Chemi-Verse™ CLK2 Kinase Assay Kit

#82146
96 reactions

Description

The Chemi-Verse™ CLK2 Kinase Assay Kit is designed to measure CLK2 (CDC like kinase 2) 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 recombinant CLK2 kinase (amino acids 137-end), kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Background

CDC-like kinase 2 (CLK2) is part of the dual-specificity protein kinase (DSK) family that phosphorylate serine/arginine rich (SR) proteins that are part of the spliceosomal complex and also regulate non-splicing proteins. It is involved in cell cycle progression, apoptosis, the DNA replication checkpoint, and regulation of telomere length. Dysfunction in pathways regulated by CLK2 can result in inflammatory and neurodegenerative diseases such as Alzheimer's disease, in hyperglycemia, and in cancer. CLK2 is upregulated in breast cancer, non-small cell lung cancer (NSCLC), glioblastoma, and GSC (glioma stem-like cell). CLK2 also contributes to fatty acid oxidation and ketogenesis and is involved in fatty liver disease. Recent findings demonstrated that CLK kinases activate PTP-1B (protein-tyrosine phosphatase) family members, and this phosphatase may be an important cellular target of CLK. The use of Lorecivivint, a CLK2 inhibitor, in a clinical trial for osteoarthritis was found to be effective and safe. The development of CLK2 inhibitors and a deeper understanding of its role in the multiple pathways where it is involved will prove crucial for the treatment of CLK2-linked diseases.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
40197	CLK2, GST-Tag*	2.5 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	50 µl	-20°C
79696	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	

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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.

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.

1. Thaw **5x Kinase Assay Buffer 1**, **500 µM ATP,** and **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 5**x Kinase Assay Buffer 1** + 0.5 µl of **500 µM ATP** + 0.5 µl of MBP (5 mg/ml) + 5.5 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.

   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 **CLK2 kinase** on ice. Briefly spin the tube to recover its full content.
10. Dilute the protein kinase (10 µl/well) to 2.5 ng/µl with 1x Kinase Assay Buffer 1.

    Note: The concentration of protein is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly. This kinase is particularly sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use the thawed protein and do not re-use the diluted kinase.
11. Initiate the reaction by adding 10 µl of diluted kinase to the wells designated “Positive Control” and "Test Inhibitor".
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.
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 45 minutes.
19. Immediately read in a luminometer or a microplate reader capable of reading luminescence.

20. The "Blank" value should be subtracted from all other readings.

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 CLK2 (2.5 ng/µl)	-	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**

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

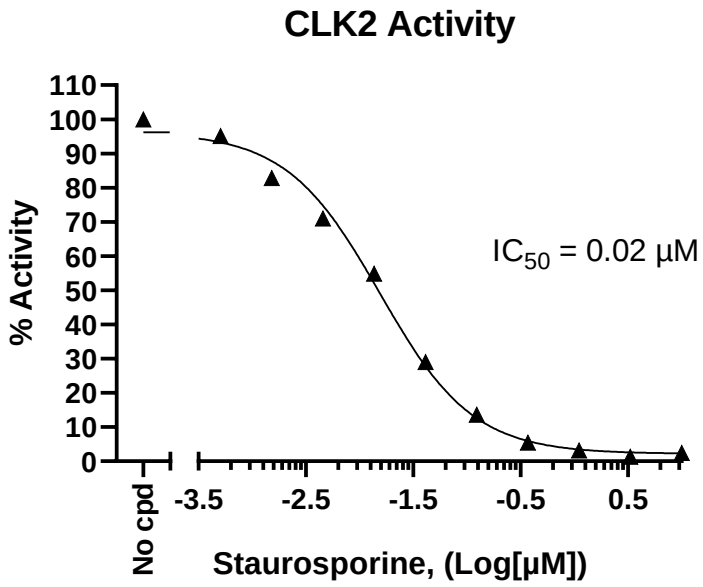

Figure 1: Inhibition of CLK2 kinase activity by Staurosporine. The inhibition of CLK2 kinase activity was measured in the presence of increasing concentrations of Staurosporine (Selleckchem S1421). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (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.

Troubleshooting Guide

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

References

Song M., *et al.*, 2023 *Signal Transduction and Targeted Therapy* 8: 148.

Related Products

Products	*Catalog #*	*Size*
CLK1, GST-Tag Recombinant	40196	10 µg
CLK3, GST-Tag Recombinant	40198	10 µg
PTP1B (PTPN1) Full Length, GST-Tag Recombinant	30009	50 µg
Fluorogenic PTP1B (Catalytic Domain) Assay Kit	79764	96 reactions
PTP1B (Catalytic Domain) Colorimetric Assay Kit	30019	96 reactions
Chemi-Verse™ CLK1 Kinase Assay Kit	82145	96 reactions
Chemi-Verse™ CLK3 Kinase Assay Kit	82150	96 reactions