



ISO 13485:2016 ISO 9001:2015

Ver.260801

Lactic Acid (LA) Content Assay Kit

BC2230 (50T/48S)

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

Product Description

L-Lactic acid is an important intermediate product in biological metabolism, which is closely related to sugar metabolism, lipid metabolism, protein metabolism and intracellular energy metabolism. Lactic acid content is an important indicator for assessing carbohydrate metabolism and aerobic metabolism. Lactic acid produces pyruvic acid under the action of lactate dehydrogenase, and NAD^+ is reduced to produce NADH and H^+ . H^+ is transferred to PMS to produce PMSH_2 and PMSH_2 reduce MTT to form purple substance which has a characteristic absorption peak at 570nm.

Kit components

Reagent	Volume	Storage
Extraction Solution I	60mL	2-8°C
Extraction Solution II	10mL	2-8°C
Reagent I	10mL	2-8°C
Reagent II	45μL	2-8°C
Reagent III	12mL	2-8°C
Reagent IV	Powder	-20°C
Reagent V	40mL	2-8°C
Standard	Powder	2-8°C

Solution Preparation:

- 1. Reagent II working solution:** Before use, throw the liquid off the bottom of the tube (use a handheld centrifuge); Reagent II is diluted according to the ratio of Reagent I (V): Reagent II (V) = 450μL: 2μL (452μL in total, about 3T). Prepare when the solution will be used.
- 2. Reagent III:** Yellow solution, which should be kept away from light.
- 3. Reagent IV:** Before use, add 4mL of distilled water and mix well. It can be stored at -20°C for 4 weeks after packaging. This reagent needs to avoid repeated freezing and thawing, and strictly avoid light.
- 4. Reagent IV working solution:** Dilute according to the ratio of Reagent III (V) : Reagent IV (V) = 540μL : 180μL (720μL, 3T).
- 5. Standard solution:** Before use, 1.04mL distilled water was added to prepare 100μmol/mL L-Lactic acid standard solution. Store at 2-8°C for 4 weeks.
- 6. 2μmol/mL standard solution:** Before use, 20μL of 100μmol/mL standard solution and 980μL of distilled water were mixed and diluted to 2μmol/mL standard solution.

Reagents and Equipment Required but Not Provided

Scale, mortar/homogenizer/cell ultrasonic crusher, centrifuge, spectrophotometer, 1mL glass cuvette, water bath, distilled water, normal saline.

Protocol

1. Tissue sample: Add Extract solution I according to the ratio of tissue mass (g) : Extract solution I volume (mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0mL of Extract solution I), after ice bath homogenization, centrifuge at room temperature, 12000rpm for 10 minutes, take 0.8mL of supernatant and Add 0.15mL Extract solution II slowly until no bubbles. Centrifuge at room temperature, 12000rpm for 10 minutes, take supernatant for test.
2. Cells: The ratio of bacteria/cell amount (10^6): the volume of normal saline (mL) is 500~1000:1(it is suggested to take about 5 million bacteria/cells and add 1mL of normal saline). Bacteria/cell is split by ultrasonic (placed on ice, 300 W, work time 3 s, interval 7s, total time 3 minutes). Centrifuge at room temperature, 12000rpm for 10 minutes, take the supernatant for test.
3. Serum (plasma) sample: Add 1mL Extract solution I to 100 μ L serum(plasma). Centrifuge at room temperature, 12000rpm for 10 minutes, take 0.8mL of supernatant and Add 0.15mL Extract solution II slowly until no bubbles. Centrifuge at room temperature, 12000rpm for 10 minutes, take supernatant for test.

- Note:** (1) The Extract solution II needs to be added slowly, and a large number of bubbles will be generated after addition. It is recommended to use 2mL EP tube for operation.
- (2) If the protein content in the sample is trace and the color is very light, it can also be directly homogenized, centrifuged and detected with normal saline.
- (3) The ultrasonic time of bacterial samples can be prolonged appropriately.

Determination Procedures

1. Preheat the spectrophotometer more than 30 minutes, adjust wavelength to 570nm, set zero with distilled water.
2. Reagent II working solution and reagent IV working solution can be incubated at 37°C for 5minutes.
3. Operation table (Add reagents in 1.5mL EP tube according to the following table)

Reagent(μ L)	Test tube(T)	Standard tube(S)	Blank tube(B)
Sample	30	-	-
Standard	-	30	-
Distilled water	-	-	30
Reagent II (Working solution)	150	150	150
Reagent IV (Working solution)	240	240	240
Mix thoroughly in centrifuge tube, react 10 minutes at 37°C water bath.			
Reagent V	600	600	600
Mix thoroughly in centrifuge tube and determine absorbance at 570nm, record A_T , A_S , A_B , $\Delta A_T = A_T - A_B$, $\Delta A_S = A_S - A_B$. (The blank tube and standard tube only need to be measured 1-2 times)			

Calculations

1. Calculate by sample protein concentration:

$$\text{L-LA content } (\mu\text{mol/mg prot}) = C \times \Delta A_T \div \Delta A_S \times V_S \div (V_S \times C_{pr}) \times F = 2 \times \Delta A_T \div \Delta A_S \times F$$

2. Calculate by sample mass:

$$\text{L-LA content } (\mu\text{mol/g mass}) = C \times \Delta A_T \div \Delta A_S \times (V_{sp} + V_{II}) \div (W \times V_{sp} \div V_I) \times F = 2.375 \times \Delta A_T \div \Delta A_S \div W \times F$$

3. Calculate by the number of bacteria or cells:

$$\text{L-LA content } (\mu\text{mol}/10^6 \text{ cell}) = C \times \Delta A_T \div \Delta A_S \times (V_{sp} + V_{II}) \div (N \times V_{sp} \div V_I) \times F = 2.375 \times \Delta A_T \div \Delta A_S \div N \times F$$

4. Calculate by liquid volume:

$$\text{L-LA content } (\mu\text{mol/mL}) = C \times \Delta A_T \div \Delta A_S \times (V_{sp} + V_{II}) \div [V_L \times V_{sp} \div (V_I + V_L)] \times F = 26.125 \times C \times \Delta A_T \div \Delta A_S \times F$$

C: Standard concentration, 2 μ mol/mL;

V_S: Sample volume, 0.03

mL; W: Sample mass, g;

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

V_{sp}: Supernatant volume, 0.8mL;

V_{II}: Extract solution II volume, 0.15mL;

V_I: Extract solution I volume, 1mL;

N: Number of cells, count by 10⁶;

V_L: Liquid sample volume, 0.1mL;

F: Dilution ratio.

Note:

1. If $A_T > 1.5$ or $\Delta A_T > 1$, the sample can be diluted with distilled water and then determined. If $\Delta A_T < 0.02$, the sample size can be appropriately increased for determination, and the calculation formula can be modified synchronously.
2. Extract solution I contains a protein precipitant and therefore the supernatant cannot be used for protein concentration determination. If protein content is to be determined, a separate sample is required.