ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

BMPR2 Kinase Assay

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Scientific Background:

BMPR2 is a member of the bone morphogenetic protein (BMP) receptor family of transmembrane serine/threonine kinases. The ligands of this receptor are BMPs that are involved in endochondral bone formation and embryogenesis (1). The loss of interaction and lack of phosphorylation of TCTEL1 by BMPR2 may contribute to the pathogenesis of primary pulmonary hypertension (PPH) and BMPR2 also plays an essential role in human T-cell differentiation (2).

1. Machado, R. D. et.al: Functional interaction between BMPR-II and Tctex-1, a light chain of dynein, is isoform-specific and disrupted by mutations underlying primary pulmonary hypertension. Hum. Molec. Genet. 12: 3277-3286, 2003.

2. Cejalvo, T. et.al: Bone morphogenetic protein-2/4 signalling pathway components are expressed in the human thymus and inhibit early T-cell development. Immunology 121: 94-104, 2007.

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.

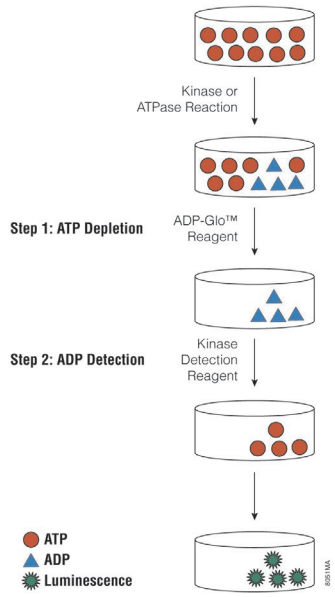

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

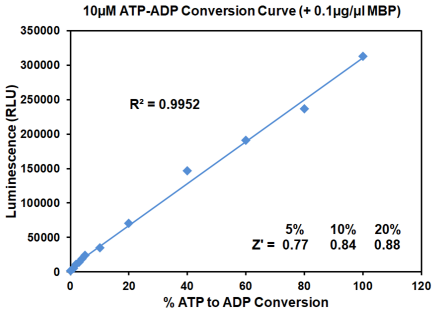

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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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	150	75	37.50	18.75	9.38	4.69	0
Luminescence	223,198	127,391	86,679	50,721	22,999	11,383	3,178
S/B	70	40	27	16	7	4	1
% Conversion	100	56	37	20	8	2	0

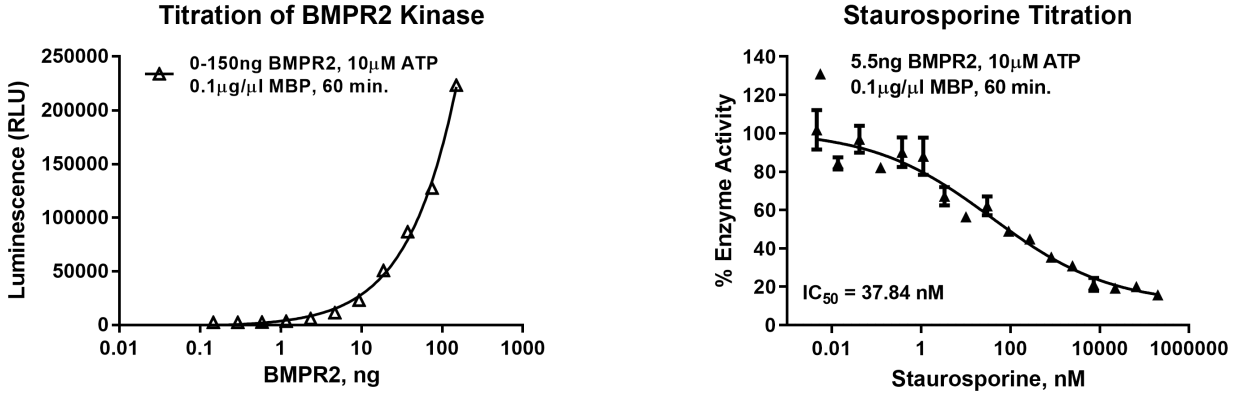

Figure 3. BMPR2 Kinase Assay Development. (A) BMPR2 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 5.5ng of BMPR2 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
BMPR2 Kinase Enzyme System	10µg	VA7387
	1mg	VA7388
ADP-Glo™ + BMPR2 Kinase Enzyme System	1 Each	VA7389