



Pyruvate Fluorometric Microplate Assay Kit User Manual

Catalog # CAK8004

(Version 1.2A)

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

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

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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, generating a compound that reacts with the probe, which can be detected fluorometrically (Ex/Em 535/587).

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Black Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C, keep in dark
Probe	Powder x 1	-20 °C, keep in dark
Probe Diluent	1 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Fluorescence microplate reader to read fluorescence at Ex/Em = 535/587
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml Assay Buffer to generate 50 mmol/L of standard stock solution, store at 4 °C for 2 weeks or -20 °C for 3-4 months after reconstitution. Then dilute to 2500 $\mu\text{mol/L}$ standard top solution by adding 50 μl stock solution into 950 μl distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 2500/1250/625/312/156/78/39 $\mu\text{mol/L}$.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use. Aliquot & store at -20 °C. Use within 1 month.

Probe: Warm Probe Diluent to RT prior to use to melt frozen Probe Diluent; then add 1 ml Probe Diluent to dissolve. Store at -20 °C, protect from light and moisture. Use within 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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.

VI. ASSAY PROCEDURE

Warm the solution to room temperature before use.

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	170 μ l	170 μ l	170 μ l
Sample	10 μ l	--	--
Standard	--	10 μ l	--
Distilled water	--	--	10 μ l
Probe	10 μ l	10 μ l	10 μ l
Enzyme	10 μ l	10 μ l	10 μ l
Mix, put it in the oven, 37 °C for 15 minutes, protected from light, record fluorescence measured at Ex/Em = 535/587 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(\text{RFU}_{\text{Sample}} - \text{RFU}_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n (\mu\text{mol/L})$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard RFU and concentration

2.1 According to the quantity of cells or bacteria

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{RFU}_{\text{Sample}} - \text{RFU}_{\text{Blank}})}{(\text{RFU}_{\text{Standard}} - \text{RFU}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol}/10^4)$$

2.2 According to the weight of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{RFU}_{\text{Sample}} - \text{RFU}_{\text{Blank}})}{(\text{RFU}_{\text{Standard}} - \text{RFU}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol/g})$$

2.3 According to the volume of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{RFU}_{\text{Sample}} - \text{RFU}_{\text{Blank}})}{(\text{RFU}_{\text{Standard}} - \text{RFU}_{\text{Blank}}) \times V_{\text{Sample}}} (\mu\text{mol/ml})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard} : the standard concentration, $\mu\text{mol/L}$

V_{Standard} : the volume of standard in assay procedure, μl

V_{Sample} : the volume of sample in assay procedure, μl

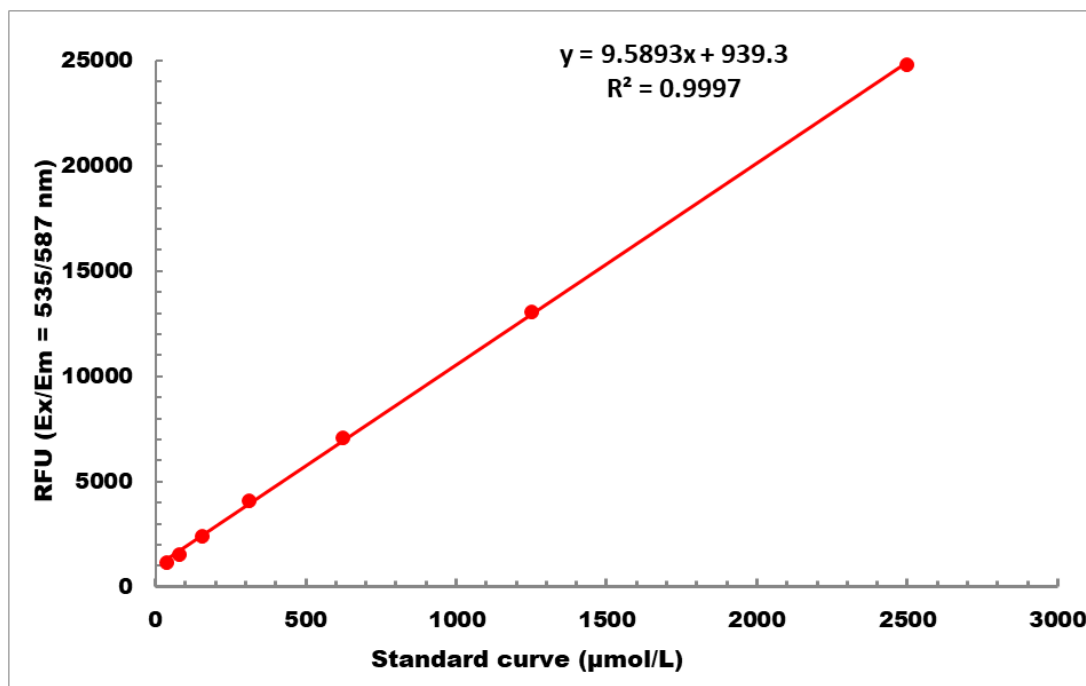
V_{Assay} : the volume of Assay Buffer, μl

W: the weight of sample, g

N: the quantity of cell or bacteria, 10^4

VIII. TYPICAL DATA

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



Detection Range: 25 μmol/L -2500μmol/L