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# Pyruvate(PA) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: AK0397

Size:100T/96S

## **Components:**

Extract: Liquid 100 mL ×1. Storage at 4°C. Solution I: Liquid 5 mL×1. Storage at 4°C. Solution II: Liquid 25 mL ×1. Storage at 4°C.

Sodium pyruvate standard solution: Liquid 1 mL ×1, 1 mg/mL, storage at 4°C.

### **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<sup>4</sup>): 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). Stand for 30 minutes. Centrifuge at 8000 g, RT 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, stand 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 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, stand for 30 minutes. Centrifuge at 8000 g, RT for 10 minutes. Take the supernatant for test.
- 4. Preparation of standard: dilute standard with distilled water to 100, 50, 25, 12.5, 6.25, 3. 125, 1.5625, 0  $\mu$ g/mL.

### **II. Determination Procedure**





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1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 520 nm and set the counter to zero with distilled water.

2. Add 75  $\mu$ L standard solution or samples and 25  $\mu$ L Solution I in the micro glass cuvette or 96 well flat-bottom plate, mix thoroughly. Stand for 2 minutes, then add 125  $\mu$ L Solution II. Mix thoroughly. Determination of absorbance A at 520 nm.

# III. Calculation of Pyruvate content:

- 1. Establish the standard curve according to the standard concentration and the measured value; y is the sodium pyruvate content ( $\mu g/mL$ ), x is the absorption value.
- 2. Calculate by volume of serum (plasma)

Pyruvate content ( $\mu g/mL$ )=( $y \times V1$ )÷[( $V3 \times V1 \div (V2 + V3)$ ] = $y \times 11$ 

3. Calculate by protein concentration

Pyruvate content ( $\mu g/mg \text{ prot}$ )= $(y \times V1) \div (V1 \times Cpr) = y \div Cpr$ 

4. Calculate by sample weight

Pyruvate content ( $\mu g/g$  fresh weight)=( $y \times V1$ )÷( $W \times V1$ ÷V2)=y÷W

5. Calculate by bacterial or cell density

Pyruvate content ( $\mu g/10^4 \text{ cell}$ ) = ( $y \times V1$ )÷( $500 \times V1 \div V2$ )=  $y \div 500$ 

V1: Sample volume, 0.075 mL;

V2: Extract solution volume, 1 mL;

V3: Serum (plasma) volume, 0.1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

500: Total number of bacteria or cells, 5 million.

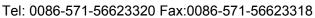
#### Note:

If the absorbance value exceeds the linear range, the sample size can be increased or diluted before the determination.

### **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

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#### **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:**

AK0516/AK0515 Hexokinase(HK) Activity Assay Kit

AK0540/AK0539 Pyruvate Kinase(PK) Activity Assay Kit

AK0542/AK0541 Phosphofructokinase(PFK) Activity Assay Kit

AK0394/AK0393 Phosphoglycerate Kinase(PGK) Activity Assay Kit

# **Technical Specifications:**

Detection limit:  $0.1510 \ \mu g/mL$ 

Linear range: 0.78125-50 μg/mL