

Pyruvate (PA) Content Assay Kit

Note: The reagents have been changed, so please be aware of and follow this instruction strictly.

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC2205

Size: 100T/96S

Components:

Extract: Liquid 110 mL × 1. Storage at 2-8°C.

Reagent I: Liquid 3 mL × 1. Storage at 2-8°C.

Reagent II: Liquid 15 mL × 1. Storage at 2-8°C.

Standard solution: Liquid 1 mL × 1, storage at 2-8°C. Sodium pyruvate, 20 μmol/mL.

Preparation of 0.125 μmol/mL standard solution : 50 μL 20 μmol/mL standard solution and 450 μL distilled water mix to obtain 2 μmol/mL standard solution ; then 50 μL 2 μmol/mL standard solution and 750 μL distilled water mix to form 0.125 μmol/mL standard solution.

Product Description

Pyruvate connects glucose, fatty acid and amino acid metabolism through acetyl CoA and plays an important pivotal role.

Pyruvate reacts with 2, 4-dinitrophenylhydrazine to produce pyruvate-2, 4-dinitrophenylhydrazone, which is fuchsia-red in alkaline solution.

Reagents and Equipment Required but Not Provided.

Table centrifuge, transferpettor, spectrophotometer/microplate reader, micro glass cuvette/96 well flat-bottom plate, ice, mortar/homogenizer and distilled water.

Procedure

I. Extraction of Pyruvate:

1. Bacteria or cells:

Collect bacteria or cells into the centrifuge tube, and discard the supernatant after centrifugation. According to the bacteria or cells (10^4) : the extract volume (mL) is 500-1000:1. (It is recommended that add 1 mL of the extract to 5 million bacteria or cells). Ultrasound breaks up bacteria or cells (power 20% or 200W, ultrasonic of 3s, interval of 10s, repeat 30 times). Static for 30 minutes. Centrifuge at 8000 g, room temperature for 10 minutes. Take the supernatant for test.

2. Tissue:

According to the tissue weight (g) : the extract volume (mL) is 1:5-10. (It is recommended that add 1 mL of extract to 0.1 g tissue). Homogenate in ice bath. Static for 30 minutes, then centrifuge at room temperature, 8000 g for 10 minutes. Take the supernatant for test.

3. Serum (plasma) sample:

According to the serum (plasma) volume : the extract solution is 1:5-10. (It is recommended that add 1 mL of extract into 0.1 mL of serum (plasma), then homogenate in ice bath. Static for 30

minutes. Centrifuge at 8000 g, room temperature for 10 minutes. Take the supernatant for test.

Determination Procedure

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 520 nm and the spectrophotometer set the counter to zero with distilled water.
2. Operation table: (The following reagents is added into 1.5 mL EP tube or 96-well plate.)

Reagent Name (μL)	Test tube (A _T)	Standard tube (A _S)	Blank tube (A _B)
Sample	75	-	-
Standard Solution	-	75	-
H ₂ O	-	-	75
Reagent I	25	25	25
Mix and react for 2min at 25°C			
Reagent II	125	125	125

After fully mix, the absorbance value is measured at 520 nm wavelength, which is recorded as A_T, A_S and A_B. $\Delta A_T = A_T - A_B$, $\Delta A_S = A_S - A_B$, Blank tube and standard tube only need to do 1-2 times.

Calculation of Pyruvate content:

1. Calculate by volume of serum (plasma)

$$\text{Pyruvate content } (\mu\text{mol/mL}) = \Delta A_T \div \Delta A_S \times C_S \times (V_E + V_L) \div V_L = 0.1375 \times \Delta A_T \div \Delta A_S$$

2. Calculate by protein concentration

$$\text{Pyruvate content } (\mu\text{mol /mg prot}) = \Delta A_T \div \Delta A_S \times C_S \times V_S \div (V_S \times C_{pr}) = 0.125 \times \Delta A_T \div \Delta A_S \div C_{pr}$$

3. Calculate by sample weight

$$\text{Pyruvate content } (\mu\text{mol /g weight}) = \Delta A_T \div \Delta A_S \times C_S \times V_S \div W = 0.125 \times \Delta A_T \div \Delta A_S \div W$$

4. Calculate by bacterial or cell density

$$\text{Pyruvate content } (\mu\text{mol /}10^4 \text{ cell}) = \Delta A_T \div \Delta A_S \times C_S \times V_S \div N = 0.125 \times \Delta A_T \div \Delta A_S \div N$$

C_S: Concentration of standard solution, 0.125μmol/mL

V_E: Extract solution volume, 1 mL;

V_L: Serum (plasma) volume, 0.1 mL;

C_{pr}: Sample protein concentration, mg/mL;

W: Sample weight, g;

N: the amount of cell, 10⁴ cell as a unit.

Note:

1. If the measured absorbance value exceeds the linear range absorbance value, the sample size can be increased or the sample can be diluted and then measured.
2. There are protein denaturation components in the extract solution, and if the protein concentration

calculation is used, another sample is required for extraction and determination.

Experimental example:

1. Weigh about 0.1171g of rabbit liver, add 1mL of extract, homogenize in an ice bath, let stand for 30min, centrifuge at 8000g for 10min at room temperature, and take the supernatant for measurement. The supernatant was taken for measurement. After that, the procedure was followed, and 96 well flat-bottom plate was used to measure and calculate $\Delta A_t = A_t - A_b = 0.544 - 0.085 = 0.459$, $\Delta A_s = A_s - A_b = 0.436 - 0.085 = 0.351$, and pyruvic acid content was calculated as follows:

$$PA (\mu\text{mol/g mass}) = 0.125 \times \Delta A_t \div \Delta A_s \div W = 1.396 \mu\text{mol/g mass}.$$
2. Weigh about 0.1094g of acacia, add 1mL of extract, homogenize in an ice bath, let stand for 30min, centrifuge at 8000g for 10min at room temperature, take the supernatant and dilute it 2 times with distilled water and leave it to be measured. After that, according to the determination steps, measured with a 96 well flat-bottom plate to calculate $\Delta A_t = A_t - A_b = 0.552 - 0.085 = 0.467$, $\Delta A_s = A_s - A_b = 0.436 - 0.085 = 0.351$, calculated pyruvic acid content was obtained:

$$PA (\mu\text{mol/g mass}) = 0.125 \times \Delta A_t \div \Delta A_s \div W \times \text{dilution} = 3.040 \mu\text{mol/g mass}$$
3. 50 μ L of rabbit serum was taken and then operated in accordance with the assay procedure. 96 well flat-bottom plate was used to calculate $\Delta A_t = A_t - A_b = 0.125 - 0.085 = 0.040$, $\Delta A_s = A_s - A_b = 0.436 - 0.085 = 0.351$, and pyruvic acid was calculated as follows:

$$PA (\mu\text{mol/mL}) = 0.1375 \times \Delta A_t \div \Delta A_s = 0.016 \mu\text{mol/mL}.$$

Recent Product Citation:

[1] Yao R, Yang Y, Lian S, et al. Effects of acute cold stress on liver O-GlcNAcylation and glycometabolism in mice[J]. International journal of molecular sciences, 2018, 19(9): 2815.

[2] Meixi Peng, Dan Yang, Yixuan Hou, et al. Intracellular citrate accumulation by oxidized ATM-mediated metabolism reprogramming via PFKFB3 and CS enhances hypoxic breast cancer cell invasion and metastasis. Cell Death and Disease. March 2019; (IF5.959)

[3] Xiaofen Fu, Pengsong Li, Lei Zhang, et al. Understanding the stress responses of Kluyveromyces marxianus after an arrest during high-temperature ethanol fermentation based on integration of RNA-Seq and metabolite data. Applied Microbiology and Biotechnology. March 2019; 103(6):2715-2729. (IF3.67)

[4] Luo M, Luo Y, Mao N, et al. Cancer-Associated Fibroblasts Accelerate Malignant Progression of Non-Small Cell Lung Cancer via Connexin 43-Formed Unidirectional Gap Junctional Intercellular Communication. Cellular Physiology and Biochemistry. November 2018

References:

[1] Venkatesh C, Ramalingam K. Lactic acid, pyruvic acid and lactate/pyruvate ratio in the Anoplocephalid tapeworm *Stilesia globipunctata* infecting sheep (*Ovis aries*)[J]. *Veterinary parasitology*, 2007, 144(1-2): 176-179.

Related Products:

BC0740/BC0745	Hexokinase(HK) Activity Assay Kit
BC0540/BC0545	Pyruvate Kinase(PK) Activity Assay Kit
BC0530/BC0535	Phosphofruktokinase(PFK) Activity Assay Kit
BC2250/BC2255	Phosphoglycerate Kinase(PGK) Activity Assay Kit

Technical Specifications:

Detection limit: 0.001 $\mu\text{mol/mL}$

Linear range: 0.0015-0.25 $\mu\text{mol/mL}$