

# PRODUCT INFORMATION & MANUAL

# Pyruvic Acid Assay Kit (Colorimetric) *NBP3-25792*

For research use only. Not for diagnostic or therapeutic procedures.

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# Pyruvic Acid Assay Kit (Colorimetric)

Catalog No: NBP3-25792

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.006 µmol/mL

Detection range: 0.006-2.0 µmol/mL

Average intra-assay CV (%): 1.3

Average inter-assay CV (%): 1.5

Average recovery rate (%): 100

▲ This kit is for research use only.

Instructions should be followed strictly, changes of operation may result in unreliable results.

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

# **General information**

#### Intended use

This kit can be used to measure pyruvate content of serum, plasma, tissue samples.

#### Background

Pyruvic acid is the simplest of the α-keto acids, with a carboxylic acid and a ketone functional group. Pyruvic acid can be made from glucose through glycolysis, converted back to carbohydrates (such as glucose) via gluconeogenesis, or to fatty acids through a reaction with acetyl-CoA. It can also be used to construct the amino acid alanine and can be converted into ethanol or lactic acid via fermentation. Pyruvic acid supplies energy to cells through the citric acid cycle (also known as the Krebs cycle) when oxygen is present (aerobic respiration), and alternatively ferments to produce lactate when oxygen is lacking (lactic acid fermentation).

#### ▲ Detection principle

Pyruvic acid can react with chromogenic agent and the product is reddish brown in alkaline solution. The depth of color is directly proportional to the pyruvate content. The pyruvate content can be calculated by measuring the OD value at 505 nm.

## ▲ Kit components & storage

ltem	Component	Specification	Storage
Reagent 1	Clarificant	12 mL × 1 vial	2-8°C , 12 months
Reagent 2	Chromogenic Agent	60 mL ×1 vial	2-8℃,12 months, shading light
Reagent 3	Alkali Reagent	50 mL × 6 vials	2-8°C , 12 months
Reagent 4	2 µmol/mL Sodium Pyruvate Standard	1.6 mL × 2 vials	2-8℃ , 12 months

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

## ▲ Materials prepared by users

## **Instruments**

Spectrophotometer (505 nm), Micropipettor, Vortex mixer, Incubator

#### **A** Reagents

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

## ▲ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

#### ▲ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

# **Pre-assay preparation**

#### ▲ Reagent preparation

Preparation of 0.2 µmol/mL sodium pyruvate standard solution:

Dillute reagent 4 with double distilled water for 10 times. Prepared the fresh solution before use.

#### ▲ Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

# Black strength (\*

## ▲ Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.006-2.0  $\mu$ mol/mL).

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

Sample type	Dilution factor	
Human serum	1	
Mouse serum	1	
Mouse plasma	1	
10% Mouse liver tissue homogenization	1	
10% Rat kidney tissue homogenization	1	
10% Rat heart tissue homogenization	1	

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

# **Assay protocol**

#### ▲ For serum or plasma sample

#### **Detailed operation steps**

 Blank tube: Add 0.1 mL of double distilled water to 5 mL EP tube. Standard tube: Add 0.1 mL of 0.2 µmol/mL sodium pyruvate standard solution to 5 mL EP tube.

Sample tube: Add 0.1 mL of sample to 5 mL EP tube.

- 2. Add 0.5 mL of reagent 2 to each tube and mix fully with a vortex mixer.
- 3. Incubate the tubes at 37°C for 10 min.
- 4. Add 2.5 mL of reagent 3 into each tube. Mix fully with vortex mixer for 5 sec, then incubate the tubes at room temperature for 5 min.
- 5. Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 505 nm with 1 cm optical path cuvette.

#### **Summary operation table**

	Blank tube	Standard tube	Sample tube			
Double distilled water (mL)	0.1					
0.2 µmol/mL Sodium pyruvate standard solution (mL)		0.1				
Sample (mL)			0.1			
Reagent 2 (mL)	0.5	0.5	0.5			
Mix fully, incubate at 37°C for 10 min						
Reagent 3 (mL)	2.5	2.5	2.5			
Mix fully, then incubate the tubes at room temperature for 5 min. Set the spectrophotometer to zero and measure the OD value of each tube.						

#### ▲ For tissue sample

#### **Detailed operation steps**

- Blank tube: Add 0.1 mL of double distilled water to 5 mL EP tube. Standard tube: Add 0.1 mL of 0.2 µmol/mL sodium pyruvate standard solution to 5 mL EP tube. Sample tube: Add 0.1 mL of sample to 5 mL EP tube.
- 2. Add 0.1 mL of reagent 1 to each tube and mix fully with a vortex mixer.
- 3. Add 0.5 mL of reagent 2 to each tube and mix fully with a vortex mixer.
- 4. Incubate the tubes at  $37^{\circ}$ C for 10 min.
- 5. Add 2.5 mL of reagent 3 into each tube. Mix fully with vortex mixer for 5 sec, then incubate the tubes at room temperature for 5 min.
- 6. Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 505 nm with 1 cm optical path cuvette.

#### Summary operation table

	Blank tube	Standard tube	Sample tube			
Double distilled water (mL)	0.1					
0.2 µmol/mL Sodium pyruvate standard solution (mL)		0.1				
Sample (mL)			0.1			
Reagent 1 (mL)	0.1	0.1	0.1			
Reagent 2 (mL)	0.5	0.5	0.5			
Mix fully, incubate at 37 °C for 10 min						
Reagent 3 (mL)	2.5	2.5	2.5			
Mix fully, then incubate the tubes at room temperature for 5 min. Set the spectrophotometer to zero and measure the OD value of each tube.						

#### ▲ Calculation

1. Serum (plasma) sample:

Pyruvate content(
$$\mu$$
mol/mL) =  $\frac{\Delta A_1}{\Delta A_2}$  × c × f

2. Tissue sample:

Pyruvate content(µmol/mgprot) = 
$$\frac{\Delta A_1}{\Delta A_2} \times c \times f \div C_{pr}$$

#### Note:

- $\Delta A_1: OD_{Sample} OD_{Blank}$
- $\Delta A_2: OD_{Standard} OD_{Blank}$
- c: Concentration of standard, 0.2 µmol/mL.
- f: Dilution factor of sample before test.
- C<sub>pr</sub>: Concentration of protein in sample (mgprot/mL)

# Appendix I Data

#### **Example analysis**

Take 0.1 mL of human serum, carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.174, the average OD value of the blank is 0.014, the average OD value of the standard is 0.089, and the calculation result is:

Pyruvate content( $\mu$ mol/mL) =  $\frac{0.174-0.014}{0.089-0.014}$  × 0.2 × 1= 0.43  $\mu$ mol/mL

# **Appendix II Sample preparation**

The following sample pretreatment methods are for reference only.

#### ▲ Serum

Collect fresh blood and stand at  $25^{\circ}$ C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at  $4^{\circ}$ C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at  $-80^{\circ}$ C for a month.

#### ▲ Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4°C . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

#### Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

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#### Note:

- 1. Homogenized medium: PBS (0.01 M, pH 7.4) or 0.9% NaCl.
- 2. Homogenized method:
- (1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm<sup>3</sup>), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min. Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.
- (2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

# **Appendix III References**

1. Pithukpakorn M. Disorders of pyruvate metabolism and the tricarboxylic acid cycle[J]. Molecular Genetics Metabolism, 2005, 85(4): 243-246.