Pyruvate Kinase Assay Kit

Catalog No.: abx298840

Size: 96 tests

Detection Range: 0.08 U/L – 6.79 U/L

Sensitivity: 0.08 U/L

Storage: Store all components in the dark at -20°C.

Application: For detection and quantification of pyruvate kinase activity in serum, plasma, tissue, and cell samples.

Introduction

Pyruvate kinase plays an important physiological role; it is an important enzyme in the glycolysis pathway because it catalyzes the conversion of phosphoenolpyruvate to enolpyruvate and ATP. Pyruvate kinase is also known as phosphopyruvate kinase or phosphotransferase. In the presence of adenosine diphosphate, pyruvate kinase catalyzes the conversion of phosphoenolpyruvate to pyruvate. Lactate dehydrogenase catalyzes pyruvic acid with NADH to produce lactic acid and NAD^+.

Abbexa's Pyruvate Kinase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Pyruvate Kinase activity. The activity of Pyruvate Kinase can be calculated by measuring the absorbance at 340 nm.

Kit components

1. 96-well microplate
2. Buffer solution: 2 x 10 ml
3. Substrate A: 2 vials
4. Substrate B: 2 vials
5. Enzyme Solution: 2 x 1.2 ml
6. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (340 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl)
4. Pipette and pipette tips
5. Vials/tubes
6. Timer
7. Sonicating water bath
8. Centrifuge
9. Vortex mixer
10. Incubator

Protocol

A. Preparation of samples and reagents

1. Reagents

Bring all reagents to room temperature before use

- **Substrate A working solution:** Dissolve a vial of Substrate A powder with 1.2 ml of double distilled water. Preserve on ice in the dark for use. It can be stored in the dark at -20°C for up to 3 days.
- **Substrate B working solution:** Dissolve a vial of Substrate B powder with 10 ml of Buffer Solution. The prepared solution can be stored in the dark at -20°C for up to 3 days.
- **Enzyme working solution:** Mix Substrate A working solution and Enzyme Solution at a 1:1 ratio. Prepare the needed amount just before use to ensure it is fresh. The prepared solution should be used on the same day.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles. Fresh samples are recommended.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 700-1000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh 0.02-1 g of tissue. For each 1 g of tissue, add 9 ml of pre-chilled normal saline (0.9% NaCl). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication, on ice. Centrifuge the homogenate at 10000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. If a precipitate appears, centrifuge again. The protein concentration in the supernatant should be determined separately.
- **Cell sample:** Collect $1x10^6$ cells into a centrifuge tube and add 0.2 ml of normal saline (0.9% NaCl). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication, on ice. Centrifuge at 10000 × g for 10 min and discard the supernatant. Take the supernatant into a new pre-cooled centrifuge tube and analyze immediately. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Rat Kidney Tissue Homogenate	4-6
10% Rat Lung Tissue Homogenate	4-6
10% Rat Liver Tissue Homogenate	4-6
10% Rat Brain Tissue Homogenate	4-6
10% Mouse Liver Tissue Homogenate	4-6
10% Mouse Heart Tissue Homogenate	4-6
Rat Serum	1
Rat Plasma	1
Mouse Serum	1
Mouse Plasma	1
Human Serum	1
Dog Plasma	1
HL-60 Cell	1
293T Cell	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell samples.
- The diluent is normal saline (0.9% NaCl).

B. Assay Procedure

1. Set sample wells on the microplate and record their positions. *Add the solution to the bottom of each well without touching the side walls. Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
2. Add 10 µl of sample into the sample wells
3. Add 150 µl of Substrate B working solution and 40 µl of Enzyme working solution into each well.
4. Measure the OD value of each well at 20 seconds (A_1) and at 3 mins 20 seconds (A_2) at 340nm.
5. if the value A_2 is low, extend the detection time appropriately.

C. Calculation of Results

1. Serum and plasma samples:

One unit is defined as the amount of enzyme that 1 mmol of NADH is consumed by 1 L of liquid sample per minute at room temperature.

$$\text{PK activity (U/L)} = \frac{\Delta A_{340}}{6220 \times d} \times \frac{V_{Total}}{V_{Sample} \times T} \times f \times 1000$$

2. Tissues and cell samples:

One unit is defined as the amount of enzyme that 1 mmol of NADH is consumed by 1 g of sample protein by per minute at room temperature.

$$\text{PK activity (U/L)} = \frac{\Delta A_{340}}{6220 \times d} \times \frac{V_{Total}}{V_{Sample} \times C_{pr} \times T} \times f \times 1000$$

where:

ΔA_{340}	$A_1 - A_2$
6220	The molar extinction coefficient of NADH, L/molcm
d	Optical path, 0.6 cm
V_{Total}	The total volume of the reaction system, 0.2 ml
V_{Sample}	The volume of the sample, 0.01 ml
C_{pr}	Concentration of the protein in sample, gprot/L
T	The time of reaction, 3 minutes
f	Dilution factor of sample before assay
1000	Unit conversion: 1 mol/L = 1000 mmol/L