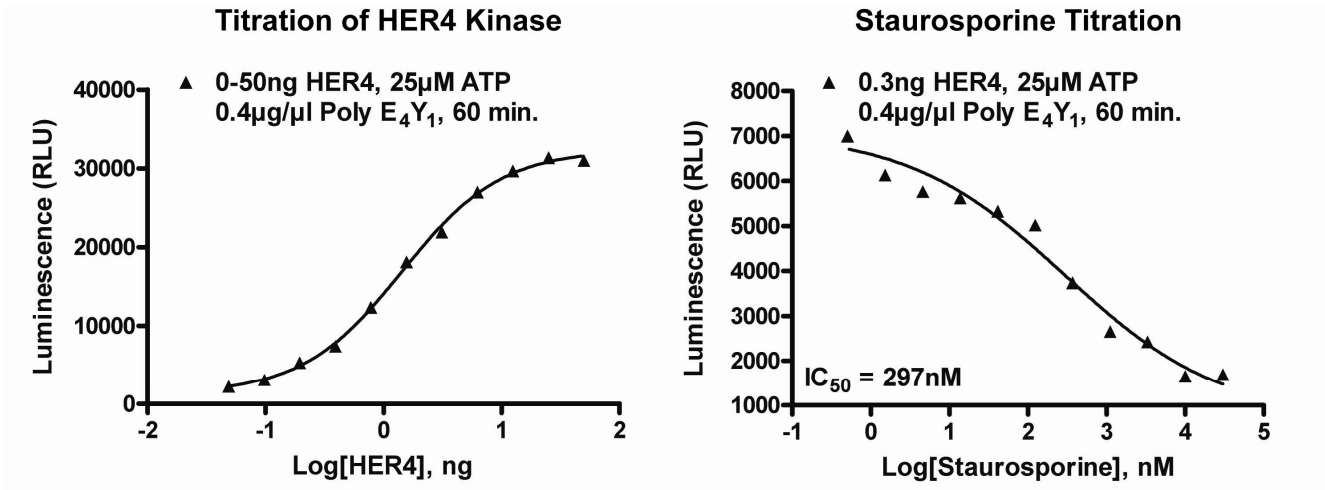

Figure 3. HER4 Kinase Assay Development: (A) HER4 enzyme was titrated using 25µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 0.3ng of HER4 to determine the potency of the inhibitor (IC_{50}).

Assay Components and Ordering Information:

Products	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
HER4 Kinase Enzyme System	Promega	V3101
ADP-Glo + HER4 Kinase Enzyme System	Promega	V9391

HER4 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 2mM $MnCl_2$; 50µM DTT.

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