# Pyruvate (PA) Content Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Catalog Number:** BC2200

**Size:** 50T/48S

**Components:**

**Extract**: Liquid 60 mL ×1. Storage at 2-8℃.

**Reagent I**: Liquid 7 mL×1. Storage at 2-8℃.

**Reagent II**: Liquid 30 mL ×1. Storage at 2-8℃.

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

**Preparation of 0.125 μmol/mL standard solution** : 50μL 20μmol/mL standard solution and 450μL distilled water mix to obtain 2μmol/mL standard solution ; then 50 μL 2μmo/mL standard solution and 750μL 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, water-bath, spectrophotometer, 1 mL glass cuvette, transferpettor, mortar/ homogenizer, ice 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 for more than 30 minutes, adjust the wavelength to 520 nm and set the counter to zero with distilled water.
2. Operation table: (The following reagents is added into 1.5 mL EP tube.)

| Reagent Name（μL） | Test tube ($A_T$) | Standard tube ($A_S$) | Blank tube ($A_B$) |
|---|---|---|---|
| Sample | 300 | - | - |
| Standard Solution | - | 300 | - |
| $H_2O$ | - | - | 300 |
| Reagent I | 100 | 100 | 100 |
| Mix and react for 2min at 25℃ | | | |
| Reagent II | 500 | 500 | 500 |
| 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)
   Pyruvate content (μmol/mL)= $\Delta A_T \div \Delta A_S \times C_S \times (V_E+V_L) \div V_L = 1.375 \times \Delta A_T \div \Delta A_S$
2. Calculate by protein concentration
   Pyruvate content (μmol /mg prot) =$\Delta A_T \div \Delta A_S \times C_S \times V_S \div (V_S \times Cpr) = 0.125 \times \Delta A_T \div \Delta A_S \div Cpr$
3. Calculate by sample weight
   Pyruvate content (μmol /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
   Pyruvate content (μmol /$10^4$ 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
   $V_S$: Sample volume, 0.3 mL;
   $V_E$: Extract solution volume, 1 mL;
   $V_L$: Serum (plasma) volume, 0.1 mL;
   Cpr: Sample protein concentration, mg/mL;
   W: Sample weight, g;
   N: the amount of cell, $10^4$ 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.

3. 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 1mL glass cuvette was used to measure and calculate ΔAt=At-Ab=0.570-0.101=0.469, ΔAs=As-Ab=0.541-0.101 =0.440, and pyruvic acid content was calculated as follows:
   PA (μmol/g mass)=0.125 × ΔAt÷ΔAs÷W = 1.138 μmol/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 1mL glass cuvette to calculate ΔAt= At - Ab = 0.886- 0.101 = 0.785, ΔAs = As- Ab = 0.541-0.101 = 0.440, calculated pyruvic acid content was obtained:
   PA (μmol/g mass) = 0.125 × ΔAt ÷ ΔAs÷ W × dilution = 4.077 μmol/g mass
3. 50μL of rabbit serum was taken and then operated in accordance with the assay procedure. 1mL glass cuvette was used to calculate ΔAt=At-Ab=0.146-0.101=0.045, ΔAs=As-Ab=0.541-0.101=0.440, and pyruvic acid was calculated as follows:
   PA (μmol/mL)=1.375 × ΔAt ÷ ΔAs =0.14μmol/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 PFKP 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 | Phosphofructokinase(PFK) Activity Assay Kit |
| BC2250/BC2255 | Phosphoglycerate Kinase(PGK) Activity Assay Kit |

**Technical Specifications:**

Detection limit: 0.001 μmol/mL

Linear range: 0.0015-0.25 μmol/mL



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