Ver.260401

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 produces $PMSH_2$ and $PMSH_2$ reduce MTT to form purple substance which has a characteristic absorption peak at 570 nm.

Kit components

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

Note:

Reagent II: 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 II (V): distilled water (V) =10μL: 450μL for adding samples in the following operating table.

Reagent IV: Before use, add 8mL distilled water to each bottle and mix well. After subpacking, store at -20°C to avoid repeated freezing and thawing. Store at -20°C for 4 weeks.

Standard: Before use, 1.04mL distilled water was added to prepare 100μmol/mL standard. Store at 2- 8°C for 4 weeks.

Reagents and Equipment Required but Not Provided

Scale, mortar/homogenizer, centrifuge, spectrophotometer, 1mL glass cuvette, constant temperature water bath, ethanol (>98%, AR), distilled water.

Protocol

I. Sample Preparation.

1. Bacteria/Cells

Accordance ratio cell amount (10^6): Extract solution I (mL)=5~10:1. (Suggested 5 million cells with 1mL Extract solution I). Breaking cells (300W, work time 3 seconds, interval 7 seconds for 3 minutes) by ultrasonic on ice bath. 12000×g centrifuge for 10 minutes at 4°C. Add 0.15mL Extract solution II to 0.8mL supernatant slowly. Blend slowly until no bubbles. 12000×g centrifuge for 10 minutes at 4°C. Supernatant is used for test.

2. 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 minutes at 4°C. Add 0.15mL Extract solution II slowly to 0.8mL supernatant. Blend slowly until no bubbles. Then 12000×g centrifuge for 10 minutes at 4°C. Supernatant 1 second for test.

3. Serum (plasma) sample:

Add 1mL Extract solution I to 100μL serum (plasma). 12000×g centrifuge for 10 minutes at 4°C. Add 0.15mL Extract solution II to 0.8mL supernatant slowly. Blend slowly until no bubbles. Centrifuge for 10 minutes at 12000×g. Supernatant is used for test.

Note: The Extraction solution II needs to be added slowly, and a large number of bubbles will be generated after addition. It is recommended to use 2mL Centrifuge tube for operation.

II. Determination Procedures

- Preheat spectrophotometer for 30 minutes, adjust wavelength to 570nm, set zero with ethanol.
- Standard working solution: 100μmol/mL standard was diluted with distilled water to be 1, 0.625, 0.3125. 0.15625, 0.078. 0.039μmol/mL for test.
- Add reagents according to the following table:

Reagent(μL)	Test tube(T)	Control tube(C)	Standard tube(S)	Blank tube(B)
Sample	50	50	-	-
Standard	-	-	50	-
Distilled water	-	50	-	50
Reagent I	200	200	200	200
Reagent II (After dilution)	50	-	50	50
Reagent IV	100	100	1000	100
Mix thoroughly in centrifuge tube, react 20 minutes at 37°C water bath.				
Reagent V	30	30	30	30
Reagent III	300	300	300	300
Mix thoroughly in centrifuge tube. Avoiding light react 20 minutes at 37°C, 10000 rpm centrifuge for 10 minutes at 25°C. Remove supernatant and retain sediment.				
Ethanol (μL)	1000	1000	1000	1000
Fully dissolved sediments, and determine absorbance at 570 nm, record A_T, A_C, A_S, A_B, Calculate $\Delta A_T = A_T - A_C$. $\Delta A_S = A_S - A_B$, (The blank tube and standard curve only need to be measured 1-2 times).				

III. Calculations

1. Drawing of standard curve.

   Standard solution concentration as x axis and its corresponding absorption value (ΔA_S) as y axis, the standard equation is y= kx+b. Bring ΔA; into the formula to get x (μmol/mL).

2. Calculation of Lactate content.

A. Protein concentration:

LA (μmol/mg prot) = $x \times V_S \div (V_S \times Cpr) = x \div Cpr$.

B. Sample weight

LA (μmol /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

LA (μmol /10^6 cell) = $x \times (V_{SP}+V_{II}) \div (N \times V_{SP} \div V_I) = 1.1875 \times x \div N$.

D. Liquid volume

LA (μmol /mL) = $x \times (Vsp+ V_{II}) \div [V_L \times Vsp \div (V_I+V_L) = 13.0625 \times x$.

V_S: Sample volume, 0.05mL
W: Sample weight, g
Cpr: Sample protein concentration, mg/mL (Protein concentration needs to be Self- determined)
V_{SP}: Supernatant volume, 0.8mL
V_{II}: Extract solution II, 0.15mL
V_I: Extract solution I, 1mL
N: Number of cells, in millions.
V_L: Liquid sample volume, 0.1mL

Note:

1. If the measured light absorption value exceeds the linear range, you can increase the sample size or dilute the sample before testing.
2. Extract 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.