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Data Sheet

TAK1-TAB1 Kinase Assay Kit

Catalog #78037
96 reactions

Background: TAK1 is a serine/threonine protein kinase that mediates signaling by TGFβ and morphogenetic protein (BMP). 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 (NF-κB). TAK1 can also activate MAPK8/JNK and MAP2K4/MKK4 and thus play a role in the cell response to environmental stress. It has been implicated in various inflammation disorders and cancers, including breast and pancreatic cancer.

Description: The *TAK1-TAB1 Kinase Assay Kit* is designed to measure TAK1-TAB1 kinase activity for screening and profiling applications using ADP-Glo® Kinase Assay as a detection reagent. The *TAK1-TAB1 Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant TAK1-TAB1 enzyme, MBP, ATP, and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40279	TAK1-TAB1, GST-tag	5 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer	1.5 ml	-20°C	
79686	ATP (500 µM)	50 µl	-20°C	
40535	MBP (5 mg/ml)	50 µl	-20°C	
79696	96-well plate, white	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
ADP-Glo® Kinase Assay (Promega #V6930)
Dithiothreitol (DTT 0.5 M)
Microplate reader capable of reading luminescence
Adjustable micropipettor and sterile tips
30°C incubator

APPLICATIONS: Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months when stored as recommended.

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REFERENCES:

1. Zhu, J., *et al.* 2015. "Expression of TAK1/TAB1 expression in non-small cell lung carcinoma and adjacent normal tissues and their clinical significance." *International Journal of Clinical and Experimental Pathology* **8(12):** 15801-15807.
2. Sakurai, H. 2012. "Targeting of TAK1 in inflammatory disorders and cancer." *Trends in Pharmacological Sciences* **33(10):** 522-530.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Thaw **5x Kinase assay buffer**, **ATP (500 µM),** and **MBP (5 mg/ml).** Add 30 µl of 0.5 M DTT to **5x Kinase assay buffer**.

2) Prepare the master mixture (12.5 µl per well): N wells x (11.5 µl **1x Kinase assay buffer** + 0.5 µl **ATP (500 µM)** + 0.5 µl **MBP (5 mg/ml)**). Add 12.5 µl to every well.

	Positive Control	**Test Inhibitor**	**Blank**
1x Kinase assay buffer	11.5 µl	11.5 µl	11.5 µl
ATP (500 µM)	0.5 µl	0.5 µl	0.5 µl
MBP (5 mg/ml)	0.5 µl	0.5 µl	0.5 µl
Test Inhibitor	–	2.5 µl	–
Inhibitor buffer (10% DMSO in water)	2.5 µl	–	2.5 µl
1x Kinase buffer	–	–	10 µl
TAK1-TAB1 (5 ng/µl)	10 µl	10 µl	–
Total	25 µl	25 µl	25 µl

3) Add 2.5 µl of Inhibitor solution of each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 2.5 µl of the same solution without inhibitor (“Inhibitor buffer”). Maintain the same level of DMSO in the controls as the test sample.
Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor at 10 µM that is dissolved in 100% DMSO, dilute 1 mM inhibitor with water to make 100 µM inhibitor in 10% DMSO(aq). Then, add 2.5 µl of the 100 µM solution into the 25 µl assay to make a 1% DMSO concentration in the final reaction mixture

4) Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 µl of 5x Kinase assay buffer with 2400 µl water. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.

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5) To the wells designated as "Blank," add 10 µl of **1x Kinase assay buffer**.

6) Thaw **TAK1-TAB1** on ice. Upon first thaw, briefly spin tube containing protein to recover full content of the tube. Calculate the amount of **TAK1-TAB1** required for the assay and dilute to 5 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted material in aliquots at -80°C. *Note: TAK1-TAB1 is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7) Initiate reaction by adding 10 µl of diluted **TAK1-TAB1** to the wells designated "Positive Control" and "Test Inhibitor." Incubate at 30˚C for 45 minutes.

8) Thaw ADP-Glo reagent.

9) After the 45 minutes reaction, add 25 µl of ADP-Glo reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 45 minutes.

10) Thaw Kinase Detection reagent.

11) After the 45 minutes incubation, add 50 µl of Kinase Detection reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for another 45 minutes.

12) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without protein (typically we set this value as 100).

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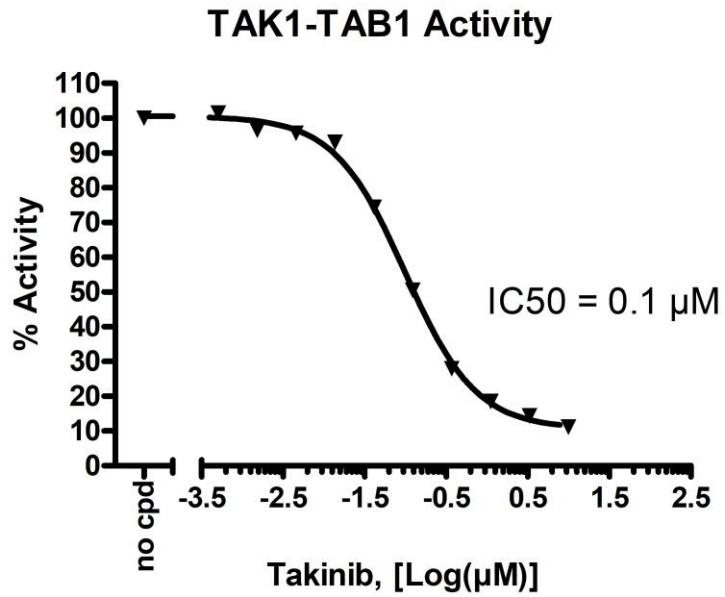
Example of Assay Results:



Inhibition of TAK1-TAB1 by Takinib (Medchem Express #HY-103490), measured using the *TAK1-TAB1 Kinase Assay Kit* (BPS Bioscience #78037). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
TAK1-TAB1, GST-tag	40279	5 µg
MBP, His-tag	40535	100 µg
ATP (500 µM) 50 µl	79686	200 µl
5X Kinase assay buffer	79334	10 ml

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