Pyruvate Assay Kit

Cat #: orb1500010 (manual)

Visible Spectrophotometer

Size: 50T /48S

Product composition and storage conditions :

No.	Specifications	Storage conditions
Extraction solution - ES06	50ml×1	Store at 4℃
orb1500010-A	8ml×1	Store at 4℃, keep away from light
orb1500010-B	35ml×1	Store at 4℃
orb1500010-Standard	1ml×1(1mg/ml)	Store at 4℃

Introduction

Significance: Pyruvate plays a pivotal role in the metabolism of glucose, fatty acids and amino acids via acetyl CoA.

Principle: Pyruvate reacts with 2,4-dinitrophenylhydrazine acid to form pyruvate-2,4-dinitrophenylhydrazone, which is cherry red in alkaline solution.

Own suppliers: Visible spectrophotometer, centrifuge, water bath, adjustable pipette, 1 ml glass colorimeter, mortar, ice and distilled water.

Sample processing (pyruvate extraction) :

1. Bacteria or culture cells: collect bacteria or cells into a centrifuge tube, centrifuge and discard the supernatant; According to the number of bacteria or cells (10^4) : extraction solution ES06 volume (mL) is 500 ~ 1000:1 ratio (5 million bacteria or cells are recommended to add 1mL Extraction solution ES06) ,the bacteria or cells were broken by ultrasonic wave (ice bath, power 20% or 200 W, ultrasonic 3 s, interval 10 s, repeat 30 times) , leave standstill for 30 min, centrifuge for 10 min at 8000 g and 25℃, and take supernatant for test.

2. Tissue: According to the tissue mass (g):the volume of extracting solution ES06 is the ratio of one to 5~10 (it is recommended to weigh about 0.1 g of tissue and add 1 mL of extracting solution ES06), homogenize in an ice bath, leave standstill for 30 min, centrifuge for 10 min at 8000 g and 25℃, and take supernatant for test.

3. Serum /plasma sample: According to the volume of serum/plasma (ml): the volume of extracting solution ES06 is the ratio of 1: 5~10 (it is recommended to take 0.1 ml serum/plasma and add 1 ml extracting solution ES06), homogenize in an ice bath, leave standstill for 30 min, centrifuge for 10 min at 8000 g and 25℃, and take supernatant for test.

Measurement operation:

1. Preheat the spectrophotometer for at least 30 minutes, adjust the wavelength to 520nm, and set it to zero with the distilled water.

2. Preparation of standard: Dilute the standard to 25,12.5, 6.25, 3.125, 1.5625, 0.78125 and 0 μ g/mL with distilled water.

3. Follow the table as below:

Reagent name	**Measuring tube (ul)**	**Standard tube (ul)**
Sample	300	
Standard		300
orb1500010-A	100	100
Mix well and allow to stand for 2 min		
orb1500010-B	500	500
Mix well, and measure the light absorption value A of each tube at the wavelength of 520nm.		

Calculation of pyruvate content:

1. Draw the standard curve: take the concentration of each standard solution as the x-axis, take the A standard as the y-axis as the standard curve, and get Equation y = kx+b; The Δ A determination was carried into the equation to find the x μg/mL value.

2. Calculated by serum (plasma) volume

Pyruvate content (μg/mL) = (x×V1)÷[V1÷(V2+V3)×V3]=11x

3. Calculate the protein concentration

Pyruvate content (μg/mg prot) =(x×V1)÷(V1×Cpr)=x÷Cpr

4. Calculated by the sample weight

Pyruvate content (μg/g fresh weight) = (x×V1)÷(W×V1÷V2)=x÷W

5. Calculated by the density of bacteria or cells

Pyruvate content (μg/104 cell) = (x×V1)÷(500×V1÷V2)= x÷500

Note: V1: Sample volume added to reaction system, 0.3 ml; V2: Extraction solution volume added, 1 ml; V3: serum (plasma) volume added, 0.1 ml; Cpr: Sample protein concentration, mg/ml; W: Sample weight, g; 500: Total number of bacteria or cells, 5 million.

Note: If the absorption value of the determination exceeds the linear range of absorption value, can increase the sample size or dilute the sample before the determination.