Pyruvic Acid Assay Kit Instruction

Catalog number:BC052

I. Principle of Measurement

Pyruvic acid reacts with chromogenic agent and in the alkaline solution, the product would be in tawny color and thus the absorbance at certain wavelength can be measured and the pyruvic acid concentration can be calculated based on it.

II. Reagent Compositions

Reagent I: 1 Bottle×10ml. Preserved at RT.

Reagent II: 1 Bottle×50ml. Preserved at RT in darkness.

Reagent III: 2 Bottles×70ml. Preserved at RT.

Reagent IV: 1 Bottle×1ml. Preserved at 4°C.

Preparation of 0.2umol/mLPyruvic Acid Standard Solution:

Dilute reagent IV solution with double distilled water (DDW) with 1:9 ratio.

III. Procedures

1. Serum Sample

a. Pre-Treatment

Unnecessary. Serum samples can be measured directly.

b. Procedures

Compositions (ml)	Blank	Standard	Sample
DDW	0.1		
0.2mM Pyruvic Acid		0.1	
Sample			0.1

Reagent II	0.5	0.5	0.5
Mix thoroughly and warm the mixture at 37° C for 10 min.			
Reagent III	2.5	2.5	2.5

Set aside the mixture at RT for 5 min. Zero the spectrophotometer with DDW and record the optical density (OD) value of each tube at 505 nm with 1 cm path length.

2. Tissue Samples

a. Pre-Treatment

Weigh the tissues precisely and add physiological saline with the ratio 1 g to 9 ml. Homogenize the mixture in an ice water bath and centrifuge the homogenate at 2,500 rpm for 10 min. Extract the supernatant for further measurement.

b. Procedures

Compositions (ml)	Blank	Standard	Sample
DDW	0.1		
0.2mM Pyruvic Acid		0.1	
Sample			0.1
Reagent II	0.5	0.5	0.5
Mix thoroughly and warm the mixture at 37° C for 10 min.			
Reagent III	2.5	2.5	2.5

Set aside the mixture at RT for 5 min. Zero the spectrophotometer with DDW and record the optical density (OD) value of each tube at 505 nm with 1 cm path length.

IV. Calculation Formula and Examples

1. Serum Samples

a. Formula

$$\frac{C_{pyruvic\ acid}}{mM} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{0.2mM}$$

b. Example

Rate serum was taken and measured, the OD values measured were 0.008, 0.096 and 0.068 respectively.

$$\frac{C_{pyruvic\ acid}}{mM} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{0.2mM} = \frac{0.069 - 0.008}{0.096 - 0.008} \times 0.2 = 0.143mM$$

2. Tissue Samples

a. Formula

$$\frac{C_{pyruvic\ acid}}{\mu mol/ml} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{0.2mM} \div \frac{C_{protein}}{mg/ml}$$

b. Example

i. Rat hepatic tissue was homogenized to 10% homogenate and the homogenate was measured. The OD values were0.033, 0.093 and 0.170 respectively. Also, the protein concentration for 10% homogenate was 19.993mg/ml

$$\frac{C_{pyruvic\ acid}}{\mu mol/ml} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{0.2mM} \div \frac{C_{protein}}{mg/ml} = \frac{0.170 - 0.033}{0.093 - 0.033} \times 0.2 \div 19.993$$
$$= 0.023 \mu mol/ml$$

ii. Rat heart tissue was homogenized to 10% homogenate and the homogenate was measured. The OD values were0.033, 0.093 and 0.092 respectively. Also, the protein concentration for 10% homogenate was 10.201mg/ml.

$$\frac{C_{pyruvic\ acid}}{\mu mol/ml} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{0.2mM} \div \frac{C_{protein}}{mg/ml} = \frac{0.092 - 0.033}{0.093 - 0.033} \times 0.2 \div 10.201$$
$$= 0.019 \mu mol/ml$$

iii. Rat brain tissue was homogenized to 10% homogenate and the homogenate was measured. The OD values were0.037, 0.099 and 0.079 respectively. Also, the protein concentration for 10% homogenate was 7.199mg/ml.

$$\frac{C_{pyruvic\ acid}}{\mu mol/ml} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{0.2mM} \div \frac{C_{protein}}{mg/ml} = \frac{0.079 - 0.037}{0.099 - 0.037} \times 0.2 \div 7.199$$
$$= 0.019 \mu mol/ml$$