

## Pyruvate (PA) Content Assay kit (enzymatic method)

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer/microplate reader

**Cat No:** BC5265

**Size:** 100T/48S

### Components:

**Extract Solution:** Liquid 60 mL×1, Storage at 2-8°C;

**Reagent I:** Liquid 25 mL×1, Storage at 2-8°C;

**Reagent II:** Powder×1, Storage at -20°C. Before use, add 1.25mL distilled water to dissolve the powder thoroughly. Unused reagents can be stored in aliquots at -20°C for 4 weeks, avoiding repeated freezing and thawing;

**Reagent III:** Liquid 13μL×1, Storage at 2-8°C; Mix Reagent III: distilled water = 1μL: 100μL (101 μL, about 6T) according to the dosage before use, and prepare for use now.

**Reagent IV:** Liquid 4 mL×1, Storage at 2-8°C;

**Standard solution:** Liquid 1 mL×1, Storage at 2-8°C; 20μmol/mL Standard solution of sodium pyruvate.

### Product Description:

Pyruvate plays an important role in the metabolism of glucose, fatty acids and amino acids through acetyl CoA.

At pH=7.5, pyruvate reacts with NADH catalyzed by LDH to produce NAD<sup>+</sup> and lactic acid. Under 1-mPMS, WST-1 reacts with NADH to produce water-soluble Formazan. Pyruvate content can be calculated by detecting the absorption value at 450nm.

### Required but Not Provided:

Ultraviolet spectrophotometer, desk centrifuge, water-bath/ constant temperature incubator, adjustable pipette, transferpeltor, mortar/homogenizer, 1 mL glass cuvette, ice and distilled water.

### Protocol

#### I. Extraction of Pyruvate:

##### 1. Bacteria or cells:

Collect bacteria or cells into the centrifuge tube, and discard the supernatant after centrifugation. According to the bacteria or cells (10<sup>6</sup>) : the extract volume (mL) is 5-10:1. (It is recommended that add 1 mL of the extract to 5×10<sup>6</sup> bacteria or cells). Ultrasound breaks up bacteria or cells (power 200W, ultrasonic of 3s, interval of 10s, repeat 30 times). Static for 30 minutes. Centrifuge at 8000 g, room temperature for 10 minutes. Take the supernatant for test.

##### 2. Tissue:

According to the tissue weight (g): the extract volume (mL) is 1:5-10. (It is recommended that

add 1 mL of extract to 0.1 g tissue). Homogenate in ice bath. Static for 30 minutes, then centrifuge at room temperature, 8000 g for 10 minutes. Take the supernatant for test.

### 3. Serum (plasma) sample:

According to the serum (plasma) volume: the extract solution is 1:5-10. (It is recommended that add 1mL of extract into 0.1 mL of serum (plasma), then homogenate in ice bath. Static for 30 minutes. Centrifuge at 8000 g, room temperature for 10 minutes. Take the supernatant for test.

## II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 450 nm, set the counter to zero with distilled water.
2. Preheat Reagent I in 37°C for 20 minutes.
3. Standard tube measurement: 20 $\mu$ mol/mL sodium pyruvate standard solution was diluted with distilled water to obtain 1、0.625、0.5、0.3125、0.25、0.15625 $\mu$ mol/mL standard solution for reserve.
4. Standard dilution table

Number	Predilution concentration ( $\mu$ mol/mL)	Standard liquid volume ( $\mu$ L)	distilled water volume ( $\mu$ L)	Diluted concentration ( $\mu$ mol/mL)
1	20	50	950	1
2	1	625	375	0.625
3	0.625	800	200	0.5
4	0.5	625	375	0.3125
5	0.3125	800	200	0.25
6	0.25	625	375	0.15625

Remarks: 20 $\mu$ L of standard solution was required for each standard tube.

### 5. Operation table: (The following reagents were added to a 1.5mLEP tube)

Reagent Name ( $\mu$ L)	Test tube (A <sub>T</sub> )	Control tube (A <sub>C</sub> )	Standard tube (A <sub>S</sub> )	Blank tube I (A <sub>B1</sub> )	Blank tube II (A <sub>B2</sub> )
Supernatant	20	20	-	-	-
Standard solution	-	-	20	-	-
distilled water	-	-	-	20	<b>40</b>
Reagent I	155	180	155	155	155
Reagent II	10	-	10	10	10
Reagent III	15	-	15	15	15
After mixing, react at 37°C for 30min					
Reagent IV	20	20	20	20	-

After mixing, reaction at 37°C for 30minutes (avoiding light). Absorb 200  $\mu$ L into micro glass cuvette or 96-well plate and measure the absorbance at 450 nm. calculate  $\Delta A_T = (A_{B1} - A_{B2}) - (A_T - A_C)$ ,  $\Delta A_S = A_B - A_S$ . (Standard curve and blank tube only need to be measured 1-2 times).

### III. Calculation of pyruvate content in soil:

#### 1. Standard curve drawing:

According to the concentration of the standard tube (x,  $\mu$ mol/mL) and the absorbance  $\Delta A_S$  (y,  $\Delta A_S$ ), the standard curve was established. According to the standard curve, the determination of  $\Delta A$  is substituted into the equation to obtain x ( $\mu$ mol/mL).

#### 2. Calculate by volume of serum (plasma)

$$\text{Pyruvate content } (\mu\text{mol/mL}) = x \times V_{sv} \div V_{sv} = x$$

#### 3. Calculate by protein concentration

$$\text{Pyruvate content } (\mu\text{mol /mg prot}) = x \times V_{sv} \div (Cpr \times V_{sv}) = x \div Cpr$$

#### 4. Calculate by sample weight

$$\text{Pyruvate content } (\mu\text{mol /g weight}) = x \times V_{ev} \div W = x \div W$$

#### 5. Calculate by bacterial or cell density

$$\text{Pyruvate content } (\mu\text{mol /}10^6 \text{ cell}) = x \times V_{ev} \div N = x \div N$$

$V_{sv}$ : Sample volume, 0.02mL

$V_{ev}$ : Extract solution volume, 1 mL

W: Sample weight, g

N : Total number of bacteria or cells,  $10^6$  cells as a unit

#### Note:

- If the absorbance value exceeds the linear range, the sample size can be increased or diluted with distilled water before the determination.

#### Experimental example:

- Weigh about 0.1g rat lung, add 1mL extract solution, grind in ice bath, 8000g, centrifuge at 4 °C for 10min, take the supernatant to be tested. Use 1mL glass cuvette to measure  $\Delta A_T = (A_{B1} - A_{B2}) - (A_T - A_C) = (1.156 - 0.045) - (0.595 - 0.271) = 0.787$ , Standard curve  $y = 0.8502x + 0.0217$ ,  $x = 0.900 \mu\text{mol/mL}$ , pyruvate content calculated:

$$\text{PA content } (\mu\text{mol /g weight}) = x \div W = 9.00 \mu\text{mol /g weight}$$

- Take 0.1g iris leaf, add 1mL extract solution, grind in ice bath, 8000g, centrifuge at 4 °C for 10min, take the supernatant to be tested. Use 1mL glass cuvette to measure  $\Delta A_T = (A_{B1} - A_{B2}) - (A_T - A_C) = (1.156 - 0.045) - (0.545 - 0.207) = 0.773$ , Standard curve  $y = 0.8502x + 0.0217$ ,  $x = 0.884 \mu\text{mol/mL}$ , pyruvate content calculated:

$$\text{PA content } (\mu\text{mol /g weight}) = x \div W = 8.837 \text{ mol/g weight}$$

3. Take 0.02 mL of horse serum to test using 1mL glass cuvette. Calculate  $\Delta A_T = (A_{B1} - A_{B2}) - (A_T - A_C) = (1.156 - 0.045) - (1.139 - 0.113) = 0.085$ , Standard curve  $y = 0.8502x + 0.0217$ ,  $x = 0.074 \mu\text{mol/mL}$ , pyruvate content calculated:

$$\text{PA content } (\mu\text{mol/mL}) = x = 0.074 \mu\text{mol/mL}$$

4. Take  $5 \times 10^4$  cells, add 1mL extract solution, grind in ice bath, 8000g, centrifuge at 4 °C for 10min, take the supernatant to be tested. Use 1mL glass cuvette to measure  $\Delta A_T = (A_{B1} - A_{B2}) - (A_T - A_C) = (1.156 - 0.045) - (0.770 - 0.098) = 0.439$ , Standard curve  $y = 0.8502x + 0.0217$ ,  $x = 0.491 \mu\text{mol/mL}$ , pyruvate content calculated:

$$\text{PA content } (\mu\text{mol} / 10^6 \text{ cell}) = x \div N = 0.098 \mu\text{mol} / 10^6 \text{ cell}$$

#### Related Products:

BC0380/BC0385	Pyruvate Dehydrogenase (PDH) Activity Assay Kit
BC0710/BC0715	$\alpha$ -Ketoglutarate Dehydrogenase ( $\alpha$ -KGDH) Activity Assay Kit
BC0950/BC0955	Succinate Dehydrogenase (SDH) Activity Assay Kit