Description

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

Background

Red blood cells rely entirely on glycolysis for ATP production, making the erythroid pyruvate kinase isoform PKR (PKLR Var1) essential for maintaining cellular energy and integrity; reduced PKR activity lowers ATP and causes accumulation of glycolytic intermediates such as 2,3-diphosphoglycerate (2,3-DPG), which increases hemoglobin S (HbS) polymerization, promotes sickling, and contributes to chronic hemolytic anemia. PK deficiency—the most common cause of nonspherocytic hemolytic anemia—shares this metabolic signature of elevated 2,3-DPG and reduced ATP, similar to sickle RBCs, contributing to anemia, dehydration, and increased sickling propensity. With more than 290 PKLR mutations identified, several linked to acute pain in sickle cell disease (SCD), these metabolic disturbances provide a strong rationale for targeting PKR. The tetrameric R form of red cell PK (PKR) has naturally low affinity for PEP, which is increased upon binding of fructose-1,6-bisphosphate (FBP), its primary allosteric activator; FBP increases PEP affinity, promotes tetramerization, and stabilizes PKR in its active form. Recently developed oral allosteric activators—mitapivat and etavopivat—bind a pocket at the dimer–dimer interface distinct from the FBP site and increase PKR activity in both wild-type and mutant enzymes. Etavopivat and mitapivat have been shown to decrease 2,3-DPG, increase ATP, raise hemoglobin-oxygen affinity, and reduce sickling in sickle RBCs, confirming the therapeutic potential of PKR activation.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
40500-KC1	PKR (PKLR Var1), 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 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 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).
- 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 AC_{50} 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).

1. Thaw **PKR**, **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 **PKR** to 0.3 ng/µl with 1x PK Assay Buffer and place it at RT for 30 minutes (20 µl/well).

4. Add 20 µl of **diluted PKR** to every well.

5. Prepare the **Test Activator** (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.

   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 with 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 µl of **Test Activator** to each well labeled “Test Activator”.

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

8. Preincubate the 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 µl/well).

10. Dilute **20 mM ADP** 4-fold with 1x PK Assay Buffer to prepare a 5 mM ADP solution (5 µl/well).

11. Prepare a **Master Mix** (25 µl/well, except “Blank” and “Negative Control” wells): N wells x (15 µl of 1x PK Assay Buffer + 5 µl of 5 mM ADP + 5 µl of 1.25 mM PEP).

Note: The Km value for PKR with PEP varies depending on its conformation. The active tetrameric form has a low Km value, while the less active dimeric form has a high Km value. The final PEP concentration in the reaction is 125 µM which is below the known Km 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 µl/”Blank” well): N wells x (20 µl of 1x PK Assay Buffer + 5 µl of 1.25 mM PEP).

13. Prepare a **Negative Control Master Mix** (25 µl/ “Negative Control” well): N wells x (20 µl of 1x PK Assay Buffer + 5 µl of 5 mM ADP).

14. Initiate the reaction by adding 25 µl of the **Master Mix** to the wells designated “Positive Control” and "Test Activator".

15. Add 25 µl of the **Blank Master Mix** to the “Blank” wells.

16. Add 25 µl 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 PKR (0.3 ng/µl)	20 µl	20 µl	20 µl	20 µl
Test Activator	-	-	-	5 µl
Diluent Buffer	5 µl	5 µl	5 µl	-
Pre-incubate at RT for 20 minutes				
Master Mix	-	-	25 µl	25 µl
Blank Master Mix	25 µl	-	-	-
Negative Control Master Mix	-	25 µl	-	-
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 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

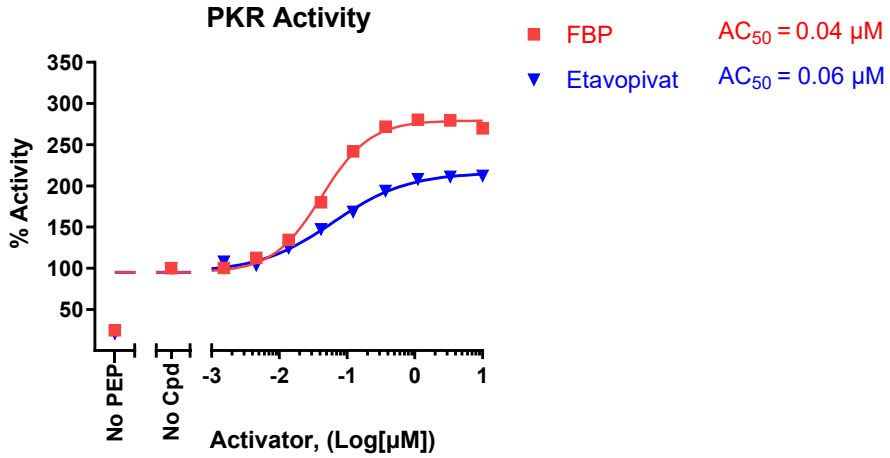

Figure 1: Activation of PKR kinase by the activators FBP and Etavopivat.
PKR kinase activity was measured in the presence of increasing concentrations of FBP (#87808-KC100) or Etavopivat (Sigma #AMBH9A903628). The "Blank" value was subtracted from all other values. Results are expressed as the percentage 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
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