TTK (MPS-1) Kinase Assay Kit

#78356
96 reactions

Description

TTK, also known as Monopolar Spindle 1 Kinase (MPS-1), is a dual specific kinase (tyrosine and serine/threonine) that plays an important role in the cell division cycle by regulating spindle assembly checkpoint during mitosis. Recent studies showed that inhibition of TTK induces aberrant mitosis and apoptosis in various cancer cells, suggesting that TTK is a potential cancer therapeutic target. The TTK Kinase Assay Kit is designed to measure TTK kinase activity for screening and profiling applications using ADP-Glo® Kinase Assay as a detection reagent. The TTK Kinase Assay Kit comes in a convenient 96-well format, with enough purified recombinant TTK enzyme, MBP substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Applications

- Studying enzyme kinetics.
- Screen small molecular inhibitors for drug discovery and high throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
40291	TTK (MPS-1)*	10 µg	-80°C
79334	5x Kinase Buffer 1	1.5 ml	-20°C
79686	ATP (500 µM)	100 µl	-20°C
78514	MBP (5 mg/ml)	100 µl	-80°C
79696	96-well plate, white	1	Room Temp

**The concentration of TTK (MPS-1) is lot-specific and will be indicated on the tube containing the enzyme*

Materials Required but Not Supplied

Name	Catalog #
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	

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

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Assay Protocol

All samples and controls should be tested in duplicate.

1. Thaw 5x Kinase Buffer 1, ATP and TTK substrate (MBP).
   Add DTT to 5x Kinase Buffer 1 to make a 10 mM DTT concentration (e.g., add 10 µl of 1 M DTT to 1 ml of 5x Kinase Buffer 1). *Note: Discard any remaining 5X Kinase assay buffer containing DTT.*

2. Prepare the Master Mix (12.5 µl per well): N wells x (3 µl of 5x Kinase Buffer 1 + 1 µl of MBP (5mg/ml) + 0.5 µl of ATP (500 µM) + 8 µl of water). Add 12.5 µl to every well.

3. Prepare 3 ml of 1x Kinase Buffer 1 by mixing 600 µl of 5x Kinase Buffer 1 (containing DTT) with 2.4 ml of water. 3 ml of 1x Kinase Buffer 1 is sufficient for 100 reactions.

4. Add 2.5 µl of 10X Test Inhibitor to each well labeled as “Test Inhibitor”. For the “Positive Control" and “Blank”, add 2.5 µl of the same diluent solution without inhibitor (Diluent Solution). The diluent solution contains buffer and the same concentration of solvent (for example DMSO) as the test Inhibitor solution.

   For example, dissolve Test Compound in DMSO; dilute in the stock solution of test Compound into 1x Kinase Buffer 1 to make a 10x concentration solution containing a maximum of 10% DMSO. At this step the compound concentration is 10-fold higher than the desired final concentration.
   For the “Positive Control” and “Blank”, use 10% DMSO in 1x Kinase Buffer 1 with no inhibitor (if the compound was dissolved in DMSO) or use 1x kinase buffer if the compound is aqueous and was diluted in kinase buffer. The final concentration of DMSO in the assay should not exceed 1%.

5. To the wells designated as "Blank", add 10 µl of 1x Kinase Buffer 1.

6. Thaw TTK (MPS-1) enzyme on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube. Dilute TTK (MPS-1) to 10 ng/µl with 1x Kinase Buffer 1.

   Note: TTK (MPS-1) enzymes are sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Although we do not recommend it, if not using all the wells of the assay at once, calculate the amount required for the assay, dilute only the amount sufficient for the assay and aliquot the remaining undiluted TTK (MPS-1) enzyme. Store single use aliquots at -80°C. Do not re-use thawed aliquots and do not re-use the diluted enzyme.

7. Initiate the reaction by adding 10 µl of diluted TTK (MPS-1) enzyme to the wells designated “Positive Control” and "Test Inhibitor Control". Incubate at 30°C for 45 minutes.

Component	Blank	Positive Control	Test Inhibitor
Master Mix	12.5 µl	12.5 µl	12.5 µl
Test Inhibitor			2.5 µl
Diluent Solution (No inhibitor)	2.5 µl	2.5 µl	
1x Kinase buffer	10 µl		
TTK (MPS-1) (10 ng/µl)		10 µl	10 µl
Total	25 µl	25 µl	25 µl

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8. Thaw the ADP-Glo reagent.

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

10. Thaw the 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 the plate in a luminometer or microtiter-plate capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

Example Results

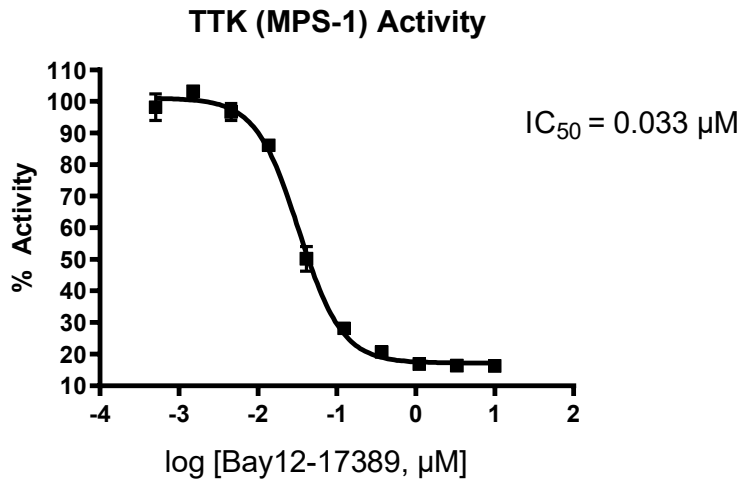

Figure 1: Inhibition of TTK enzyme by BAY 12-17389. TTK activity was measured in the presence on increasing concentrations of BAY-12-17389 using the TTK kinase assay kit (BPS Bioscience, #78356). The Blank value was subtracted from all values. Results are expressed as percent of positive control (no inhibitor - set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

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Related Products

Products	*Catalog #*	*Size*
SPHK1 Assay Kit	78026	96 reactions
Sphingosine Kinase 1, His-Tag	40610	20 µg
PIM1 Assay Kit	79885	96 reactions
PIM1, GST-Tag	41107	10 µg
BTK Assay Kit	79568	96 reactions
BTK, GST-His-Tag	40405	10 µg
MNK1 Kinase Assay Kit	78032	96 reactions
MNK1 (T385D), GST-Tag	40078	10 µg

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