ADP-Glo™ Kinase Assay Application Notes

SER-THR KINASE SERIES: TAK1-TAB1

TAK1-TAB1 Kinase Assay

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Scientific Background:

TAK1 is a serine/threonine protein kinase that mediates signaling by TGFβ and morphogenetic protein (BMP) (1). In response to IL-1, TAK1 forms a kinase complex with TAB1 and this complex is required for the activation of nuclear factor kappa B (NfκB). TAK1 can also activate MAPK8/JNK and MAP2K4/MKK4 and thus play a role in the cell response to environmental stress. Tak1 is essential for thymocyte development and activation and deletion of TAK1 prevents maturation of single-positive thymocytes displaying CD4 or CD8 (2). Thymocytes lacking TAK1 fail to activate NfκB and JNK and are prone to apoptosis upon stimulation.

1. Yamaguchi, K. et al: Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. Science 270: 2008-2011, 1995.

2. Liu, H.-H. et al: Essential role of TAK1 in thymocyte development and activation. Proc. Nat. Acad. Sci. 103: 11677-11682, 2006.

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.

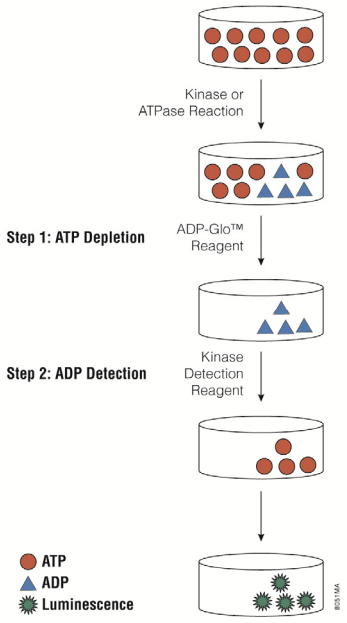


Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

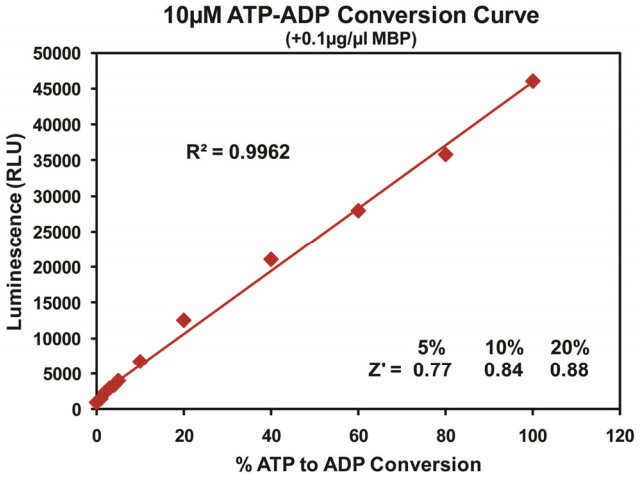


Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

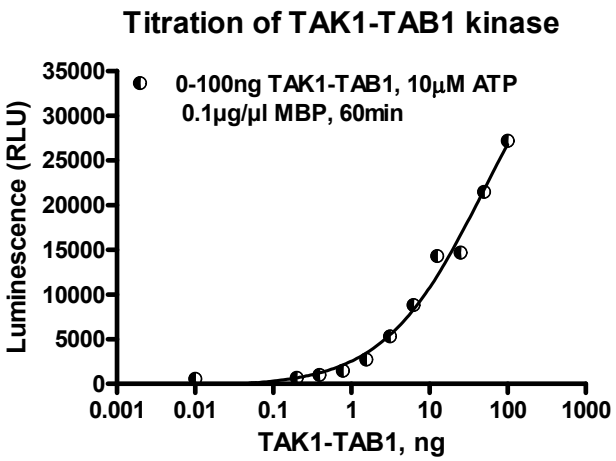
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html

Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 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. TAK1-TAB1 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.

TAK1-TAB1, ng	100	50	25	13	6.3	3.1	1.6	0.8	0
RLU	27195	21483	14699	14298	8815	5311	2730	1483	564
S/B	48	38	26	25	16	9	5	3	1
% Conversion	34	27	18	18	10	6	2	0.8	0



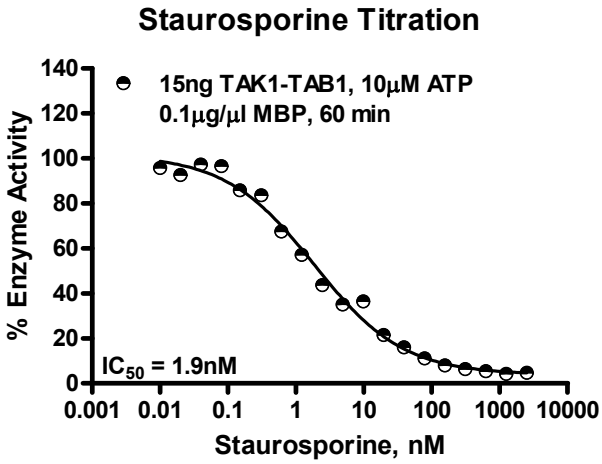


Figure 3. TAK1-TAB1 Kinase Assay Development. **(A) TAK1-TAB1 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 15ng of TAK1-TAB1 to determine the potency of the inhibitor (IC_{50}).**

Assay Components and Ordering Information:

Products

	Company	Cat.#
ADP-Glo™ Kinase Assay	Promega	V9101
TAK1-TAB1 Kinase Enzyme System	Promega	V4088
ADP-Glo™ + TAK1-TAB1 Kinase Enzyme System	Promega	V4089

TAK1-TAB1 Kinase Buffer: 40mM Tris,7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.

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