Pyruvate (PA) Content Assay kit (enzymatic method)

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

Operation Equipment: Spectrophotometer

Cat No: BC5260

Size: 50T/24S

Components:

Extract Solution：Liquid 30 mL×1, Storage at 2-8°C;

Reagent I: Liquid 60 mL×1, Storage at 2-8°C;

Reagent II: Powder×1, Storage at -20°C. Before use, add 3.6mL 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 37 μL×1, Storage at 2-8°C; Mix Reagent III: distilled water = 10μL: 1mL (1.01 mL, about 13T) according to the dosage before use, and prepare for use now.

Reagent IV: Liquid 8 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^+ 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, transferpettor, 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 (10^6) : the extract volume (mL) is 5-10:1. (It is recommended that add 1 mL of the extract to 5×10^6 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 for 30 minutes, adjust wavelength to 450 nm, set the counter to zero with distilled water.
2. Preheat Reagent I in 37℃ for 20 minutes.
3. Standard tube measurement: 20μmol/mL sodium pyruvate standard solution was diluted with distilled water to obtain 0.5, 0.25, 0.125, 0.0625, 0.03125μmol/mL standard solution for reserve.
4. Standard dilution table

Number	Predilution concentration（μmol/mL）	Standard liquid volume（μL）	distilled water volume（μL）	Diluted concentration（μmol/mL）
1	20	50	950	1
2	1	500	500	0.5
3	0.5	500	500	0.25
4	0.25	500	500	0.125
5	0.125	500	500	0.0625
6	0.0625	500	500	0.03125

Remarks: 100μL of standard solution was required for each standard tube.

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

Reagent Name (μL)	Test tube (A_T)	Control tube (A_C)	Standard tube (A_S)	Blank tube (A_B)
Supernatant	100	100	-	-
Standard solution	-	-	100	-
distilled water	-	-	-	100
Reagent I	775	**900**	775	775
Reagent II	50	-	50	50
Reagent III	75	-	75	75
After mixing, react at 37℃ for 30min				
Reagent IV	100	100	100	100
After mixing, reaction at 37℃ for 30minutes (avoiding light). The absorbance at 450nm was measured in A 1mL glass colorimetric dish, and $\Delta A_T = A_B-(A_T-A_C)$, $\Delta A_S = A_B - A_S$. (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, μmol/mL) and the absorbance ΔA_S (y, ΔA_S), the standard curve was established. According to the standard curve, the determination of ΔA is substituted into the equation to obtain x (μmol/mL).

2. Calculate by volume of serum (plasma)

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

3. Calculate by protein concentration

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

4. Calculate by sample weight

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

5. Calculate by bacterial or cell density

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

V_{sv}: Sample volume, 0.1mL

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:

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

Experimental example:

1. 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 $\Delta A_T = A_B - (A_T - A_C) =$ 1.122- （0.678-0.299） =0.743, Standard curve y=1.4748x+0.0548， x=0.467μmol/mL, pyruvate content calculated:

   PA content (μmol /g weight) = x÷W =4.667 μmol /g weight

2. 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 $\Delta A_T = A_B - (A_T - A_C) =$ 1.122-（0.572-0.203）=0.753, Standard curve y=1.4748x+0.0548， x=0.473μmol/mL, pyruvate content calculated:

   PA content (μmol /g weight) = x÷W =4.73mol/g weight

3. Take 0.1 mL of horse serum to test using 1mL glass cuvette. Calculate $\Delta A_T = A_B - (A_T - A_C) =$ =1.122-（1.142-0.126）=0.106, Standard curve y=1.4748x+0.0548，x=0.035μmol/mL, pyruvate content calculated:

   PA content (μmol /mL) = x =0.035 μmol/mL

4. Take 5×10^4 cells, add 1mL extract solution, grind in ice bath, 8000g, centrifuge at 4 °C for 10min,

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com
For research use only. Do not use for clinical, diagnostic, food, cosmetic testing and other purposes.

take the supernatant to be tested. Use 1mL glass cuvette to measure $\Delta A_T=A_B-(A_T-A_C)=1.122-(0.949-0.113)=0.286$, Standard curve $y=1.4748x+0.0548$, $x=0.157$ μmol/mL, pyruvate content calculated:

PA content (μmol / 10^6 cell) = x÷N = 0.031 μmol/10^6 cell

Related Products：

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

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com
For research use only. Do not use for clinical, diagnostic, food, cosmetic testing and other purposes.