Chemi-Verse™ MLK4 Kinase Assay Kit

#82563
96 reactions

Description

The Chemi-Verse™ MLK4 Kinase Assay Kit is designed to measure MLK4 (Mixed-Lineage Kinase 4) serine/threonine kinase activity for screening and profiling applications using ADP-Glo™ as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified MLK4, kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Background

MLK4 (mixed-lineage kinase 4) is a member of the serine/threonine kinase MLK family of proteins. It is involved in DNA damage, and it is often mutated or overexpressed in several types of cancer. It has been described as tumor suppressor and oncogene in different studies. In ovarian cancer it inhibits the MAPK (mitogen-activated protein kinase) signaling pathway and thus reduces the invasion potential of the cancer cells, while in melanoma it was described to phosphorylate MEK1 (MAP kinase kinase 1) and MKK4/7 (MAK kinase 4) and activate ERK and JNK (c-Jun N-terminal kinase) pathways. In triple negative breast cancer (TNBC) it can contribute to the development of drug resistance. In a cellular model and in a xenograft model, the knockdown of MLK4 increased the sensitivity of cells to doxorubicin and etoposide. The use of inhibitors in combinatory therapy may thus present a new avenue of cancer therapy.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
41105	MLK4, GST-Tag*	10 µg	-80°C
79334	5x Kinase Buffer 1	1.5 ml	-20°C
79686	500 µM ATP	50 µl	-20°C
78514	Myelin Basic Protein (MBP), 5 mg/ml	100 µl	-20°C
82545	White 96-well plate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

Name	Ordering Information
ADP-Glo™ Kinase Assay	Promega #V6930
DTT (Dithiothreitol), 1M, optional	
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.

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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.

Assay Principle

The **ADP-Glo™ Kinase Assay (Promega #V6930)** quantifies the amount of ADP produced by a kinase upon phosphorylation of a substrate. First, addition of the ADP-Glo™ reagent terminates the reaction and quenches the remaining ATP. Second, the addition of the Kinase Detection reagent converts the produced ADP to ATP. The newly generated ATP is quantified by a luciferase reaction. The luminescent signal correlates with the amount of ADP generated by the kinase and is linear to 1 mM ATP.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Blank", "Positive Control" and "Test Inhibitor" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Staurosporine (#27002) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

1. Thaw **5x Kinase Assay Buffer 1**, **500 µM ATP,** and **Myelin basic protein (MBP), 5 mg/ml.**

   *Optional: If desired, make **5x Kinase Assay Buffer 1** with 10 mM DTT.*

2. Prepare 3 ml of **1x Kinase Assay Buffer 1** by mixing 600 µl of **5x Kinase Assay Buffer 1** with 2,400 µl of distilled water.

   *Note: Three (3 ml) of **1x Kinase Assay Buffer 1** is sufficient for 100 reactions.*

3. Prepare a **Master Mix** (12.5 µl/well): N wells x (6 µl of 5x Kinase Assay Buffer 1 + 0.5 µl of 500 µM ATP + 1 µl of Myelin basic protein (MBP), 5 mg/ml + 5 µl of distilled water).

4. Add 12.5 µl of Master Mix to every well.

5. Prepare the **Test Inhibitor** (2.5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

   5.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in 1x Kinase Assay Buffer 1, 10-fold more concentrated than the desired final concentrations.

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For the positive and negative controls, use 1x Kinase Assay Buffer 1 (Diluent Solution).

OR

5.2 If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x Kinase Assay Buffer 1 to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Kinase Assay Buffer 1 to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Kinase Assay Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

6. Add 2.5 µl of Test Inhibitor to each well labeled “Test Inhibitor”.

7. Add 2.5 µl of Diluent Solution to the “Positive Control" and “Blank” wells.

8. Add 10 µl of 1x Kinase Assay Buffer 1 to the "Blank" wells.

9. Thaw **MLK4 Kinase** on ice. Briefly spin the tube to recover its full content.

10. Dilute the protein kinase (10 µl/well) to 10 ng/µl with **1x Kinase Assay Buffer 1**.

11. Initiate the reaction by adding 10 µl of diluted kinase to the wells designated “Positive Control” and "Test Inhibitor".

Component	**Blank**	**Positive Control**	**Test Inhibitor**
Master Mix	12.5 µl	12.5 µl	12.5 µl
Test Inhibitor	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	-
1x Kinase Assay Buffer 1	10 µl	-	-
Diluted MLK4 (10 ng/µl)	-	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**

12. Incubate at 30°C for 45 minutes.

13. Thaw the ADP-Glo™ reagent.

14. At the end of the 45-minute reaction, add 25 µl of ADP-Glo™ reagent to each well.

15. Cover the plate with aluminum foil and incubate at Room Temperature (RT) for 45 minutes.

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16. Thaw the Kinase Detection Reagent.

17. Add 50 µl of Kinase Detection reagent to each well.

18. Cover the plate with aluminum foil and incubate at RT for another 45 minutes.

19. Immediately read in a luminometer or a microplate reader capable of reading luminescence.

20. The "Blank" value is subtracted from all other readings.

Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, 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: 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).

Example Results

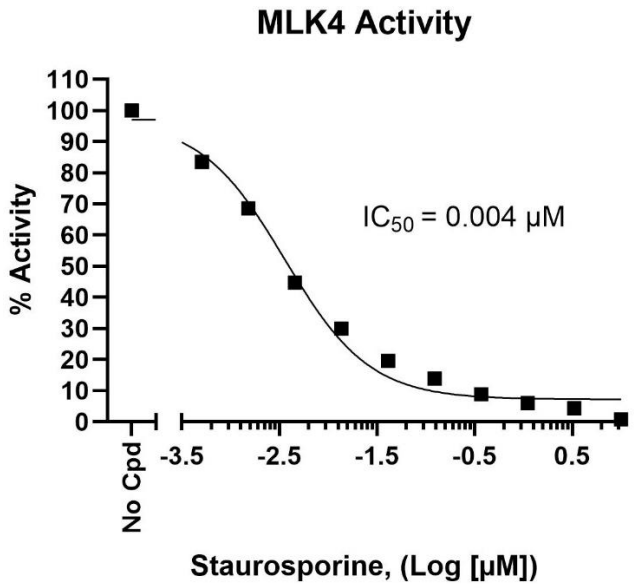

Figure 1: Inhibition of MLK4 kinase activity by the inhibitor Staurosporine.
MLK4 kinase activity was measured in the presence of increasing concentrations of Staurosporine (#27002). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of 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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Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

Mehlich D., *et al.*, 2921 *Cell Death Dis* 12(12):1111.
Gong M., *et al.*, 2023 *PLoS One* 18(8):e0290462.

Related Products

Products	*Catalog #*	*Size*
MLK1, GST-Tag Recombinant	41102	10 µg
MLK2, GST-Tag Recombinant	41103	10 µg
MLK3, GST-Tag Recombinant	41104	10 µg

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