Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

CheKine™ Micro Phosphoenolpyruvate (PEP) Content Assay Kit

Cat #: KTB1132 Size: 48 T/48 S 96 T/96 S

	Micro Phosphoenolpyruvate (PEP) Content Assay Kit		
REF	**Cat #:** KTB1132	LOT	**Lot #:** Refer to product label
	Detection range: 10-1000 nmol/mL		**Sensitivity:** 10 nmol/mL
	Applicable samples: Animal and Plant Tissues, Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months		

Assay Principle

Phosphoenolpyruvate (PEP) is a key metabolic intermediate in living organisms. It plays an indispensable role in crucial metabolic pathways such as glycolysis, gluconeogenesis, and the synthesis of aromatic amino acids, being of great significance for maintaining the balance of cellular energy metabolism and material synthesis. CheKine™ Micro Phosphoenolpyruvate (PEP) Content Assay Kit can be used to detect biological samples such as animal and plant tissues, cells, plasma, serum or other liquid samples. In the sample, PEP undergoes a transphosphorylation reaction with adenosine diphosphate (ADP) under the catalysis of pyruvate kinase (PK), generating pyruvate and adenosine triphosphate (ATP). Subsequently, pyruvate is oxidized under the action of pyruvate oxidase, accompanied by the reduction of flavin adenine dinucleotide (FAD), producing reduced flavin adenine dinucleotide ($FADH_2$). The generated $FADH_2$ is further oxidized to regenerate FAD and release hydrogen peroxide (H_2O_2). Finally, in the catalytic system of peroxidase, H_2O_2 undergoes a coupled color reaction with 4-aminoantipyrine (4-AAP) and N-ethyl-N-(2-hydroxy-3-propanesulfonyl)-3-methylaniline (TOOS), generating stable red quinone imine compounds. By detecting the absorbance value at 554 nm, the content of PEP in the sample can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60×2 mL	4℃
Reagent Ⅰ	3.2 mL	6.4 mL	4℃
Reagent Ⅱ	Powder×2 vials	Powder×2 vials	4℃
Reagent Ⅲ	35 μL	70 μL	4℃
Reagent Ⅳ	3.2 mL	6.4 mL	4℃
Reagent Ⅴ	350 μL	700 μL	4℃
Standard	Powder×2 vials	Powder×2 vials	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 554 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water, PBS
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent Ⅰ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Working Reagent Ⅱ: Prepared before use. For 48 T, add 200 μL of deionized water to one vial of Reagent Ⅱ. For 96 T, add 400 μL of deionized water to one vial of Reagent Ⅱ. Dissolve the reagent thoroughly and set aside for use; Unused reagents should be stored at 4℃ for up to 2 weeks.

Reagent Ⅲ: Ready to use as supplied; Centrifuge briefly at low speed before use, and allow it to equilibrate to room temperature; Store at 4℃.

Working Solution Ⅰ: Prepared before use. Mix Reagent Ⅰ, Working Reagent Ⅱ and Reagent Ⅲ in a ratio of 90:10:1, and prepare this mixture immediately before use.

Reagent Ⅳ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent Ⅴ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Working Solution Ⅱ: Prepared before use. Mix Reagent Ⅳ and Reagent Ⅴ in a ratio of 90:10, and prepare this mixture immediately before use.

Standard: Prepared before use. Take one vial and add 1 mL of deionized water. Dissolve the contents thoroughly to prepare a 20 μmol/mL Standard solution. Unused reagents should be stored at 4℃ for up to 2 weeks. Using 20 μmol/mL Standard solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (nmol/mL)
Std.1	60 μL of 20 μmol/mL Standard	1,140	1,000
Std.2	300 μL of Std.1 (1,000 nmol/mL)	200	600
Std.3	400 μL of Std.1 (1,000 nmol/mL)	600	400
Std.4	500 μL of Std.3 (400 nmol/mL)	500	200
Std.5	500 μL of Std.4 (200 nmol/mL)	500	100
Std.6	500 μL of Std.5 (100 nmol/mL)	500	50
Std.7	100 μL of Std.6 (50 nmol/mL)	400	10

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for 1 month.

1. Animal and plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice. Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay.

2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times).

Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay.

3. Plasma, Serum or other Liquid samples: Test directly.

Note: If the protein concentration of the sample needs to be determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 554 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Test Well (μL)	Standard Well (μL)
Sample	50	0
Standard	0	50
Working Solution Ⅰ	50	50
Mix well, incubate at 37℃for 10 min, and record the absorbance A_1 at 554 nm		
Working Solution Ⅱ	50	50

3. Mix well, incubate at 37℃for 60 min, and record the absorbance A2 at 554 nm. The Test Well is marked as A_{Test}, the Standard Well is marked as $A_{Standard}$. Finally calculate $\Delta A_{Test}=A_{2Test}-A_{1Test}$, $\Delta A_{Standard}=A_{2Standard}-A_{1Standard}$.

Note: The Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If $\Delta A_{Test}<0.005$, increase the sample quantity. If ΔA_{Test} is above the $\Delta A_{Standard}$ value of the 1,000 nmol/mL standard, the sample can be appropriately diluted with Extraction Buffer.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve and obtain the standard equation. The determination of ΔA_{Test} is substituted into the equation to get y (nmol/mL).

2. Calculation of PEP content

(1) Calculated by protein concentration

PEP (nmol/mg prot)=$(V_{Sample}\times y)\div(V_{Sample}\times Cpr)\times n$**$=y\div Cpr\times n$**

(2) Calculated by fresh weight of samples

PEP (nmol/g fresh weight)=$(V_{Sample}\times y)\div(W\times V_{Sample}\div V_{Total\ sample})\times n$**$=y\div W\times n$**

(3) Calculated by number of cells

PEP (nmol/10^4)=$(V_{Sample}\times y)\div(N\times V_{Sample}\div V_{Total\ sample})\times n$**$=y\div N\times n$**

(4) Calculated by volume of liquid samples

PEP (nmol/mL)**$=y\times n$**

V_{Sample}: Added the sample volume, 0.05 mL; $V_{Total\ sample}$: Added Extraction Buffer volume, 1 mL; Cpr: Sample protein concentration, mg/mL; n: The sample dilution factor; W: Sample weight, g; N: The number of cells, with the unit of 10^4 (for example, if the number of cells is 5×10^6, then N=500).

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

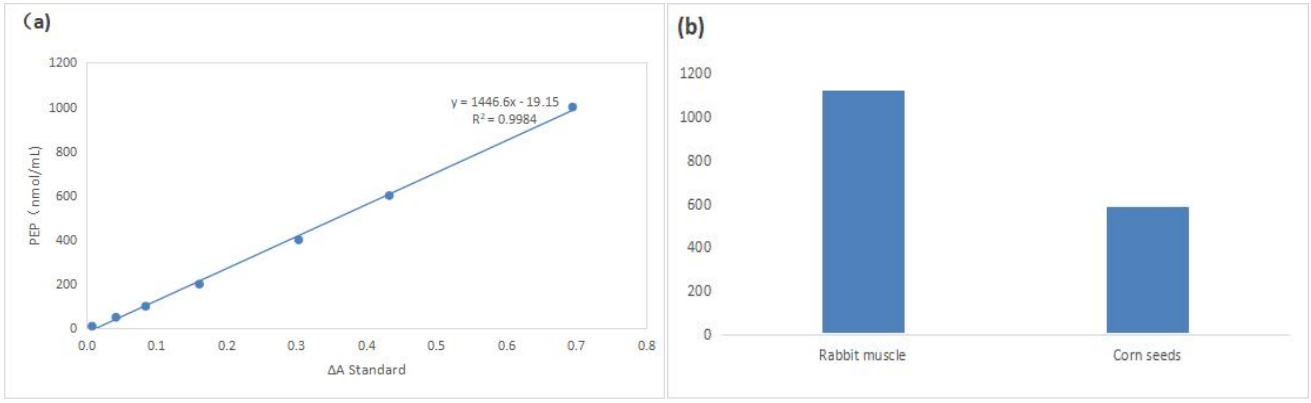

Figure 1. (a) Standard curve of PEP. (b) Determination of PEP content in rabbit muscle and corn seeds by this kit.

Recommended Products

Catalog No.	Product Name
KTB1016	CheKine™ Micro ATP Content Assay Kit
KTB1120	CheKine™ Micro Pyruvate Kinase (PK) Assay Kit
KTB1121	CheKine™ Micro Pyruvate Acid (PA) Assay Kit
KTB1126	CheKine™ Micro Phosphoenolpyruvate Carboxykinase (PEPCK) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.