Ver.240823

Pyruvate (PA) Assay Kit

BC10005-01 (50 Tests/48 Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Pyruvate connects glucose, fatty acid and amino acid metabolism through acetyl CoA and plays an important pivotal role.

Pyruvate reacts with 2, 4-dinitrophenylhydrazine to produce pyruvate-2,4-dinitrophenylhydrazone, which is fuchsia-red in alkaline solution.

Kit components

Reagent	Volume	Storage
Extract Solution	60 mL × 1	2-8°C
Reagent I	7 mL × 1	2-8°C
Reagent II	30 mL × 1	2-8°C
Standard Solution (sodium pyruvate 20μmol/mL)	1 mL × 1	2-8°C
Preparation of 0.125 μmol/mL standard solution : 50μL 20μmol/mL standard solution and 450μL distilled water mix to obtain 2μmol/mL standard solution ; then 50 μL 2μmol/mL standard solution and 750μL distilled water mix to form 0.125 μmol/mL standard solution.		

Reagents and Equipment Required but Not Provided

Table centrifuge, water-bath, spectrophotometer, 1 mL glass cuvette, transferpettor, mortar/homogenizer, ice and distilled water.

Procedure

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 (10^4) : the extract solution volume (mL) is 500-1000:1. (It is recommended that add 1 mL of the extract solution to 5 million bacteria or cells). Ultrasound breaks up bacteria or cells (power 20% or 200W, ultrasonic of 3second, interval of 10second, 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 solution volume (mL) is 1:5-10. (It is recommended that add 1mL of extract solution 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 solution 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.

Determination Procedure

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 520nm and set the counter to zero with distilled water.
2. Operation table: (The following reagents is added into 1.5 mL EP tube.)

Reagent Name（μL）	Test tube (A_T)	Standard tube (A_S)	Blank tube(A_B)
Sample	300	-	-
Standard Solution	-	300	-
H_2O	-	-	300
Reagent I	100	100	100
Mix and react for 2 minutes at 25℃			
Reagent II	500	500	500
After fully mix, the absorbance value is measured at 520 nm wavelength, which is recorded as A_T, A_S and A_B. $\Delta A_T = A_T - A_B$, $\Delta A_S = A_S - A_B$, Blank tube and standard tube only need to do 1-2 times.			

Calculation of Pyruvate content

1. Calculate by volume of serum (plasma)

   Pyruvate content (μmol/mL)= $\Delta A_T \div \Delta A_S \times C_S \times (V_E+V_L) \div V_L$

   $= 1.375 \times \Delta A_T \div \Delta A_S$

2. Calculate by protein concentration

   Pyruvate content (μmol /mg prot) =$\Delta A_T \div \Delta A_S \times C_S \times V_S \div (V_S \times Cpr)$

   $= 0.125 \times \Delta A_T \div \Delta A_S \div Cpr$

3. Calculate by sample weight

   Pyruvate content (μmol /g weight) =$\Delta A_T \div \Delta A_S \times C_S \times V_S \div W$

   $=0.125 \times \Delta A_T \div \Delta A_S \div W$

4. Calculate by bacterial or cell density

   Pyruvate content (μmol /10^4 cell)＝$\Delta A_T \div \Delta A_S \times C_S \times V_S \div N$

   $= 0.125 \times \Delta A_T \div \Delta A_S \div N$

C_S: Concentration of standard solution, 0.125μmol/mL
V_S: Sample volume, 0.3 mL
V_E: Extract solution volume, 1 mL
V_L: Serum (plasma) volume, 0.1 mL
Cpr: Sample protein concentration, mg/mL
W: Sample weight, g
N: the amount of cell, 10^4 cell as a unit

Note:

1. If the measured absorbance value exceeds the linear range absorbance value, the sample size can be Increased or the sample can be diluted and then measured.
2. There are protein denaturation components in the extract solution, and if the protein concentration Calculation is used, another sample is required for extraction and determination.