(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

# Pyruvic Acid Assay Kit

Catalog No.: BC00001

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:


✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎Tel: 0086-27-87002838

Website: www.enkilife.com


**Shelf life:** Please refer to the label on the outer package.

**Techsupport:** In order to provide you with better service, please inform us the lot number on the label of the outer package.

## Basic Information

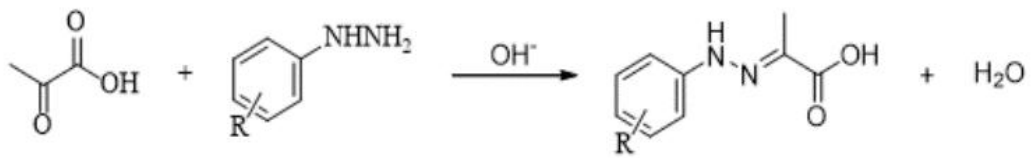
| | |
|---|---|
| **Product Name** | Pyruvic Acid Assay Kit |
| **Detection Method** | Colorimetric |
| **Sample Type** | Tissue, serum, plasma |
| **Assay Type** | Quantitative |
| **Detection Instrument** | Microplate reader (480-520 nm, optimal detection wavelength 505 nm) |
| **Range** | 0.125-2mM |
| **Sensitivity** | 0.0254mM |

## Product Introduction

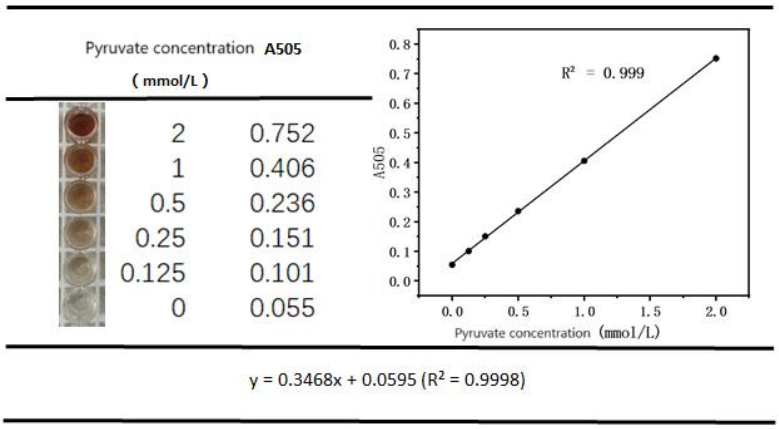
Pyruvate is an important intermediate in the sugar metabolism of all biological cells and the mutual transformation of various substances in the body. Because the molecule contains activated ketone and carboxyl groups, it is widely used as a basic chemical raw material in various fields such as chemistry, pharmaceuticals, food, agriculture and environmental protection.

## Principle

Pyruvic acid reacts with a color developer, and the reaction product is reddish brown in an alkaline solution. The depth of the color is proportional to the pyruvic acid content, and the pyruvic acid content can be calculated by colorimetry. When this kit detects tissue samples, the total protein concentration needs to be determined. It is recommended to use the protein concentration determination kit (BCA method) (BC00006) produced by EnkiLife.




The figure below shows the standard curve of pyruvate determination by this kit:




## Components

| No. | Components | Size (100T) | Storage |
|---|---|---|---|
| Reagent 1 | Clarifying Agent | 0.6 mL | -20℃, store at 2-8℃ after opening. |
| Reagent 2 | Color Developer | 3 mL | -20℃, protect from light, store at 2-8℃ after opening. |
| Reagent 3 | Alkaline Reagent | 10 mL | -20℃, store at 2-8℃ after opening. |
| Reagent 4 | 2 μmol/mL Sodium Pyruvate Standard | 0.8mL/vial, 2 vials | -20℃, store at 2-8℃ after opening. |
| Consumable 1 | Microplate | 1 plate | RT |
| Consumable 2 | Plate Sealer | 2 pieces | RT |

## Storage

The unopened kit can be stored at -20℃ for 12 months, and after opening, it can be stored at 2-8℃ for 6 months.

## Preparation

- **Sample handling**

1. Serum and plasma samples: can be measured directly.

Tissue samples: Take 0.020-1.0 g of fresh tissue blocks, add physiological saline (0.9%

NaCl) or PBS (0.01 M, pH 7.4) at a weight (g): volume (mL) ratio of 1:9, homogenize, centrifuge at 4°C, 10,000×g for 10 min, take the supernatant and place it on ice for testing. Keep part of the supernatant for protein concentration determination.

2. Dilution of samples: Before formal testing, 2-3 samples with large expected differences need to be selected and diluted into different concentrations for preliminary experiments.

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

- **Preparation of the kit**

1. Before testing, equilibrate the reagents in the kit to room temperature.
2. Dilution of different concentrations of standards: dilute reagent 4 with double distilled water in half to different concentrations such as 2, 1, 0.5, 0.25, 0.125, 0 (blank well) mmol/L

## Operation process

1. Standard wells: Take 15 μL of different concentrations of standards and add them to the plate wells; Measurement wells: Take 15 μL of samples to be tested and add them to the plate wells;
2. Add 50 μL of Reagent 2 to the standard and measurement wells from step (1).
3. Vibrate the plate on the microplate reader for 10 s and incubate at 37°C for 10 min.
4. Add 150 μL of Reagent 3 to the standard and measurement wells in step (3).
5. Vibrate the plate on the microplate reader for 10 s, let stand at room temperature for 5 min, and measure the OD value at 505 nm with a microplate reader.

| | Standard Well | Measurement Well |
|---|---|---|
| Different concentrations of standard solutions (μL) | 15 | -- |
| Sample to be tested (μL) | -- | 15 |
| Reagent 2 (μL) | 50 | 50 |
| Vibrate the plate on the microplate reader for 10 s and incubate at 37°C for 10 min. | | |
| Reagent 3 (μL) | 150 | 150 |
| Vibrate the plate on the microplate reader for 10 s, let stand at room temperature for 5 min, and measure the OD value at 505 nm with a microplate reader. | | |

## Calculation

Standard fitting curve: y = ax + b

Normal serum (plasma) sample, pyruvate calculation formula: pyruvate content (mmol/L) = (ΔA505 - b) ÷ a × f

In tissues, pyruvate is calculated by the formula: Pyruvate content (μmol/mgprot) = (ΔA505 - b) ÷ a × f ÷ Cpr

**Annotation:**

y: OD value of standard well - OD value of blank well (OD value when the concentration of standard is 0)

x: concentration corresponding to the absorbance

a: slope of the curve

b: intercept of the curve

△A 505 : Sample OD value - blank OD value (OD value when the standard concentration is 0)

f: dilution factor of the sample before adding it to the detection system

Cpr: protein concentration of the sample to be tested (mg/mL)

## Notes

1. The optimal detection wavelength for the microplate reader is 505 nm, and detection within the range of 480 nm to 520 nm is acceptable.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.