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L-Lactic Acid（L-LA）Content Fluorometric Assay Kit

L-Lactic Acid (L-LA) Content Fluorometric Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Fluorescent microplate reader

Catalog Number: AK1032

Size: 50T/48S ; 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact SunLong staff in time.

Reagent name

50T Size

100T Size

Preservation Condition

Extract Solution I

Liquid 60 mL×1 Liquid 110 mL×1

2-8℃

Extract Solution II

Liquid 10mL×1

Liquid 20 mL×1

2-8℃

Reagent I

Liquid 6 mL×1

Liquid 10 mL×1

2-8℃

Reagent II

Liquid 0.2 mL×1 Liquid 0.3 mL×1

2-8℃

Reagent III

Liquid 0.2 mL×1 Liquid 0.3 mL×1

2-8℃

Reagent IV

Powder×2

Powder×4

2-8℃

Standard

Powder×1

Powder×1

2-8℃

Black 96-well plate (Cat#YA0502)

1 piece
1 piece
-
Sealing film (Cat#YA0249)
2 sheets
2 sheets
-
Solution preparation:
1.
Reagent IV: Before use, add 0.075 mL of DMSO one of the bottle. After dissolving, aliquot according to the sample volume. Store at room temperature in the dark for immediate use, and store the remaining vials at -20℃ in the dark. It can be stored for 12 weeks. Avoid repeated freezing and thawing. (The reagent is a freeze-dried reagent, and there may be a large difference or even a small amount of reagent between different bottles. This phenomenon does not affect the use, and the actual quality is the same.)
2.
Working liquid: Before use, the working liquid is prepared according to the sample size in the ratio of Reagent I: Reagent II: Reagent III: Reagent IV=74μL: 2μL: 2μL: 2μL (80μL, 1T).
3.
Standard: Before use, add 1.04 mL distilled water to prepare 100μmol/mL L-Lactic Acid standard solution for use; store at 2-8°C for 4 weeks..

Product Description：

L-Lactic acid is an important intermediate product in the metabolic process of organisms, closely related to sugar metabolism, lipid metabolism, protein metabolism and intracellular energy metabolism. The content of L-Lactic acid is an important indicator for evaluating glycogen metabolism and aerobic metabolism. L-Lactic acid is converted into pyruvate and H2O2 under the action of lactate oxidase. H2O2 reacts with a fluorescent probe under the action of enzymes to form a strong fluorescent product. The fluorescence intensity at the excitation wavelength of 535nm and the emission wavelength of 587nm is proportional to the content of H2O2. This kit calculates the content of L-Lactic acid in the sample by measuring the amount of H2O2 generated.

Reagents and Equipment Required but Not Provided：

Microplate Reader (Including fluorescence detection function), centrifuge, water bath/constant temperature incubator/metal bath, adjustable pipette, all black 96-well plate (fully black, 1 piece included in the box), sealing film (2 sheets included in the box), DMSO (AR≥98%), normal saline, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Operation procedure

I. Sample preparation (The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)
1.
Tissue sample: According to the proportion of tissue mass (g): Extract solution I (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of Extract solution I and fully homogenized on ice bath. After ice

bath homogenization, centrifuge at 12000g for 10 min at room temperature, take 0.8mL supernatant, then slowly add 0.15mL of extract II, and gently mix until no bubbles are produced.

Centrifuge at room temperature for 10min at 12000g, and then take the supernatant for testing.

2. Cell/bacteria sample: The ratio of bacteria/cell amount (106): normal saline (mL) is 5~10:1(It is suggested to take about 5×106 bacteria/cells and add 1 mL of Extract solution). Disrupt the cells by ultrasonic (power 300w, ultrasonic for 3s, 7s interval, totally 3min), then centrifuge at 12000g for 10 min at room temperature, take the supernatant and put it on ice for testing.

3.

Serum (plasma) sample: Add 100 μL of liquid to 1 mL Extraction solution I, centrifuge at 12000g for 10min at room temperature, take 0.8mL supernatant, then slowly add 0.15mL of extract II, and gently mix until no bubbles are produced. Centrifuge at room temperature for 10min at 12000g, and then take the supernatant for testing.

Note: (1) The extract solution II should be added slowly. After adding, a large number of bubbles will be produced. It is recommended to use a 2mL EP tube for the operation.

(2) If the protein content in the sample is very low and the color is very light, the sample can be directly homogenized with normal saline, centrifuged, and then tested.

(3) For bacterial samples, the ultrasonic time can be appropriately extended.

II. Determination procedure：

1. Preheat Fluorescent microplate reader for 30 min, adjust wavelength to Ex530/ Em587nm.

2. Before the experiment, preheat the working liquid to 37℃.

3. Dilution of the standard: Dilute the 100 μmol/mL (100 000 nmol/mL) standard solution with distilled water to obtain standard solutions of 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 nmol/mL for use.

4. Operation Table: (Add the following reagents to each of the black 96-well plate)

Reagent(μL)

Test tube(T)

Standard tube(S)

Blank tube(B)

Sample

20

-

-

Standard

-

20

-

Distilled water

-

-

20

Working liquid

80
80
80

Mix thoroughly, react at 37℃ in the dark for 10min, measure the fluorescence value at Ex535/ Em587 nm, and record it as RFUT, RFUS, and RFUB. Calculate ΔRFUT = RFUT - RFUB, ΔRFUS = RFUS - RFUB.

(Standard curve and blank tube only need to be prepared 1-2 times). Note: The dilution factor of the samples can reference to the experimental examples.

III. Calculation：

1. Standard curve: Taking the concentration of each standard solution (x, nmol/mL) and its corresponding ΔRFUS (y, ΔRFUS), draw a standard curve, and bring ΔRFUT (y, ΔRFUT) into the equation to get sample concentration (x, nmol/mL).

2. Calculation of L-LA content

(1) Calculate by sample protein concentration

L-LA content (nmol/mg prot) = x×VS÷(VS×Cpr)×F = x÷Cpr×F

(2) Calculate by sample mass

L-LAcontent (nmol/g mass) = x×(VU+VEII)÷(W×VU÷VEI)×F = 1.1875×x÷W×F

(3) Calculate by the number of bacteria or cells

L-LA content (nmol/106 cell) = x×VE÷N×F = x÷N×F

(4) Calculate by liquid volume

L-LA content (nmol/mL) = x×(VU+VEII)÷[VL×VU÷(VEI+VL)]×F = 13.0625×x×F

VS: Sample volume, 0.01mL; W: Sample mass, g; Cpr: Sample protein concentration, mg/mL, the protein concentration needs to be determined by yourself; VU: Volume of the supernatant during extraction, 0.8mL; VEII: Extract solution II volume, 0.15mL; VEI: Extract solution I volume, 1mL; VE: Normal saline volume, 1mL; N: The total number of cells or bacteria, count by 106; VL: Liquid sample volume, 0.1mL; F: Dilution multiples;

Note:

1. If the ΔRFU of the sample is too low, you can appropriately extend the reaction time or increase the sample; if the ΔRFU exceeds the linear range, you can dilute the sample with distilled water for measurement, and remember to simultaneously modify the calculation formula.

2. The extract solution contains a protein precipitant, so the supernatant cannot be used for protein concentration measurement. If you need to determine the protein content, you need to take another sample.

3. The fluorescence values (RFU) obtained by different microplate readers may vary, which is a normal phenomenon. As long as the reagent preparation and experimental operation are correct, the standard curve is linear, and the detection results are accurate and effective.

Experimental example:

1. Take 100μmol/mL standard solution, dilute to 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125nmol/mL. Measure the fluorescence values of the blank tube and standard tube, and obtain RFUB = 825.3, establish a standard curve based on the fluorescence values of each standard tube, y= 210.75x - 13.14，R2=0.9988.

2. The test results for different types of samples are as follows:

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Sample
Mass/Volume/Cell count
Dilution multiples
RFUT
ΔRFUT
L-LA content
Unit
Rat heart
0.1032g
320
3498.6
2673.3
46936.8 nmol/g mass
Rat muscle
0.1024g
320
3562.5
2737.2
48428.7 nmol/g mass
Rat plasma
0.1mL
40
4268.0
3442.7
8567.9 nmol/mL
HepG2 cell
10×106
8
5621.0
4795.7
18.3 nmol/106 cell