



Pyruvate Microplate Assay Kit

User Manual

Catalog # CAK1016

(Version 2.2D)

Detection and Quantification of Pyruvate (PA) Content in Urine,
Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and
Other biological fluids Samples.

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

I. INTRODUCTION.....2

II. KIT COMPONENTS.....3

III. MATERIALS REQUIRED BUT NOT PROVIDED.....3

IV. SAMPLE PREPARATION.....4

V. ASSAY PROCEDURE.....5

VI. CALCULATION.....6

VII. TYPICAL DATA.....7

VIII. TECHNICAL SUPPORT.....7

IX. NOTES.....7

I. INTRODUCTION

Pyruvate (Pyruvic acid) is an alpha-keto acid that plays a central role in various biochemical pathways. As a product of fermentation, pyruvic acid can be found in large quantities, especially in dark beers. This acid is also found in wine, fruits (e.g. apple) and cheese. The salt form of this acid is the active ingredient in various dietary supplements implicated in the control of body weight. The concentration of pyruvic acid in blood and urine is a useful clinical marker for certain medical conditions. Pyruvate Microplate Assay Kit provides a simple and sensitive method for monitoring pyruvate concentration in various samples. Pyruvate is oxidized by pyruvate oxidase via enzyme reactions to generate color at OD 550 nm. Since the color is proportional to pyruvate content, the pyruvate concentration can be accurately measured.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent	Powder x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Enzyme: add 1 ml Assay Buffer to dissolve before use.

Dye Reagent: add 10 ml Assay Buffer to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.1 ml into 0.9 ml distilled water, the concentration will be 5 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum, plasma samples

Add 0.1 ml serum, plasma into 1 ml Assay buffer on ice, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	90 μ l	90 μ l	90 μ l
Sample	10 μ l	--	--
Standard	--	10 μ l	--
Distilled water	--	--	10 μ l
Enzyme	10 μ l	10 μ l	10 μ l
Dye Reagent	90 μ l	90 μ l	90 μ l
Mix, put it in the oven, 37 °C for 30 minutes, measured at 550 nm and record the absorbance.			

Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) The concentrations can vary over a wide range depending on the different samples.
For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- 3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the weight of sample

$$\begin{aligned} \text{PA (mmol/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \\ &\quad \times W / V_{\text{Assay}}) \\ &= 5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

2. According to the quantity of cell or bacteria

$$\begin{aligned} \text{PA (mmol / 10}^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (N \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) \\ &= 5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

3. According to the volume of sample

$$\begin{aligned} \text{PA (mmol/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} \\ &= 5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

C_{Standard} : the standard concentration, 5 mmol/L;

C_{Protein} : the protein concentration, mg/ml;

V_{Standard} : the volume of standard, 0.01 ml;

V_{Sample} : the volume of sample, 0.01 ml;

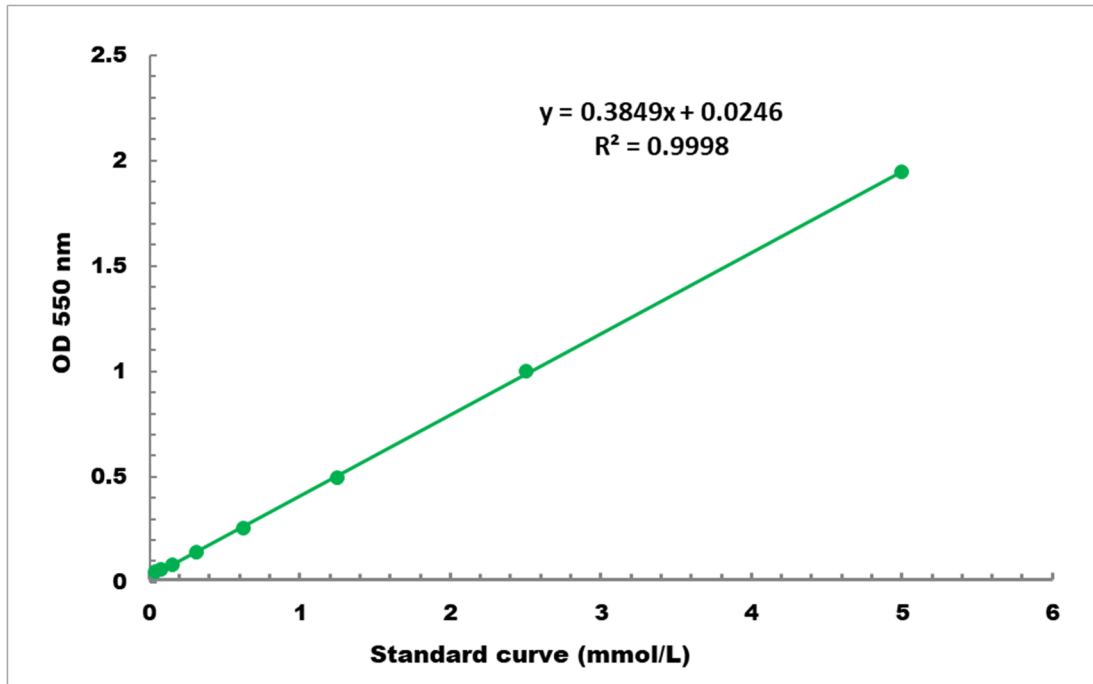
V_{Assay} : the volume of Assay buffer, 1 ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.05 mmol/L - 5 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES