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

MNK1 Kinase Assay Kit

Catalog #78032

Background: MAPK-interacting serine/threonine-protein kinase 1 (MNK1) is a member of the serine/threonine family that plays a role in mitotic cell cycle progression. Alteration of MNK1 has been shown in various cancers such as breast carcinoma and melanoma. MNK1 phosphorylates eukaryotic translation initiation factor 4E (eIF4E), enhancing phosphorylation of quiescent cells and rendering eIF4E oncogenic.

Description: The *MNK1 Kinase Assay Kit* is designed to measure MNK1 kinase activity for screening and profiling applications using ADP-Glo® Kinase Assay as a detection reagent. The *MNK1 Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant MNK1 enzyme, MBP, ATP, and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40078	MNK1	40 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)	100 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.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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

REFERENCE:

1. Chrestensen, C.A., *et al.* 2007. "MNK1 and MNK2 regulation in HER2-overexpressing breast cancer lines." *Journal of Biological Chemistry* **282(7):** 4243-4252.
2. Dreas, A., *et al.* 2017. "Mitogen-activated protein kinase (MAPK) interacting kinases 1 and 2 (MNK1 and MNK2) as targets for cancer therapy: recent progress in the development of MNK inhibitors." *Current Medicinal Chemistry* **24(28):** 3025-3053.

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 µl **1x Kinase assay buffer** + 0.5 µl **ATP (500 µM)** + 1 µl **MBP (5 mg/ml)**). Add 12.5 µl to every well.

	Positive Control	Test Inhibitor	Blank
1x Kinase assay buffer	11 µl	11 µl	11 µl
ATP (500 µM)	0.5 µl	0.5 µl	0.5 µl
MBP (5 mg/ml)	1 µl	1 µl	1 µl
Test Inhibitor	–	2.5 µl	–
Inhibitor buffer (10% DMSO in water)	2.5 µl	–	2.5 µl
1x Kinase buffer	–	–	10 µl
MNK1 (40 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 Inhibitor buffer (same solution without inhibitor, usually 10% DMSO in water). *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.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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

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

6) Thaw **MNK1** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **MNK1** required for the assay and dilute enzyme to 40 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: MNK1 enzyme 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 **MNK1** 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:

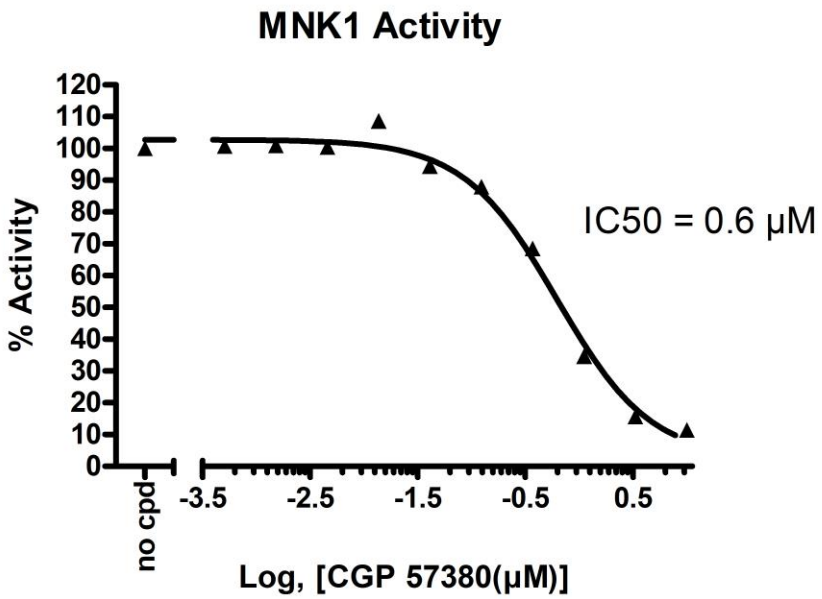
Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

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 enzyme (typically we set this value as 100).

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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

Example of Assay Results:



Inhibition of MNK1 by CGP 57380 (Sigma #454861), measured using the *MNK1 kinase assay kit* (BPS Bioscience #78032). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	**Catalog #**	**Size**
MNK1 (T385D), GST-tag	40078	10 µg
MNK2, GST-tag	40128	10
µg5X Kinase assay buffer	79334	10 ml
ATP (500 µM)	79686	200 µl
MBP, His-tag	40535	100 µg
eIF4E, GST-tag	40530	100 µg

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.