



6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet
TBK1 Assay Kit
Catalog # 79871
96 Reactions

Background: TBK1 (TANK-binding kinase 1) is a ubiquitously expressed serine/threonine kinase that belongs to the non-canonical I κ B kinase (IKKs) family. TBK1 plays a critical role in controlling Type I interferon production through the IRF3 signaling pathway, suggesting it could be a promising drug target for autoimmune diseases such as interferonopathies.

DESCRIPTION: The *TBK1 Assay Kit* is designed to measure TBK1 activity for screening and profiling applications using Kinase-Glo[®] MAX as a detection reagent. The *TBK1 Assay Kit* comes in a convenient 96-well format, with enough purified recombinant TBK1 enzyme, TBK1 substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40286	TBK1	10 μ g	-80°C	Avoid multiple freeze/ thaw cycles!
79334	5x Kinase assay buffer	1.5 ml	-20°C	
79686	ATP (500 μ M)	100 μ l	-20°C	
	TBK1 Substrate (MBP, 5 mg/ml)	200 μ l	-20°C	
79696	96-well plate, white	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Kinase-Glo MAX (Promega #V6071)
Dithiothreitol (DTT, 1 M; optional)
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.

REFERENCE:

1. Fitzgerald K. A., *et al.*, *Nature Immunol*, 2003, **4(5)**: 491-496
2. Ahmad L., *et al.*, *Trend Mol Med*. 2016, **22(6)**: 511-527

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

- 1) Thaw **5x Kinase assay buffer**, **ATP (500 μ M)**, and **TBK1 Substrate (MBP)**.
(Optional: If desired, add DTT to **5x Kinase assay buffer** to make a 10 mM concentration; e.g. add 10 μ l of 1 M DTT to 1 ml **5x Kinase assay buffer**)
- 2) Prepare the master mixture (25 μ l per well): N wells x (6 μ l **5x Kinase assay buffer** + 1 μ l **ATP (500 μ M)** + 2 μ l **TBK1 Substrate (5 mg/ml)** + 16 μ l distilled water). Add 25 μ l to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	6 μ l	6 μ l	6 μ l
ATP (500 μ M)	1 μ l	1 μ l	1 μ l
TBK1 Substrate (MBP, 5 mg/ml)	2 μ l	2 μ l	2 μ l
Water	16 μ l	16 μ l	16 μ l
Test Inhibitor	-	5 μ l	-
Inhibitor Buffer (e.g. 10% DMSO(aq))	5 μ l	-	5 μ l
1x Kinase buffer	-	-	20 μ l
TBK1 (5 ng/ μ l)	20 μ l	20 μ l	-
Total	50 μ l	50 μ l	50 μ l

- 3) Add 5 μ l of Inhibitor solution of each well labeled as "Test Inhibitor." For the "Positive Control" and "Blank," add 5 μ l of the same solution without inhibitor (Inhibitor buffer). Make certain that the DMSO concentration of the inhibitor buffer is the same as the test inhibitor. Final concentration of DMSO in the assay should be \leq 1%.
- 4) Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 μ l of **5x Kinase assay buffer** with 2400 μ l water. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.
- 5) To the wells designated as "Blank," add 20 μ l of **1x Kinase assay buffer**.
- 6) Thaw **TBK1** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **TBK1** required for the assay and dilute enzyme to \sim 5 ng/ μ l with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80° C.

Note: TBK1 enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

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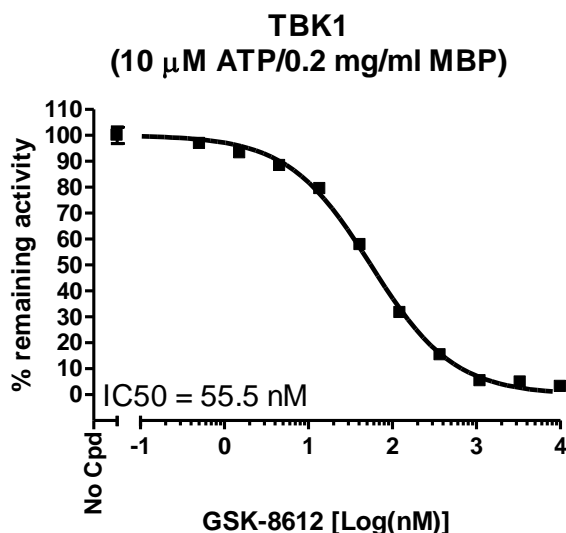
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- 7) Initiate reaction by adding 20 μ l of diluted **TBK1** to the wells designated "Positive Control" and "Test Inhibitor Control." Incubate at 30°C for 45 minutes.
- 8) Thaw Kinase-Glo Max reagent.
- 9) After the 45 minute reaction, add 50 μ l of Kinase-Glo Max reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes.
- 10) Measure luminescence using a microplate reader capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

Example of Assay Results:



Inhibition of TBK1 by GSK8612 measured using the TBK1 assay kit. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
TBK1, GST-tag (Human)	40286	10 μ g
Kinase Buffer 1	79334	10 ml
ATP (500 μ M)	79686	200 μ l
NF- κ B Reporter Kit	60614	500 rxns.
IKK β , GST-tag	40304	10 μ g

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