

Description

The PKL Kinase Inhibition Assay Kit is designed to measure the inhibition of human liver-type pyruvate kinase enzyme PKL, also known as PKLR Var 2, 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 PKL, Phosphoenolpyruvate (PEP), ADP, allosteric PK activator FBP (fructose-1,6-bisphosphate), and kinase assay buffer for 100 enzyme reactions.

Background

PKL (pyruvate kinase liver) is a pyruvate kinase isoform that catalyzes the transphosphorylation of phosphoenolpyruvate and ADP into pyruvate and ATP as part of the cell metabolism. The other isoforms are PKR, PKM1 and PKM2. This reaction is the rate-limiting step of glycolysis. PKL is mostly involved in hepatic glucose and lipid metabolism, and when dysregulated can result in MASLD (metabolic dysfunction-associated steatotic liver disease) and HCC (hepatocellular carcinoma). Interestingly, it is expressed differently according to the sex of the individual, with males having higher expression than females. The development of PKL modulators, such as small molecules, holds great promise in the treatment of PKL-linked disorders.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
40501-KC5	PKL (PKLR Var2), His-tag*	5 µg	-80°C
87804-KC1.5	5x PKM2 Assay Buffer	1.5 ml	-20°C
84188-KC4	5x Diluent Solution (no DTT)	4 ml	-20°C
82735-KC200	0.5 M DTT	200 µl	-20°C
87806-KC1	20 mM ADP	1 ml	-20°C
87807-KC500	50 mM PEP	500 µl	-20°C
87808-KC100	100 mM FBP	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
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 \(bpsbioscience.com\)](https://www.bpsbioscience.com/protein-faqs).
 - We recommend using PKL-IN-1 (#84234) or Shikonin (#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 IC₅₀ 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 for at least 20 minutes.
 - For instructions on how to prepare reagent dilutions please refer to [Serial Dilution Protocol \(bpsbioscience.com\)](https://www.bpsbioscience.com/serial-dilution-protocol).
1. Thaw **PKL**, **20 mM ADP**, **50 mM PEP**, **100 mM FBP** and **0.5 M DTT** on ice. Briefly spin the tube to recover the full content.
 2. Thaw **5x PKM2 Assay Buffer** and **5x Diluent Solution (no DTT)** at Room Temperature (RT).
 3. Prepare 10 ml of **1x Diluent Solution** by diluting 5x Diluent Solution (no DTT) 5-fold with distilled water, then add 20 µl of **0.5 M DTT**.
 4. Dilute **PKL** to 2 ng/µl with 1x Diluent Solution (25 µl/well).
 5. Add 25 µl of the **diluted PKL** to every well.
 6. 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.

6.1 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

6.2 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 Test 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 the Blank, 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%.

7. Add 5 µl of **Test Inhibitor** to each well labeled "Test Inhibitor".
8. Add 5 µl of **Diluent Buffer** to the "Blank", "Positive Control" and "Negative Control" wells.
9. Pre-incubate the plate at RT for 20 minutes with gentle agitation.
10. Dilute **50 mM PEP** 3.33-fold with distilled water to prepare 15 mM PEP (5 µl/well).
11. Prepare a **Master Mix** (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 15 mM PEP).

Notes: The default assay setup includes 1500 µM PEP in the reaction. The kit also provides FBP, which acts as an allosteric activator by binding to PKL and inducing a conformational change that increases its affinity for PEP. In the presence of FBP, the K_m of PKL for PEP is much lower. Alternatively, the inhibition assay may be performed using 10 µM FBP and a lower PEP concentration, such as 500 µM. For this alternative setup, PKL should be pre-mixed with FBP prior to the addition of the test inhibitor and initiation of the reaction (for detailed instructions on how to perform this alternative protocol step by step, see PKM2 Kinase Inhibition Assay Kit (#87809).

12. Prepare a **Blank Master Mix** (20 µl/"Blank" well): N wells x (10 µl of 5x PKM2 Assay Buffer + 5 µl of 15 mM PEP + 5 µl of distilled water).
13. 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).
14. Initiate the reaction by adding 20 µl of **Master Mix** to the wells designated "Positive Control" and "Test

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Inhibitor".

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

16. 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
Diluted PKL (2 ng/µl)	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	-	-	20 µl	20 µl
Blank Master Mix	20 µl	-	-	-
Negative Control Master Mix	-	20 µl	-	-
Total	50 µl	50 µl	50 µl	50 µl

17. Incubate the plate at RT for 30 minutes with gentle agitation.

18. 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.

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

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

21. 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

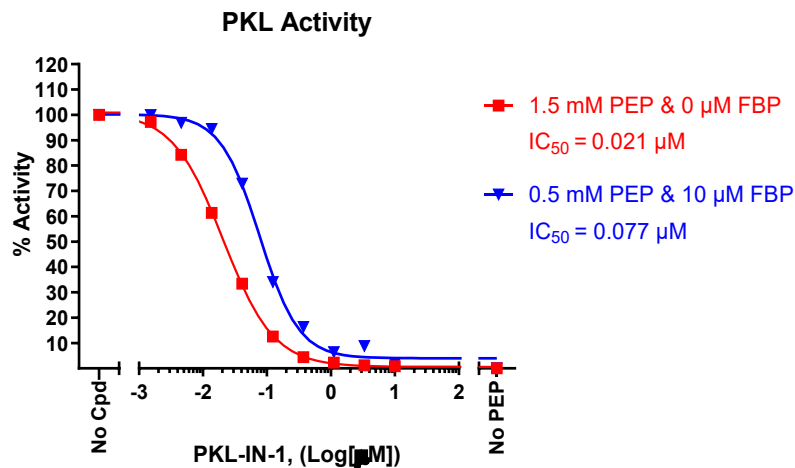


Figure 1: Inhibition of PKL kinase activity by the inhibitor PKL-IN-1.

PKL kinase activity was measured in the presence of increasing concentrations of PKL-IN-1. The “Blank” value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of activator, set at 100%).

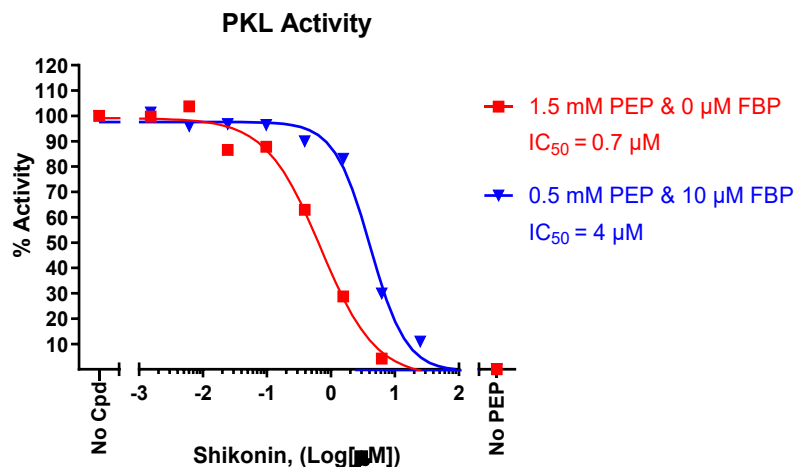


Figure 2: Inhibition of PKL kinase activity by the inhibitor Shikonin.

PKL kinase activity was measured in the presence of increasing concentrations of Shikonin. The “Blank” value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of activator, 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

Yuan M., *et al.*, 2025 *Trends in Endocrinology & Metabolism* 36 (12): 1099-1110.

Lee S., *et al.*, 2017 *Mol Sys Biol* 13 (8): 938.

Iqbal S., *et al.*, 2024 *Int. J. Mol. Sci.* 25 (14): 7968.

Version 061026