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Datasheet

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L-Lactic Acid (LA) Colorimetric Assay Kit

Cat# CC1049/CC1050 – 50/100 Assays

Storage at 4℃ for 3 months

APPLICATION

This kit can be used to measure L-Lactic Acid (LA) content in tissue, serum (plasma), cells, culture supernatant samples.

DETECTION SIGNIFICANCE

Lactic acid is an intermediate product of glucose metabolism in the body, which is mainly produced by red blood cells, striated muscle and brain tissue. The concentration of lactic acid in the blood mainly depends on the synthesis speed and metabolic rate of liver and kidney. The bidirectional conversion of lactic acid and pyruvate is regulated by lactate dehydrogenase (LDH).

DETECTION PRINCIPLE

Using NAD+ as H+ receptor, LDH catalyzes the reaction of lactic acid and NAD+ to generate pyruvic acid and NADH respectively. NBT is reduced to a kind of purple compound during the reaction. Measure the OD value at 530 nm, and the concentration of lactic acid can be calculated.

- **Method:** Colorimetric method
- **Specification:** 50/100 Assays
- **Measuring instrument:** Spectrophotometer
- **Sensitivity:** 0.05 Mmol/L
- **Detection range:** 0.05-6.0 mmol/L

KIT COMPONENTS

Item	Component	Specification, CC1049	Specification, CC1050	Storage
Reagent 1	Buffer Solution	60 mL x 1 vial	60 mL x 2 vials	2-8°C, 6 months
Reagent 2	Enzyme Stock Solution	0.6 mL x 1 vial	1.2 mL x 1 vial	2-8°C, 6 months
Preparation of the enzyme working solution: Mix reagent 2 and Reagent 1 at the ratio of 1:100 before use. The working solution should be freshly prepared and it can be stored at 4℃ for 24 hours.				
Reagent 3	Chromogenic Agent	12 mL x 1 vial	24 ml × 1 vial	2-8°C, 6 months
Reagent 4	Stop Solution	60 mL x 2 vials	60 mL x 4 vials	2-8°C, 6 months
Reagent 5	3 mmol/L Lactic Acid Standard	1 mL x 1 vial	2 mL x 1 vial	2-8°C, 6 months

EXPERIMENTAL INSTRUMENT

Test tube, Micropipettor, Vortex mixer, Water bath, Centrifuge, Spectrophotometer (530 nm).

PRETREATMENT OF SAMPL

It is recommended to take 2-3 samples which expected large difference to do pre-experiment before formal experiment.

1. **Serum sample:**

   Fresh blood was collected and placed at 25℃ for 30 min to clot the blood. Centrifuge the sample at 4 ℃ for 15 min at 2000 g, the upper yellowish clear liquid was taken as serum. Place the serum on ice for detection.

2. **Plasma sample:**

   The fresh blood was added into the test tube containing anticoagulant (heparin is recommended) and mixed upside down. Centrifuge the sample at 4℃ for 10 min at 700-1000 g, the upper yellowish transparent liquid was taken as the plasma, and the middle white interference layer (white blood cells and platelets) could not be absorbed. Place the plasma on ice for detection.

3. **Tissue samples:**

   Take 0.02-1 g tissue sample, wash with PBS (0.01 M, pH 7.4) at 2-8℃. Absorb the water with filter paper and weigh. Then add 9 times the volume of PBS according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection.

4. **Cell sample:**

   Collect the cells with cell scraper, add PBS (0.01M, pH7.4) at a ratio of cell number (10^6): PBS (μL) =1: 300-500, then treat the sample with mechanical homogenate or sonication on ice. Centrifuge at 4ºC at 1500 g for 10 min and collect the supernatant for measurement. If not detected on the same day, stored the serum at -80℃, which can be stored for a month.

5. **Culture supernatant samples:**

   Collect the fresh cell culture supernatant and centrifuge the sample at 10000 g for 10 min at 4℃. Take the supernatant and preserve it on ice for detection.

OPERATION STEPS

1. **Blank well:** add 0.02 mL of double distilled water into the 5 mL EP tube.
   Standard well: add 0.02 mL of 3 mmol/L lactic acid standard into the 5 mL EP tube.
   Sample well: add 0.02 mL of sample into the 5 mL EP tube.
2. Add 1.0 mL of enzyme working solution and 0.2 mL of reagent 3 orderly to the tubes.
3. Mix fully and incubate at 37℃ water bath for 10 min exactly.
4. Add 2 mL of reagent 4 to the tubes.
5. Mix thoroughly, set spectrophotometer to zero with double distilled water and measure the absorbance of each tube at 530 nm with 1 cm optical path quartz cuvette.

Note: The following operating table could be as a reference.

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	0.02	-	-
3 mmol/L Standard (mL)	-	0.02	-
Sample (mL)	-	-	0.02
Enzyme working solution (mL)	1.0	1.0	1.0
Reagent 3 (mL)	0.2	0.2	0.2
Mix thoroughly and incubate in 37℃ water bath for 10 min exactly.			
Reagent 4 (mL)	2.0	2.0	2.0
Mix thoroughly, set spectrophotometer to zero with double distilled water and measure the absorbance of each tube at 530 nm with 1 cm optical path quartz cuvette.			

Calculation of results

1. **For serum (plasma), culture supernatant and other liquid sample:**

   LA content (mmol/L) $=\frac{\Delta A_1}{\Delta A_2}$ X c X f

2. **For tissue and cells sample:**

   LA content (mmol/gprot) $=\frac{\Delta A_1}{\Delta A_2}$ X c X f ÷ C_{pr}

Notes

ΔA1: OD_{sample}-OD_{blank}

ΔA2: $OD_{standard}$-OD_{blank}

c: The concentration of standard, 3 mmol/L

f: Dilution factor of sample before test.

Cpr: Concentration of protein in sample, gprot/L

TECHNICAL PARAMETER

1. The sensitivity of the kit is 0.05 mmol/L.
2. The intra-assay CV is 1.1% and the inter-assay CV is 1.9%.
3. The recovery of the kit is 101%.
4. The detection range of the kit is 0.05-6.0 nmol/Ml

Notes

1. This kit is for research use only.
2. Please progress strictly with operation procedures.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.
5. Severe hemolysis or jaundice may raise the OD value.
6. If the LA content is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (E-BC-K318-M).

Appendix: Standard Curve (This is for reference only)

PRETREATMENT

Dilute 10 mmol/L lactic acid standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 6, 5, 4, 3, 2, 1, 0 mmol/L.

OPERATION TABLE

	Standard tube
Standards with different concentrations (mL)	0.02
Enzyme working solution (mL)	1.0
Reagent 3 (mL)	0.2
Mix thoroughly and incubate in 37℃ water bath for 10 min exactly.	
Reagent 4 (mL)	2.0
Mix thoroughly, set spectrophotometer to zero with double distilled water and measure the absorbance of each tube at 530 nm with 1 cm optical path quartz cuvette.	

STANDSRD CURVE