Pyruvate Microplate Assay Kit

User Manual

Catalog # FTA0016

(Version 1.2C)

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

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. Pyruvic acid can react with 2,4-dinitrophenylhydrazine. The reaction products can be measured at a colorimetric readout at 520 nm.

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
Dye Reagent	3 ml x 1	4 °C
Standard (50 µg/ml)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 520 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%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 8000g 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 8000g 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 8000g 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
Sample	75 µl	--	--
Standard	--	75 µl	--
Distilled water	--	--	75 µl
Dye Reagent	25 µl	25 µl	25 µl
Mix, stand at room temperature for 2 minutes.			
Reaction Buffer	100 µl	100 µl	100 µl
Mix, record the absorbance measured at 520nm.			

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.

VI. CALCULATION

1. According to the protein concentration of sample

$$PA\ (\mu g/mg) = (C_{Standard} \times V_{Sample}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 50 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$PA\ (\mu g/g) = (C_{Standard} \times V_{Sample}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$$

$$= 50 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

$$PA\ (\mu g/10^4) = (C_{Standard} \times V_{Sample}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$$

$$= 50 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum, plasma

$$PA\ (\mu g/ml) = (C_{Standard} \times V_{Sample}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V \times V_{Sample} / V_{Assay})$$

$$= 50 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V$$

$C_{Standard}$: the protein concentration, 50 μg/ml;

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

W: the weight of sample, g;

V: the volume of serum or plasma, ml;

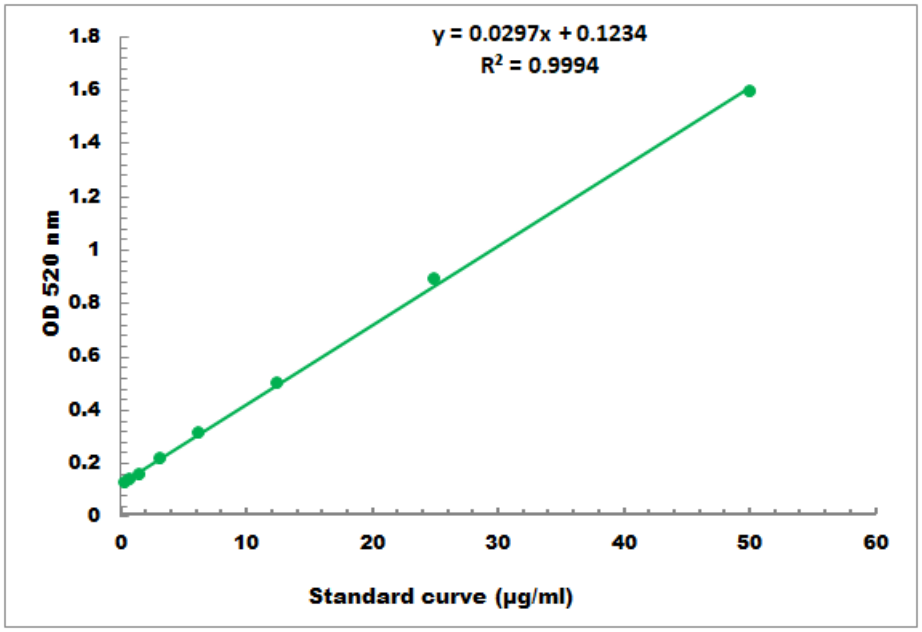
N: the quantity of cell or bacteria, $N \times 10^4$;

V_{Sample}: the volume of sample, 0.075 ml;

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

VII. TYPICAL DATA

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



Detection Range: 0.5 μg/ml - 50 μg/ml

VIII. TECHNICAL SUPPORT

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

IX. NOTES