

HER2 (A775_G776 ins YVMA) Kinase Assay

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Scientific Background:

HER2 gene encodes a cell-surface glycoprotein tyrosine kinase receptor with extensive homology to the epidermal growth factor receptor. HER2 is an oncogene and overexpression of unaltered HER2 coding sequences in NIH 3T3 cells results in cellular transformation and tumorigenesis (1). HER2 is amplified in about 30% of primary human breast malignancies and overexpression of HER2 is associated with the most aggressive tumors that show uncontrolled proliferation, resistance to apoptosis and increased motility (2).

1. Hudziak, R M. et al: Increased expression of the putative growth factor receptor p185HER2 causes transformation and tumorigenesis of NIH 3T3 cells. Proc Natl Acad Sci U S A. 1987 Oct; 84 (20):7159-63.
2. Badache, A. et al: The ErbB2 Signaling Network as a Target for Breast Cancer Therapy. J Mammary Gland Biol Neoplasia. 2006 Jan; 11(1):13-25.

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.

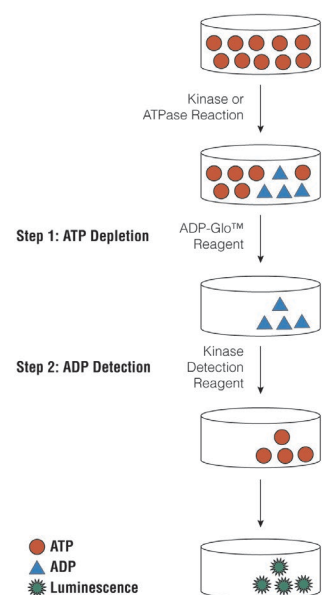


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

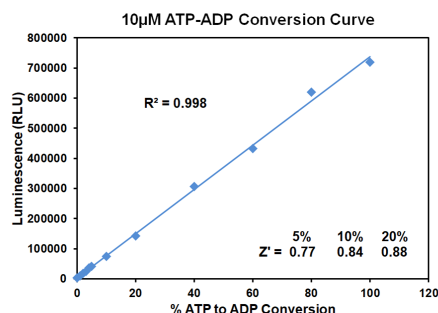


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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>

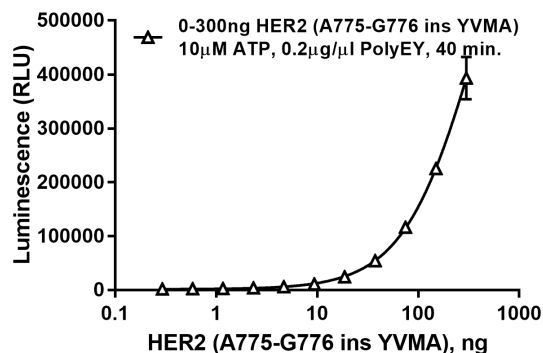
Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction buffer.
- Add to the wells of 384 low volume plate:
 - ✓ 1 μ l of inhibitor or (5% DMSO)
 - ✓ 2 μ l of enzyme (defined from table 1)
 - ✓ 2 μ l of substrate/ATP mix
- Incubate at room temperature for indicated time (See Figure 3).
- Add 5 μ l of ADP-Glo™ Reagent.
- Incubate at room temperature for 40 minutes.
- Add 10 μ l 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	393,192	225,273	116,480	54,473	24,567	10,924	5,870	3,765	2,098
S/B	187	107	56	26	12	5	3	2	1
% Conversion	53	30	15	7	3	1	0	0	0

Titration of HER2 (A775-G776 ins YVMA) Kinase



Staurosporine Titration

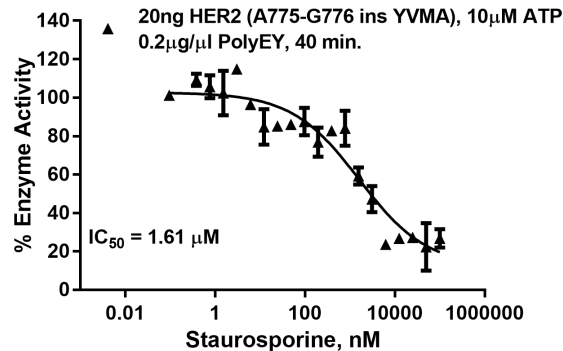


Figure 3. HER2 (A775_G776 ins YVMA) Kinase Assay Development. (A) HER2 (A775_G776 ins YVMA) enzyme was titrated using 10 μ M ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 20ng of HER2 (A775_G776 ins YVMA) to determine the potency of the inhibitor (IC₅₀).



Ordering Information:

Products	Size	Cat. #
HER2 (A775_G776 ins YVMA) Kinase Enzyme System	10 μ g	VA7189
	1mg	VA7190
ADP-Glo™ + HER2 (A775_G776 ins YVMA) Kinase Enzyme System	1 Each	VA7191