Pyruvate Acid (PA) Colorimetric Assay Kit

Cat#: orb1173222 (User Manual)

Micro Pyruvate Acid (PA) Assay Kit
Cat #: orb1173222 **Lot #:** Refer to product label
Size: 48 T/96 T
Detection range: 1.094-70 µg/mL **Sensitivity:** 0.5 µg/mL
Applicable samples: Animal and Plant Tissues, Cells or Bacteria, Plasma, Serum
Storage: Stored at 4°C for 6 months, protected from light

Assay Principle

Pyruvate Acid (PA) connects the three major metabolisms of glucose, fatty acids and amino acids through acetyl CoA, and plays an important pivotal role. Biorbyt Micro Pyruvate Acid (PA) Assay Kit is specially developed for the detection of Pyruvate Acid in a variety of biological samples such as Plasma, Serum, Animal and Plant Tissues, Cells and Bacteria. The operation is simple and convenient, and the detection is more sensitive and accurate. The Pyruvate Acid reacts with 2,4-dinitrophenylhydrazine to produce a red compound with a maximum absorption peak at 520 nm. Within a certain concentration range, the Pyruvate Acid content has a linear relationship with the absorbance at 520 nm. According to the standard curve, the Pyruvate Acid content in the sample can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60 mL×2	4°C
Chromogen A	1.5 mL	2.5 mL	4°C, protected from light
Chromogen B	6.25 mL	12.5 mL	4°C
Standard (1 mg/mL)	1 mL	1 mL	4°C

Materials Required but Not Supplied

·Microplate reader or visible spectrophotometer capable of measuring absorbance at 520 nm
·Incubator, ice maker, refrigerated centrifuge
·96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
·Deionized water
·Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.
Chromogen A: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.
Chromogen B: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.
Standard preparation:
Standard curve setting: dilute 1 mg/mL Standard with Extraction Buffer to 70, 35, 17.5, 8.75, 4.375, 2.188, 1.094 µg/mL standard solution as shown in the table below.

Num.	Volume of Standard	Volume of Extraction Buffer (µL)	The Concentration of Standard (µg/mL)
Std.1	35 µL 1 mg/mL	465	70
Std.2	200 µL of Std.1 (70 µg/mL)	200	35
Std.3	200 µL of Std.2 (35 µg/mL)	200	17.5
Std.4	200 µL of Std.3 (17.5 µg/mL)	200	8.75
Std.5	200 µL of Std.4 (8.75 µg/mL)	200	4.375
Std.6	200 µL of Std.5 (4.375 µg/mL)	200	2.188
Std.7	200 µL of Std.6 (2.188 µg/mL)	200	1.094

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80°C for one month.

1. Animal tissue samples: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize, letting stand for 30 min. Centrifuge at 4,000 g for 10 min at room temperature. Use supernatant for assay, and place it on ice to be tested.
2. Plant tissue samples: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize, then ultrasonically disrupt the plant tissue 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times), letting stand for 30 min. Centrifuge at 4,000 g for 10 min at room temperature. Use supernatant for assay, and place it on ice to be tested.
3. Cells or bacteria: Collect 5×106 bacteria or cells into the centrifuge tube, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the bacteria or cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times), letting stand for 30 min. Centrifuge at 4,000 g for 10 min at room temperature. Use supernatant for assay, and place it on ice to be tested.
4. Plasma, serum: Take 100 µL plasma (serum), add 1 mL Extraction Buffer, mix well, letting stand for 30 min. Centrifuge at 4,000 g for 10 min at room temperature. Use supernatant for assay, and place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 520 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Add the following reagents respectively into the 96-well plate or microglass cuvette:

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	75
Different Concentration of Std.	0	75	0
Extraction Buffer	75	0	0
Chromogen A	25	25	25
Mix well, letting stand for 2 min at room temperature			
Chromogen B	125	125	125

3. Mix well, then reading the values at 520 nm. Finally, calculate ΔATest=ATest-ABlank, ΔAStandard=AStandard-ABlank. (Only one blank well needs to be detected)

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve
With the concentration of the Standard Solution as the y-axis and the ΔAStandard as the x-axis, draw the standard curve. Substitute the ΔATest into the equation to obtain the y value (μg/mL).

2. Calculate the content of Pyruvate Acid in sample

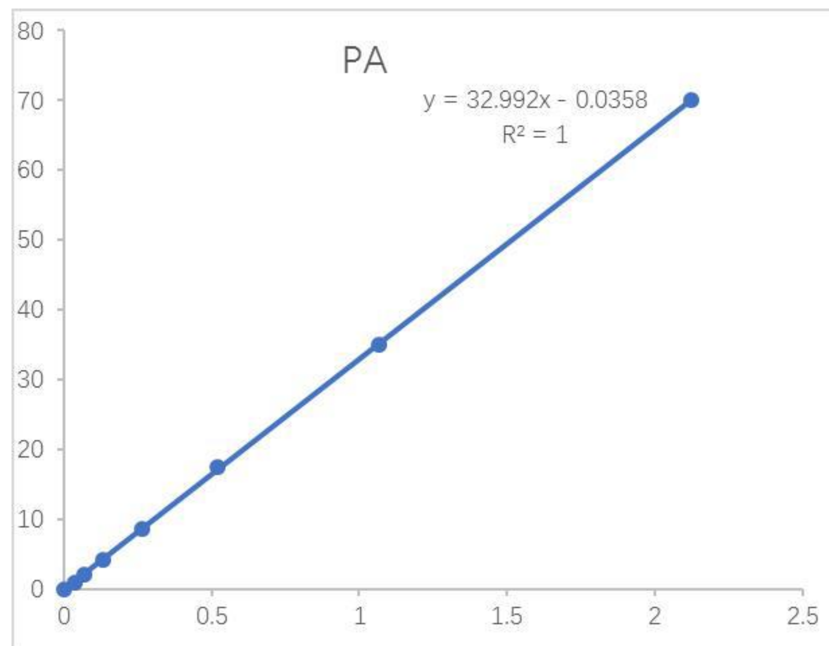
(1) By sample fresh weight
Pyruvate Acid (μg/g)=(y×VSample)÷(W×VSample÷VExtraction Buffer)×n**=y÷W×n**

(2) By liquid volume of plasma (serum)
Pyruvate Acid (μg/mL)=(y×VSample)÷(VLiquid×VSample÷VExtraction Buffer)×n**=10×y×n**

(3) By number of cells or bacteria
Pyruvate Acid (μg/104)=(y×VSample)÷(500×VSample÷VExtraction Buffer)×n**=y÷500×n**
Where: VSample: the volume of add sample volume (0.075 mL); VExtraction Buffer: the volume of add Extraction Buffer (1 mL); W: the weight of sample (g); VLiquid: the liquid sample volume of be taken (0.1 mL); 500: the number of cells or bacteria, 5×106; n: the sample dilution factor.

Typical Data

Typical standard curve:



ΔOD 520 nm

Figure 1. Standard curve of Pyruvate Acid in 96-well plate assay-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

Examples:

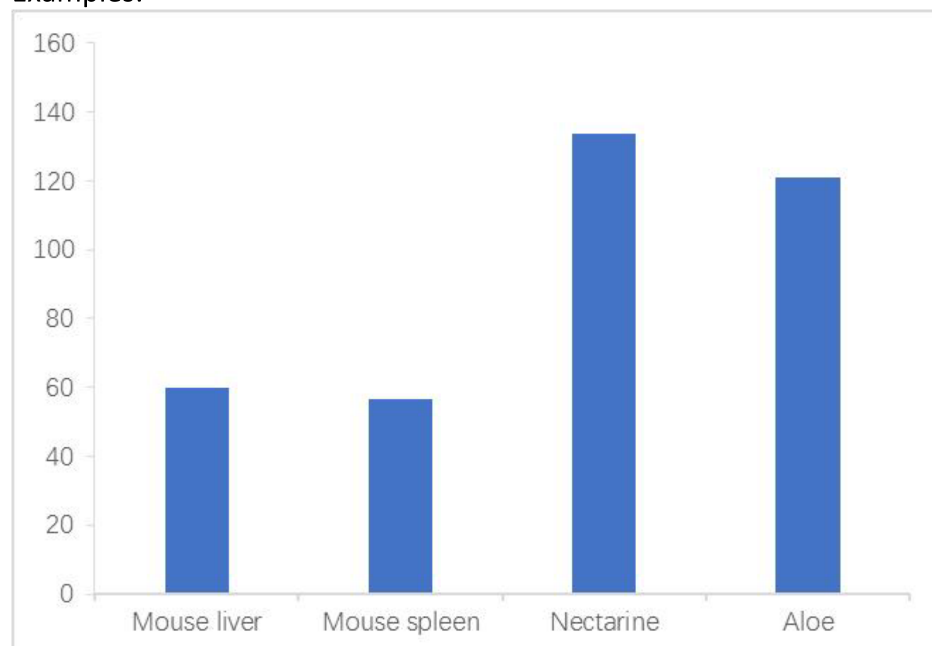


Figure 2. Pyruvate Acid concentration in mouse liver, mouse spleen, nectarine and aloe respectively. Assays were performed following kit protocol.