Description

The PKM2 Kinase Inhibition Assay Kit is designed to measure the inhibition of human PKM2 (pyruvate kinase M2), also known as PKM1/M2 isoform a, for screening and profiling applications using Kinase-Glo® Max as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified PKM2, Phosphoenolpyruvate (PEP), ADP, allosteric PKM2 activator FBP (fructose-1,6-bisphosphate), and kinase assay buffer for 100 enzyme reactions.

Background

PKM2 (pyruvate kinase M2), also known as PKM1/M2 isoform a, is an isoenzyme of the glycolytic enzyme pyruvate kinase family. It is found in differentiated tissues, including lung cells, fat tissue, and cells that require a high rate of acid nucleic synthesis, such as cancer cells. PKM2 can form either highly active tetramers or low-activity dimers. The dimeric form slows glycolysis, diverting glucose metabolism from oxidative phosphorylation toward lactate production - a hallmark of tumor cells. This low-activity dimer is strongly associated with cancer because it channels glucose intermediates into biosynthetic pathways that support cell growth. PKM2 Inhibitors target the low-activity dimeric form of PKM2 that supports cancer cell survival by enhancing glycolysis and biosynthetic pathways. Inhibitors have demonstrated effectiveness in sensitizing cancer cells to chemotherapy. The dual impact on metabolism and transcription makes PKM2 inhibitors attractive candidates for combination cancer therapies.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
50295-KC5	PKM2 (PKM2/ Variant 1), His-tag*	5 µg	-80°C
87804-KC1.5	5x PKM2 Assay Buffer	1.5 ml	-20°C
87805-KC4	5x Diluent Solution	4 ml	-20°C
87806-KC1	20 mM ADP	1 ml	-20°C
87807-KC500	50 mM PEP	500 µl	-20°C
87808-KC1	100 mM FBP	1 ml	-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
Kinase-Glo® Max	Promega #V6071
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	

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

Kinase activity is measured using Kinase-Glo® Max (Promega #V6071). The addition of the reagent results in the generation of a luminescent signal that correlates with the amount of ATP. The reagent is linear to 500 µM ATP.

Contraindications

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

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include "Blank", "Negative Control", "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 Shikonin (BPS Bioscience #83730) as inhibitor control. If not running a dose response curve for the inhibitor control, we recommend running the control at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- If a preincubation step is required, we recommend preincubating the enzyme with an inhibitor prior to adding the Master Mix 2 for at least 20 minutes.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

1. Thaw **PKM2**, **20 mM ADP, 50 mM PEP,** and **100 mM FBP** on ice**.** Thaw **5x PKM2 Assay Buffer** and **5x Diluent Solution** at Room Temperature (RT).

2. Prepare 10 ml of **1x Diluent Solution** by diluting **5x Diluent Solution** 5-fold with distilled water.

3. Dilute **PKM2** to 2.5 ng/µl with 1x Diluent Solution (20 µl/well).

4. Dilute **100 mM FBP** 1000-fold to 100 µM with 1x Diluent Solution (5 µl/well).

5. Prepare a **Master Mix 1** (25 µl/well): N wells x (20 µl of diluted PKM2 + 5 µl of 100 µM FBP).

6. Add 25 µl of Master Mix 1 to each well.

7. Prepare the **Test Inhibitor** (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 50 µl.

7.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in 1x Diluent Solution, 10-fold more concentrated than the desired final concentrations.

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

OR

7.2 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 Dilution Solution 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 Dilution Solution to keep the concentration of DMSO constant.

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

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

8. Add 5 µl of **Test Inhibitor** to each well labeled “Test Inhibitor”.

9. Add 5 µl of **1x Diluent Buffer** to the “Blank”, “Positive Control" and “Negative Control” wells.

10. Pre-incubate the plate at RT for 20 minutes.

11. Dilute **50 mM PEP** 10-fold with distilled water to prepare **5 mM PEP** (5 µl/well).

12. Prepare a **Master Mix 2** (20 µl/well, except “Blank” and “Negative Control” wells): N wells x (10 µl of 5x PKM2 Assay Buffer + 5 µl of 20 mM ADP + 5 µl of 5 mM PEP).

    Notes: The default assay setup includes 500 µM PEP and 10 µM FBP in the reaction. FBP acts as an allosteric activator that binds to PKM2, promoting a conformational change that increases its affinity for PEP. Without FBP, the Km for PKM2 with PEP is much higher, approximately 1.5 mM. Alternatively, the inhibition assay can be run without FBP but using a higher PEP concentration.

13. Prepare a **Blank Master Mix** (20 µl/”Blank” well): N wells x (10 µl of 5x PKM2 Assay Buffer + 5 µl of 5 mM PEP + 5 µl of distilled water).

14. Prepare a **Negative Control Master Mix** (20 µl/”Negative Control” well): N wells x (10 µl of 5x PKM2 Assay Buffer + 5 µl of 20 mM ADP + 5 µl of distilled water).

15. Initiate the reaction by adding 20 µl of **Master Mix 2** to the wells designated “Positive Control” and "Test Inhibitor".

16. Add 20 µl of the **Blank Master Mix** to the "Blank" wells.

17. Add 20 µl of the **Negative Control Master Mix** to the "Negative Control" wells.

Component	Blank (No ADP)	Negative Control (No PEP)	Positive Control	Test Inhibitor
Master Mix 1	25 µl	25 µl	25 µl	25 µl
Diluent Buffer	5 µl	5 µl	5 µl	-
Test Inhibitor	-	-	-	5 µl
Pre-incubate at RT for 20 minutes				
Master Mix 2	-	-	20 µl	20 µl
Blank Master Mix	20 µl	-	-	-
Negative Control Master Mix	-	20 µl	-	-
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

18. Incubate the plate at RT for 30 minutes.

19. During the incubation, thaw the Kinase-Glo® Max reagent. At the end of the 30-minute reaction, add 50 µl of Kinase-Glo® Max reagent to each well.

20. Cover the plate with aluminum foil and incubate the plate at RT for 10 minutes.

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

22. 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

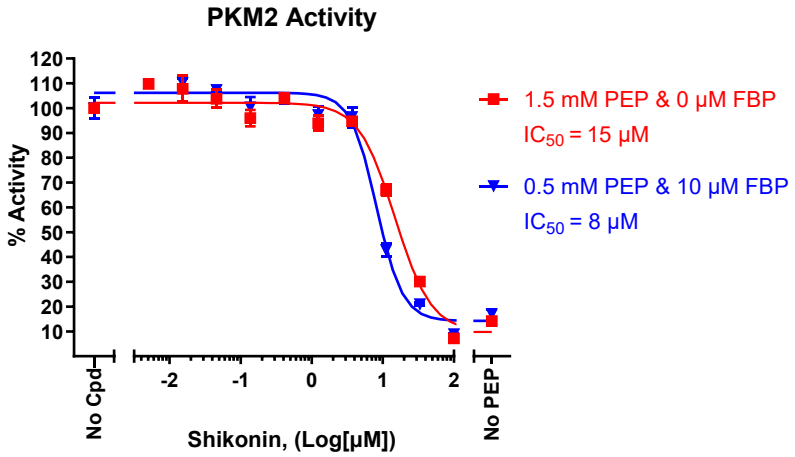

Figure1: Inhibition of PKM2 kinase activity by the inhibitor Shikonin.
PKM2 kinase activity was measured in the presence of increasing concentrations of Shikonin, at two concentrations of PEP and FBP. The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of inhibitor, set at 100%).

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References
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Wang Y., *et al.*, 2021 *Cancer Biomark.* 32(2):221-230
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