

## **Lactic Acid(LA) Content Assay Kit**

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

**Operation Equipment:** Spectrophotometer/ Microplate reader

**Cat No:** BC2235

**Size:** 100T/48S

### **Components:**

Extract solution I: 50 mL×1 bottle, store at 4°C;

Extract solution II: 8 mL×1 bottle, store at 4°C;

Reagent I: 6 mL×1 bottle, store at 4°C;

Reagent II: 34 μL×1 bottle, store at 4°C. Working solution: Accordance ratio Reagent II: Distilled water=10 μL: 450 μL. Prepare when it will be used.

Reagent III: Powder×1 bottle, store at -20°C and protect from light; Working solution: add 4 mL distilled water before use. Mix thoroughly. It can be stored for one week at -20°C. Avoid repeated freezing and thawing.

Reagent IV: Powder×1 bottle, store at 4°C and protect from light; Working solution: add 4 mL distilled water before use. Mix thoroughly. It can be stored for one week at 4°C.

Reagent V: Powder×1 bottle, store at -20°C and protect from light; Working solution: add 3 mL distilled water before use. Mix thoroughly. Store at -20°C after spacing out. Avoid repeated freezing and thawing.

Reagent VI: Liquid 2 mL×1 bottle, store at 4°C;

Standard: Powder×1 bottle, store at 4°C; Standard solution: Add 1.04 mL distilled water to 100 μmol/mL before use.

Prepare Chromogenic Solution: Accordance ratio Reagent III(V): Reagent IV (V)=1:1, mix thoroughly, Prepare when it will be used.

### **Description:**

L (+)-Lactic acid is an important intermediate product in biological metabolism. It is closely related to glucose metabolism, lipid metabolism, protein metabolism and intracellular energy metabolism. Lactic content is an important indicator for assessing carbohydrate metabolism and aerobic metabolism. L (+)-Lactic produces pyruvic acid under the action of lactate dehydrogenase, and NAD<sup>+</sup> is reduced to produce NADH and H<sup>+</sup>. H<sup>+</sup> is transferred to PMS to produces PMSH<sub>2</sub> and PMSH<sub>2</sub> reduce MTT to form purple substance, and has a characteristic absorption peak at 570 nm.

### **Required but not provided:**

Scale, mortar/homogenizer, centrifuge, spectrophotometer/microplate reader, micro glass cuvette/ 96 well flat-bottom plate, constant temperature water bath, ethanol, distilled water.

### **Procedure:**

## I. Sample Preparation.

### 1. Tissue:

Accordance ratio weight(g): Extract solution I(mL)=1: 5~10. (Suggested 0.1g tissue with 1mL Extract solution I). Homogenate on ice bath. 12000 g centrifuge for 10 min at 4°C. Add 0.15mL Extract solution II to 0.8 mL supernatant. 12000 g centrifuge for 10 min at 4°C. Supernatant is for test.

### 2. Cells:

Accordance ratio cell amount ( $10^4$ ): Extract solution I(mL)=500~1000:1. (Suggested 5 million cells with 1mL Extract solution I). Breaking cells (300W, work time 3s, interval 7s for 3 min) by ultrasonic on ice bath. 12000 g centrifuge for 10 min at 4°C. Add 0.15 mL Extract solution II to 0.8mL supernatant. 12000 g centrifuge for 10 min at 4°C. Supernatant is used for test.

### 3. Serum (plasma) sample:

Add 1 mL Extract solution I to 100  $\mu$ L serum(plasma). 12000 g centrifuge for 10 min at 4°C. Add 0.15 mL Extract solution II to 0.8 mL supernatant. Centrifuge for 10 min at 12000 g. Supernatant is used for test.

## II. Determination procedure.

1. Preheat spectrophotometer/microplate reader for 30 min, adjust wavelength to 570 nm, set zero with ethanol.

2. Standard working solution: 100 $\mu$ mol/mL standard was diluted with distilled water to be 2, 1, 0.5, 0.25, 0.125, 0.0625 $\mu$ mol/mL for test.

3. Add reagents according to the following table.

	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube (B)
Sample( $\mu$ L)	10	10	-	-
Standard( $\mu$ L)			10	-
Distilled water( $\mu$ L)	-	10	-	10
Reagent I( $\mu$ L)	40	40	40	40
Reagent II( $\mu$ L)	10	-	10	10
Reagent V( $\mu$ L)	20	20	20	20
Mix thoroughly in centrifuge tube, react 20 min at 37°C water bath.				
Reagent VI	6	6	6	6
Chromogenic Solution( $\mu$ L)	60	60	60	60
Mix thoroughly in centrifuge tube. Avoiding light react 20 min at 37°C, 10000 rpm centrifuge for 10 min at 25°C. Remove supernatant and retain sediment.				
Ethanol( $\mu$ L)	200	200	200	200
Fully dissolved sediments, and determine absorbance at 570 nm, record $A_T$ , $A_C$ , $A_S$ , $A_B$ , $\Delta A_T=A_T-A_C$ . $\Delta A_S=A_S-A_B$ .				

## III. Calculation.

### 1. Drawing of standard curve.

Standard solution concentration as x axis and its corresponding absorption value ( $\Delta A_S$ ) as y axis, the

standard equation is  $y=kx+b$ . Bring  $\Delta A_T$  into the formula to get  $x$  ( $\mu\text{mol/mL}$ ).

## 2. Calculation of Lactate content.

A. Protein concentration:

$$\text{LA } (\mu\text{mol/mg prot}) = x \times V_s \div (V_s \times C_{pr}) = x \div C_{pr}.$$

B. Sample weight

$$\text{LA } (\mu\text{mol/g weight}) = x \times (V_{sp} + V_{II}) \div (W \times V_{sp} \div V_I) = 1.1875 \times x \div W.$$

C. Cell amount

$$\text{LA } (\mu\text{mol}/10^6 \text{ cell}) = x \times (V_{sp} + V_{II}) \div (5 \times V_{sp} \div V_I) = 0.2375 \times x.$$

D. Liquid volume

$$\text{LA } (\mu\text{mol/mL}) = x \times (V_{sp} + V_{II}) \div [V_L \times V_{sp} \div (V_I + V_L)] = 13.0625 \times x$$

$V_s$ : Sample volume, 0.01 mL

$W$ : Sample weight, g

$C_{pr}$ : Sample protein concentration, mg/mL (Protein concentration needs to be Self-determined)

$V_{sp}$ : Supernatant volume, 0.8 mL

$V_{II}$ : Extract solution II, 0.15 mL

$V_I$ : Extract solution I, 1 mL

Cells amount: 5 million

$V_L$ : Liquid sample volume, 0.1 mL

### Note:

If the absorbance value exceeding 1.5 or  $\Delta A > 1.2$ , please dilute the sample volume properly and multiply the dilution multiple in the formula.

### Experimental example:

1. Take 0.1g of rabbit heart, add 1 mL of Extract solution 1, grind and centrifuge, take 0.8 mL of supernatant and add 0.15 ml of Extract solution 2, centrifuge supernatant and dilute 5 times, then operate according to the determination steps, use 96 well plate to measure and calculate  $\Delta A_T = A_T - A_C = 0.591 - 0.069 = 0.522$ , according to the standard curve  $y = 0.412x - 0.0214$ ,  $x = 1.319$ , calculate the content according to the sample mass.

$$\text{La content } (\mu\text{mol/g mass}) = 1.1875 \times x \div W \times \text{dilution ratio} = 1.1875 \times 1.319 \div 0.1 \times 5 = 78.32 \mu\text{mol/g mass}.$$

2. Take 100  $\mu\text{L}$  of mouse serum, add 1 mL of Extract solution 1, take 0.8 mL of supernatant and then add 0.15 mL of Extract solution 2, centrifugate the supernatant, and then operate according to the determination steps, use 96 well plate to measure and calculate  $\Delta A_T = A_T - A_C = 0.572 - 0.211 = 0.361$ , according to the standard curve  $y = 0.412x - 0.0214$ ,  $x = 0.928$ , calculate the content according to the liquid volume

$$\text{La content } (\mu\text{mol/mL}) = 13.0625 \times x = 13.0625 \times 0.928 = 12.122 \mu\text{mol/mL}.$$

### Recent Product Citations:

[1] Meixi Peng, Dan Yang, Yixuan Hou, et al. Intracellular citrate accumulation by oxidized ATM-mediated metabolism reprogramming via PFKFB3 and CS enhances hypoxic breast cancer cell invasion and

metastasis. Cell Death and Disease. March 2019;(IF5.959)

[2] Xiaojin Luo, Weihua Shi, Haoming Yu, et al. Wearable Carbon Nanotube-Based BioSensors on Gloves for Lactate. Sensors. October 2018;(IF3.031)

[3] Zhou F, Du J, Wang J. Albendazole inhibits HIF-1 $\alpha$ -dependent glycolysis and VEGF expression in non-small cell lung cancer cells[J]. Molecular and cellular biochemistry, 2017, 428(1-2): 171-178.

#### **References:**

Eolbergrová J, MacMillan V, Siesjö B K. The effect of moderate and marked hypercapnia upon the energy state and upon the cytoplasmic NADH/NAD<sup>+</sup> ratio of the rat brain[J]. Journal of neurochemistry, 1972, 19(11): 2497-2505.

#### **Related Products:**

BC0740/BC0745 Hexokinase(HK) Activity Assay Kit

BC0540/BC0545 Pyruvate Kinase(PK) Activity Assay Kit

BC0530/BC0535 Phosphofructokinase(PFK) Activity Assay Kit

#### **Technical Specifications:**

The detection limit: 0.0771  $\mu\text{mol/mL}$

The linear range: 0.078-5  $\mu\text{mol/mL}$