L-Lactic Acid (LA) Colorimetric Assay Kit

Cat# : AS1005, 96 tests

Storage at 4℃ and -20 ℃

INTRODUCTION

L-lactic acid is the main intermediate product of lactate metabolism in the body and is present in the blood. This kit provides a rapid and sensitive method for the determination of lactic acid: lactic acid is produced by the action of lactate dehydrogenase, and NAD+ is reduced to NADH; in order for this reaction to proceed smoothly, additional enzymes are added to further decompose pyruvate; the resulting NADH reacts with a specific color developer to produce a colored substance with a maximum absorption peak at 450 nm. The lactic acid content is calculated by measuring the increase in the amount of this substance at 450 nm.

KIT COMPOSITION AND PREPARATION

	48 T	**96 T**	
Reagent name	Specification	Specification	Preservation requirements
Extraction solution	Liquid 60 mL x 1 bottle	Liquid 120 mL x 1 bottle	4°C
Reagent 1	Liquid 1 mL x 1 pc	Liquid 2 mL x 1 pc	4°C
Reagent 2	Liquid 0.5 mL x 1 pc	Liquid 1 mL x 1 pc	4°C
Reagent 3	Liquid 10 mL x 1 bottle	Liquid 20 mL x 1 bottle	4°C
Reagent 4	Liquid 0.5 mL x 1 pc	Liquid 1 mL x 1 pc	4°C
Reagent 5	Liquid x 1 pc Shake a few times before use to make the reagent fall to the bottom part, then add **0.55** mL distilled water to dissolve	Liquid x 1 pc Shake a few times before use to make the reagent fall to the bottom part, then add **1.1** mL distilled water to dissolve	-20°C
Standards	Liquid x 1 pc	Liquid x 1 pc	4°C

INSTRUMENTS AND SUPPLIES REQUIRED

ELISA, 96-well plates, adjustable pipettes, scales, mortar and pestle, centrifuge.

DETERMINATION OF LACTIC ACID (LA) CONTENT

It is recommended that 2 samples are selected for pre-determination before the formal experiment,

to understand the situation of this batch of samples and to familiarize with the experimental process, so as to avoid wasting samples and reagents!

1. Sample preparation.

Tissue sample: 0.1 g of tissue sample, grinded with 1mL of extraction solution, transfer all the crude extract to an EP tube. Centrifuge at 12000rpm for 10min, supernatant to be tested.

[Note]: *If the sample size is increased, the extraction can be done at a ratio of 1:5~10 tissue mass (g): volume of extraction solution (mL)*

Bacterial/cellular samples: Collect the bacteria or cells into a centrifuge tube, centrifuge and discard the supernatant; add 5 million bacteria or cells to 1mL of extraction solution; ultrasound to break up the bacteria or cells (ice bath, power 20% or 200W, 3s, 10s interval, repeat 30 times); centrifuge at 4°C, 12000 g for 10 min, remove the supernatant for determination.

[Note]: *If the sample size is increased, the ratio of bacteria/cell number (104): extraction solution (mL) is 1000~5000 : 1.*

Liquid samples:

a. Nearly neutral liquid samples can be transferred directly into EP tubes by taking 1mL; centrifuge at 12,000 rpm for 10 min and leave the supernatant to be measured.

b. For acidic liquids, adjust the pH of the solution to approximately 8 with KOH (5M) and incubate for 30 minutes at room temperature. Transfer 1 mL to an EP tube; centrifuge at 12000 rpm for 10 min, supernatant to be measured.

Serum samples: clarified serum samples can be tested directly.

2. On-board testing.

1) Preheat the ELISA for more than 30 min and adjust the wavelength to 450 nm.

2) Reagents 1,2, 3 and 4 can be mixed in the ratio 20:10:130:10 to form a mixture with 170 μL of the mixture.

3) In a 96-well plate add in sequence.

Reagent name (μL)	Measurement tubes	Blank control (done once only)
Sample	20	0
Reagent 1	20	20
Reagent 2	10	10
Reagent 3	130	150
Reagent 4	10	10
Reagent 5	10	10
The reaction was immediately carried out at 37°C for 30 min at 450 nm, protected from light.		

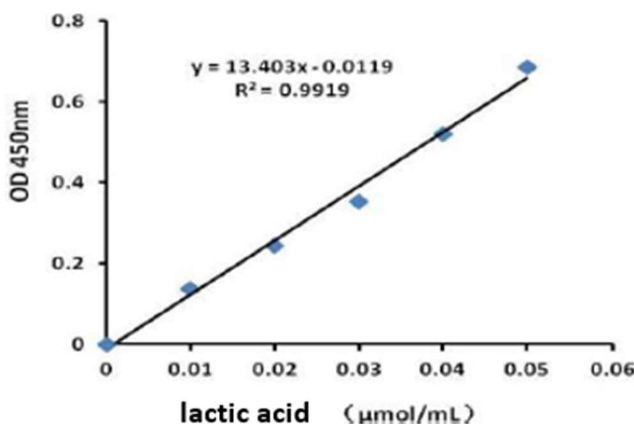
Read the absorbance value A, ΔA = A measurement - A control (each sample makes a control of its own).

[Note]:

1. If the sample itself has a strong background value (e.g. very dark or contains reducing substances such as ascorbic acid), a sample control can be added: i.e. reagent IV is replaced by distilled water and the other reagents remain unchanged, then ΔA = A assay - A control.
2. If the ΔA value is small, increase the sample volume V1 (e.g. increase to 40 µL, then reduce reagent 3 accordingly), then the changed V1 A recalculation is required by substituting the calculation formula.
3. If the ΔA value is large, or if the A determination exceeds 1.5, the sample may be diluted with distilled water; or the sample loading V1 may be reduced (e.g. to 10 µL, with a corresponding increase in reagent 3) and the dilution D and the altered V1 substituted into the equation and recalculated.

CALCULATION OF RESULTS

1. Standard curve: y = 13.403x - 0.0119; x is the molar concentration of the standard (µmoL/mL) and y is ΔA.



2. Calculated according to sample quality
   Lactic acid (µmol/g fresh weight) =
   [(ΔA+0.0119)÷13.403×V2]÷(W×V1÷V)×D=0.75×(ΔA+0.0119)÷W×D
3. Calculated by bacteria/cell
   Lactic acid content (µmol/104 cell) = [(ΔA+0.0119)÷13.403×V2]÷(500×V1÷V) ×D
   = 0.0015×(ΔA+0.0119)×D
4. By volume of liquid
   Lactic acid content (µmol/mL) = [(ΔA+0.0119)÷13.403×V2]÷V1×D = 0.75×(ΔA+0.0119)×D
5. By volume of serum
   Lactic acid content (µmol/mL) = [(ΔA+0.0119)÷13.403×V2]÷V1×D = 0.75×(ΔA+0.0119)×D

V - volume of extraction solution added, 1 mL;

V1 - volume of sample added to the reaction system, 0.02 mL.

V2 - total volume of the reaction system, 0.2 mL;

W - sample mass, g.

500 - number of cells,

D - number of dilutions, undiluted is 1;

lactic acid molecular weight Mr - 90.08.

Attachment: standard curve preparation procedure.

1. Preparation of standard master mix (30 µmol/mL): dissolve the standard in 1 mL of distilled water. (The master batch should be used within two days and stored at -20°C).
2. The master mix is diluted into six concentration gradients: 0, 0.1, 0.2, 0.3, 0.4, 0.5 µmol/mL. The concentration of the standard can also be adjusted according to the actual sample.
3. The standard curve can be produced from the results according to the tube addition table.

PRODUCT USE LIMITATION

These products are intended for research use only.