Lactic Acid assay kit

(Cat/No.:BC093 Size:50T/48S)

1. Introduction

This kit applies to measure lactic acid (LD) content in blood serum, tissue & broth, etc.
This kit can be used for laboratory research only.

2. Assay significance

Lactic acid (LD) is product of saccharide anaerobic metabolism (glycolysis). LD is produced in ossatures, muscles, brain & erythrocytes, metabolized in liver and evacuate by kidney secretion. Blood LD assay can reflect tissue oxygen supply, metabolic state & perfusion shortage. LD level increasing exists in many clinical diseases.

3. Assay principle (colorimetric method)

Use NAD+ as hydrogen acceptor, LDH catalyze lactic acid dehydrogenates to produce pyruvic acid, NAD+ converts to NADH. During this reaction, PHS transferring hydrogen causes NBT reduces to purple compound. At 530nm, this compound 's OD value appears linear correlation with lactic acid.

4. Composition & Preparation (The kit is valid for 6 month)

Reagent 1: Enzyme diluent, 60ml ×1 bottle, can be stored at 2℃~8℃ for 1 year.

Reagent 2: Enzyme stock solution, 0.6ml ×1 vial, can be stored at 2℃~ 8℃ for 6 months.

Enzyme working solution preparation: Before use, mix Reagent 2 (enzyme stock solution) and Reagent 1 (enzyme diluent) at ratio of 1:100, this working solution.

should be used soon after preparation, can be stored at 2℃~8℃ for 24 hours.

Reagent 3: Yellow limpid liquid, 6ml ×2 bottles, can be stored at 2℃~ 8℃ away from light for 6 months.

Reagent 4: White powder ×2 vials, can be cold preservated at -20℃ for 1 year.

Chromogenic agent preparation: Before use, add 1 vial of Reagent 4 powder in 1 bottle of Reagent 3 solution, wait until dissolve completely, use micropipet to transfer a bit of liquid in small centrifuge tube, turn small centrifuge tube upside down repeatedly, transfer liquid from centrifuge tube into bottle, loop these steps 2~3 times in order to mix sufficiently,

chromogenic agent is prepared, can be stored at 2℃~8℃ away from light for 2 weeks.

Reagent 5: Terminator, 60ml×2 bottles, can be stored at 2℃~8℃ for 1 years.

Reagent 6: 3mmol/L standard solution, 2ml ×1 vial, can be stored at 2℃~ 8℃ for 6 months

5. Required Equipment & Reagents

● An spectrophotometer (or ELIASA or semi-automatic bio-analysator) capable of measuring absorbance at 530nm

● Thermostatic water bath or air bath capable of controlling temperature at 37℃

Desk centrifuge

● Micropipets and tips

Vortex mixer

- A source of pure water (preferably double distilled water and double distilled water)

6. Operation Procedure

	Blank tube	Standard tube	Sample tube
Distilled water (ml)	0.02		
3mmol/L standard solution (ml)		0.02	
Sample to assay (ml)			0.02
Enzyme working solution (ml)	1	1	1
Chromogenic agent (ml)	0.2	0.2	0.2
Mix sufficiently, react in 37℃ water bath for 10 minutes.			
Terminator (ml)	2	2	2
Mix sufficiently, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 530 nm (adjust zero by distilled water).			

7. Calculation

(1) Blood serum LD assay formula:

$$\text{Blood serum LD content (mmol/L)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (3mmol/L)} \times \text{Sample dilution times before assay}$$

(2) Tissue LD assay formula:

$$\text{Tissue LD content (mmol/L)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (3mmol/L)} \div \text{Sample protein concentration (gprot*/L)}$$

Note *: gprot means gram protein.

8. Announcements

(1) Blood serum, blood plasma, tissue pieces can be stored at -20℃ for 1 month or at -70℃ for 2~3 months. Lower temperture leads to longer storage. Thawed sample or tissue should be assayed in the same day.

(2) Heavy hemolysis and choloplania will cause result increasing.

(3) Please do pretest before do formal experiments in batches, it is in order to make $OD_{Absolute}$ (=OD_{Sample}-OD_{Blank}) is between 0. 1 and 0.3. If $OD_{Absolute}$<0.1, then increase sample concentration and assay again; if $OD_{Absolute}$>0.3, then dilute sample and assay again.

APPENDIX I: Lactic Acid Standard

Dilute 6mmol/ L lactic acid (LD) standard to different concentrations in order to prepare standard curve.

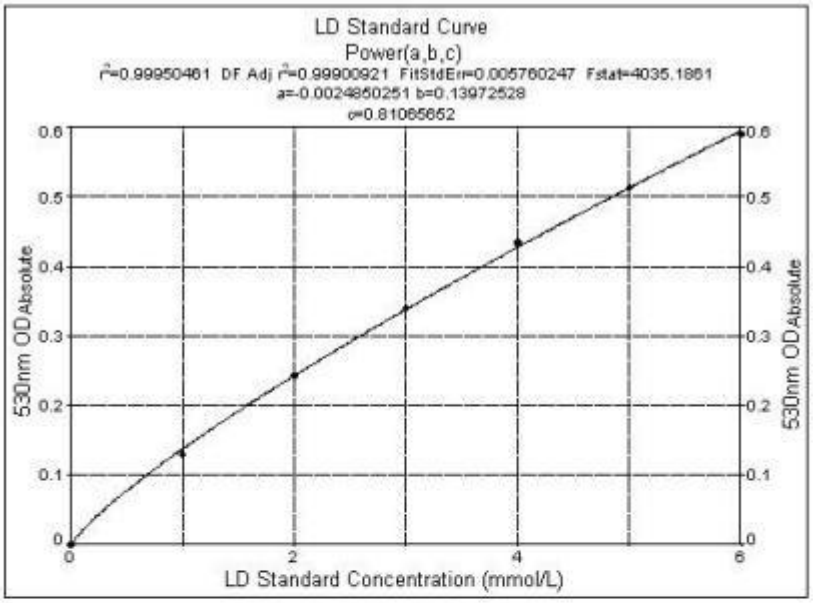
Operation table:

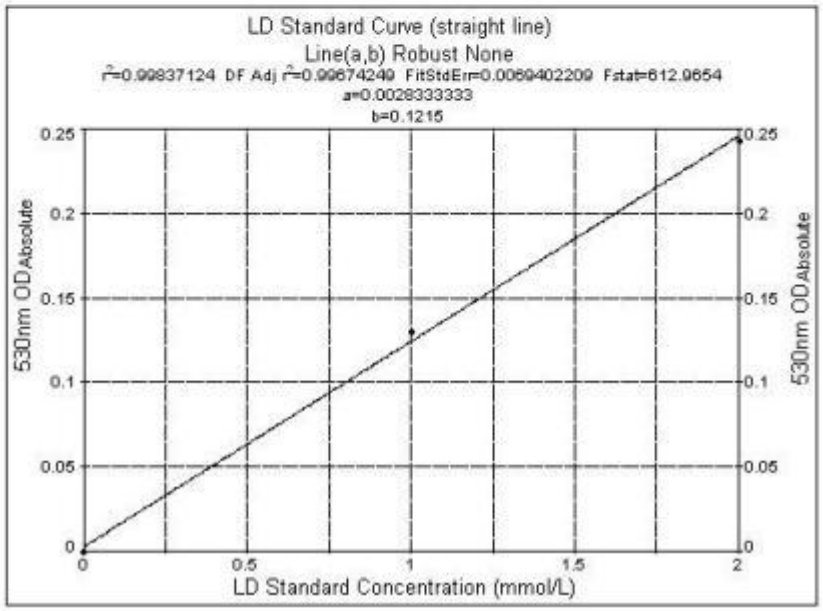
	Blank tube	Standard tube
Distilled water (ml)	0.02	
LD standard solutions of different concentrations (ml)		0.02
Enzyme working solution (ml)	1	1
Chromogenic agent (ml)	0.2	0.2
Mix sufficiently, react in 37℃ water bath for 10 minutes accurately.		
Terminator (ml)	2	2
Mix sufficiently, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 530nm (adjust zero by distilled water).		

Results:

LD standard concentration (mmol/L)	Measured OD value	$OD_{Absolute}$
0	0.078	0.000
1	0.208	0.130
2	0.321	0.243
3	0.418	0.340
4	0.512	0.434
5	0.592	0.514
6	0.667	0.589

Curves:





APPENDIX II: LD Assay in Blood serum (or plasma), Culture fluid

1. Sample pretreatment:

Dilute blood serum (or plasma) or culture fluid with physiological saline to different

concentrations. Do pretest according to operation table in order to determine optimal sampling concentration (follow 3rd point in Annoucements).

2. Operation table:

	Blank tube	Standard tube	Sample tube
Distilled water (ml)	0.02		
3mmol/L standard solution (ml)		0.02	
Sample to assay (ml)			0.02
Enzyme working solution (ml)	1	1	1
Chromogenic agent (ml)	0.2	0.2	0.2
Mix sufficiently, react in 37℃ water bath for 10 minutes.			
Terminator (ml)	2	2	2
Mix sufficiently, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 530nm (adjust zero by distilled water).			

3. Calculation:

(1) Formula:

$$\text{LD content (mmol/L)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (3mmol/L)} \times \text{Sample dilution times before assay}$$

(2) Examples:

① Take 0.02ml blood serum (already diluted with physiological saline at ratio of 1:1) to measure LD content, in results, ODBlank is 0.086, ODStandard is 0.404, ODSample is 0.321, calculate as follows:

$$\text{LD content (mmol/L)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (3mmol/L)} \times \text{Sample dilution times before assay}$$

$$= \frac{0.321 - 0.086}{0.404 - 0.086} \times 3 \times 2 = 4.434 mmol/L$$

② Take 0.02ml culture fluid (already diluted with physiological saline at ratio of 1:4) to measure LD content, in results, ODBlank is 0.086, ODStandard is 0.404, ODSample is 0.207, calculate as follows:

$$\text{LD content (mmol/L)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (3mmol/L)} \times \text{Sample dilution times before assay}$$

$$= \frac{0.207 - 0.086}{0.404 - 0.086} \times 3 \times 5 = 5.707 mmol/L$$

APPENDIX III: LD Assay in Tissue

1. Sample pretreatment:

Prepare 10% tissue homogenate according to Experimental Methodology, then dilute 10% homogenate with homogenate medium to different concentrations for pretests, this is in order to determine optimal sampling concentration (follow 3rd point in Annoucements).

2. Operation table:

	Blank tube	Standard tube	Sample tube
Distilled water (ml)	0.02		
3mmol/L standard solution (ml)		0.02	
Sample to assay (ml)			0.02
Enzyme working solution (ml)	1	1	1
Chromogenic agent (ml)	0.2	0.2	0.2
Mix sufficiently, react in 37℃ water bath for 10 minutes.			
Terminator (ml)	2	2	2
Mix sufficiently, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 530nm (adjust zero by distilled water).			

3. Calculation:

(1) Formula:

$$\text{Tissue LD content (mmol/L)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (3mmol/L)} \div \text{Sample protein concentration (gprot*/L)}$$

Note *: gprot means gram protein.

(2) Examples:

① Take 0.02ml 5% mouse liver tissue homogenate to measure LD content, in results, ODBlank is 0.086, ODStandard is 0.404, ODSample is 0.376, protein concentration in 5% mouse liver tissue homogenate is 7.006gprot/L. calculate as follows:

$$\text{Tissue LD content (mmol/L)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (3mmol/L)} \div \text{Sample protein concentration (gprot/L)}$$

$$= \frac{0.376 - 0.086}{0.404 - 0.086} \times 3 \div 7.006 = 0.391 mmol/gprot$$

② Take 0.02ml 2% swine muscle tissue homogenate to measure LD content, in results, ODBlank is 0.086, ODStandard is 0.404, ODSample is 0.167, protein concentration in 2% swine muscle tissue homogenate is 1.0191gprot/L. calculate as follows:

$$\text{Tissue LD content (mmol/L)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (3mmol/L)} \div \text{Sample protein concentration (gprot/L)}$$

$$= \frac{0.167 - 0.086}{0.404 - 0.086} \times 3 \div 1.0191 = 0.7498 mmol/gprot$$