

Description

The PKL Kinase Activation Assay Kit is designed to measure the activation 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 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 activators for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
40501-KC1	PKL (PKLR Var2), His-tag*	>1 µg	-80°C
84098-KC5	5x PK Assay Buffer	5 ml	-20°C
87806-KC250	20 mM ADP	250 µl	-20°C
87807-KC125	50 mM PEP	125 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 μ M 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 Activator” conditions.
 - We recommend maintaining the thawed protein on ice during use.
 - For detailed information on protein handling please refer to [Protein FAQs \(bpsbioscience.com\)](https://www.bpsbioscience.com).
 - We recommend using fructose-1,6-bisphosphate (FBP) (#87808-KC1) as a control activator. If not running a dose response curve for the control, we recommend running the control at 0.1X, 1X and 10X the AC₅₀ value shown in the validation data below.
 - If a preincubation step is required, we recommend preincubating the enzyme with an activator 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).
1. Thaw **PKL**, **20 mM ADP**, and **50 mM PEP** on ice. Thaw **5x PK Assay Buffer** at Room Temperature (RT).
 2. Prepare 20 ml of **1x PK Assay Buffer** by diluting **5x PK Assay Buffer** 5-fold with distilled water.
 3. Dilute **PKL** to 0.3 ng/ μ l with 1x PK Assay Buffer and place it at RT for 30 minutes (20 μ l/well).
 4. Add 20 μ l of **diluted PKL** to every well.
 5. Prepare the **Test Activator** (5 μ l/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 μ l.

5.1 If the Test Activator is water-soluble: Prepare a serial dilution in 1x PK Assay Buffer, 10-fold more concentrated than the desired final concentrations.

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

OR

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

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

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

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

6. Add 5 μ l of **Test Activator** to each well labeled "Test Activator".
7. Add 5 μ l of **Diluent Buffer** to the "Blank", "Positive Control" and "Negative Control" wells.
8. Preincubate plate at RT for 20 minutes.
9. Dilute **50 mM PEP** 40-fold with 1x PK Assay Buffer to prepare a 1.25 mM PEP solution (5 μ l/well).
10. Dilute **20 mM ADP** 4-fold with 1x PK Assay Buffer to prepare a 5 mM ADP solution (5 μ l/well).
11. Prepare a **Master Mix** (25 μ l/well, except "Blank" and "Negative Control" wells): N wells x (15 μ l of 1x PK Assay Buffer + 5 μ l of 5 mM ADP + 5 μ l of 1.25 mM PEP).

Note: The K_m value for PKR with PEP varies depending on its conformation. The active tetrameric form has a low K_m value, while the less active dimeric form has a high K_m value. The final PEP concentration in the reaction is 125 μ M which is below the known K_m value for the dimeric, low-affinity state PKR but close to the tetrameric, high-affinity state observed in the presence of the allosteric activator fructose 1,6-bisphosphate (FBP).

12. Prepare a **Blank Master Mix** (25 μ l/"Blank" well): N wells x (20 μ l of 1x PK Assay Buffer + 5 μ l of 1.25 mM PEP).
13. Prepare a **Negative Control Master Mix** (25 μ l/"Negative Control" well): N wells x (20 μ l of 1x PK Assay Buffer + 5 μ l of 5 mM ADP).
14. Initiate the reaction by adding 25 μ l of the **Master Mix** to the wells designated "Positive Control" and "Test Activator".
15. Add 25 μ l of the **Blank Master Mix** to the "Blank" wells.
16. Add 25 μ l of the **Negative Control Master Mix** to the "Negative Control" wells.

Component	Blank (No ADP)	Negative Control (No PEP)	Positive Control (No Activator)	Test Activator
Diluted PKL (0.3 ng/ μ l)	20 μ l	20 μ l	20 μ l	20 μ l
Test Activator	-	-	-	5 μ l
Diluent Buffer	5 μ l	5 μ l	5 μ l	-
Pre-incubate at RT for 20 minutes				
Master Mix	-	-	25 μ l	25 μ l
Blank Master Mix	25 μ l	-	-	-
Negative Control Master Mix	-	25 μ l	-	-
Total	50 μl	50 μl	50 μl	50 μl

17. Incubate the plate at RT for 30 minutes.
18. During the incubation, thaw the Kinase-Glo[®] Max reagent. At the end of the 30-minute reaction, add 50 μ l 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

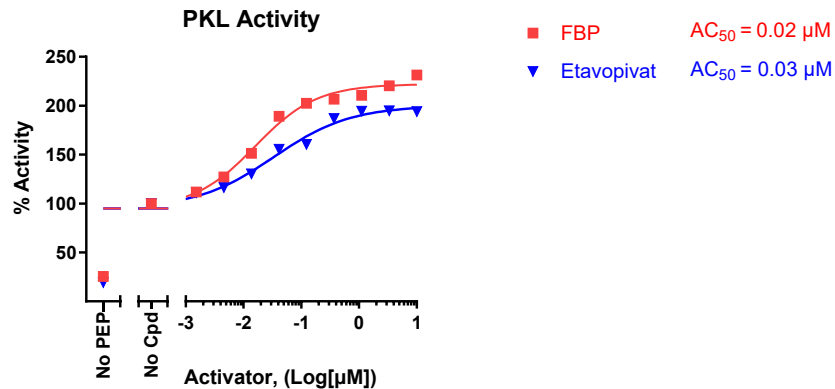


Figure 1: Activation of PKL kinase by the activators FBP and Etavopivat.

PKL kinase activity was measured in the presence of increasing concentrations of FBP (#87808-KC100) and Etavopivat (Sigma #AMBH9A903628). The “Blank” value was subtracted from all other values. Results are expressed as the percentage 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.

Version 040926