L-Lactic Acid Content Assay Kit (WST colorimetry)

Note: The reagents of this product have been changed, please pay attention to and strictly follow the instructions.

Operation Equipment: Spectrophotometer/ Microplate reader

Catalog Number: BC5345

Size: 100T/48S

Components:

Extraction reagent I: Liquid 60 mL×1. Store at 2-8℃.

Extraction reagent II: Liquid 10 mL×1. Store at 2-8℃

Reagent I: Liquid 6 mL×1. Store at 2-8℃.

Reagent II: Liquid 20 μL×1. Store at 2-8℃. Before use, prepare the Reagent II in the ratio of Reagent II (V): distilled water (V) = 10 μL: 450 μL (46T). Prepare when the solution will be used.

Reagent III: Powder ×1. Store at -20℃, Dissolve with 3mL distilled water before use. After packaging, store at -20℃ to avoid repeated freezing-thawing. Store at -20℃ for 4 weeks.

Reagent IV: Liquid 4 mL×1. Store at 2-8℃.

Standard goods: Powder ×1. Store at 2-8℃. Before use, 1.04 mL distilled water was added to form 100 μmol/mL standard solution. Standards can be stored at 2-8℃ for 12 weeks.

Product Description

Lactic acid is an important intermediate product in the process of biological metabolism, which is closely related to glucose metabolism, lipid metabolism, protein metabolism and intracellular energy metabolism. Lactic acid content is an important index to evaluate glycogen metabolism and aerobic metabolism. Under the action of Lactate dehydrogenase, pyruvate is generated from Lactic acid, and NAD^+ is reduced to produce NADH and H^+. Under the action of 1-mPMS, WST-1 reacts with NADH to produce water-soluble formazan, which has the maximum absorption peak at 450nm, based on which the content of Lactic acid can be calculated.

Reagents and Equipment Required but Not Provided.

Spectrophotometer/Microplate reader, mortar/homogenizer/cell ultrasonic crusher, centrifuge, constant temperature foster box/water-bath, centrifuge, micro quartz cuvette/96 well flat-bottom plate, ice and distilled water.

Procedure:

I. Sample preparation

1. **Tissue:** According to the proportion of tissue weight (g): Extraction reagent I volume (mL) of 1:5-10 to extract. it is suggested that 0.1 g of tissue with 1 mL of Extraction reagent I and fully homogenized on ice bath. Centrifuge at 12000 g for 10 minutes at 4℃. Take 0.8mL of supernatant, and slowly add

0.15mL of Extraction reagent II, slowly blow and mix until no bubbles occur, centrifuge at 4℃

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at 12000g for 10 minutes, take the supernatant on ice before testing.

2. **Cells:** Collect cells into the centrifuge tube, discard supernatant after centrifugation. According to the proportion of cells number (10^4): Extraction reagent I volume (mL) of 500-1000:1 to extract. It is suggested that 5 million of cell amount with 1 mL of Extraction reagent I. Split the cell with ultrasonication (placed on ice, ultrasonic power 300W, working time 3s, interval 7s, Total times:3 minutes). Centrifuge at 12000 g for 10 minutes at 4℃. Take 0.8mL of supernatant, and slowly add 0.15mL of Extraction reagent II, slowly blow and mix until no bubbles occur, centrifuge at 4℃ at 12000g for 10 minutes, take the supernatant on ice before testing.

3. **Serum (plasma) or other liquids sample:** Take 100μL liquid sample and add 1mL Extraction reagent I, centrifuge at 4℃ at 12000g for 10min, Take 0.8mL of supernatant, and slowly add 0.15mL of Extraction reagent II, slowly blow and mix until no bubbles occur, centrifuge at 4℃ at 12000g for 10 minutes, take the supernatant on ice before testing.

Note: Extraction reagent II should be added slowly, after addition will produce a lot of bubbles, it is recommended to use 2mL EP tube for operation.

II. Determination

1. Preheat the spectrophotometer or microplate reader for more than 30 minutes, adjust the wavelength to 450 nm and set spectrophotometer counter to zero with distilled water.

2. Dilution of standard solution: Dilute 100μmol/mL standard solution with distilled water to 1.25, 0.625, 0.3125, 0.15625, 0.078, 0.039, 0.020μmol/mL standard solution for reserve.

3. Standard dilution table:

Number	Concentration before dilution (μmol/mL)	Standard liquid volume (μL)	Distilled water volume (μL)	Diluted concentration (μmol/mL)
1	100	50	950	5
2	5	250	750	1.25
3	1.25	500	500	0.625
4	0.625	200	200	0.3125
5	0.3125	200	200	0.15625
6	0.15625	200	200	0.078
7	0.078	200	200	0.039
8	0.039	200	200	0.020

Each standard tube required 10μL of standard solution. (Be careful not to test absorbance directly in this step).

4.Sample Test (add Reagent in the 96 well flat-bottom plate or EP tubes):

Reagent（μL）	Test tube (T)	Control tube(C)	Standard tube (S)	Blank tube (B)

Sample	10	10	-	-
Standard	-	-	10	-
distilled water	-	10	-	10
Reagent I	40	40	40	40
Reagent II	10	-	10	10
Reagent III	20	20	20	20
Reagent IV	30	30	30	30
Thoroughly mix and react in 37°C water bath/constant temperature incubator for 30min without light				
distilled water	90	90	90	90

Mix thoroughly, read the absorbance of wavelength at 450 nm. Absorption values of blank tube, standard tube, test tube and control tube as A_B, A_S, A_T and Ac respectively. Calculation $\Delta A_T = A_T -$ Ac; $\Delta A_S = A_S - A_B$. The standard tube and blank tube only need to be measured 1-2 times.

III. Calculation of Lactic acid content:

1. Drawing of standard curves:

Taking the concentration of each standard solution as the X-axis and the corresponding absorption value (ΔA_S) as the Y-axis, the standard curve was drawn to obtain the standard equation y=kx+b, and x (μmol/mL) was obtained by inserting the ΔA_T into the formula.

2. Lactic acid calculation:

1) Protein concentration:

LA content（μmol/mg prot）= x ×V_S÷(V_S×Cpr)=x ÷ Cpr

2) Sample weight:

LA content（μmol/g weight）= x ×(V_{SV}+V_{EVII})÷(W×V_{SV}÷V_{EVI})=1.1875×x÷W

3) Cell amount:

LA content（μmol/10⁴ cell）= x ×(V_{SV}+V_{EVII})÷(N×V_{SV}÷V_{EVI})=1.1875×x÷N

4) Volume of liquid:

LA content（μmol/mL）= x ×(V_{SV}+V_{EVII})÷[V_{LS}×V_{SV}÷(V_{EVI}+ V_{LS})]=13.0625×x

V_S: Sample volume,0.01 mL;

W: Sample mass, g;

Cpr: Sample protein concentration, mg/mL. The protein concentration should be determined by yourself;

V_{SV}: supernatant volume,0.8 mL;

V_{EVI}: Extraction volume I, 1 mL;

V_{EVII}: Extraction volume II, 0.15 mL;

N: The number of bacteria/cells, In tens of thousands;

V_{LS}: Liquid sample volume,0.1 mL.

Note:

1. The ΔA_T is between 0.01 and 1.1. If the absorbance value exceeds the linear range, the sample can be diluted with distilled water and then measured again; if the absorbance value is less than the linear range, the sample size needs to be increased and then measured again. Pay attention to the synchronous calculation formula.

2. Extraction reagent I contains protein precipitator, so the supernatant cannot be used for protein concentration determination. For determination of protein content, separate tissue should be taken.

Experimental examples:

1. Take 0.1466g of mouse liver was added with 1mL Extraction reagent I, homogenized in ice bath, centrifuged, 0.8mL supernatant was added with 0.15mL Extraction reagent II. Take supernatant by centrifuge and dilute it twice with distilled water. Follow the determination procedure. After determination with 96-well plate, calculate $\Delta A_T = A_T - A_C = 0.573 - 0.091 = 0.482$. According to the standard curve $y = 0.8941x + 0.0016$, $R^2 = 0.9991$, calculate $x = 0.5373$, To calculate:

   LA content（μmol/g weight）= $1.1875 \times x \div W \times$ Dilution ratio = 8.7046μmol/g weight.

2. Take 0.1063g of sweet potato root was added with 1mL Extraction reagent I, homogenized in ice bath, centrifuged, 0.8mL supernatant was added with 0.15mL Extraction reagent II, centrifuge supernatant, and then follow the measurement procedure. After determination with 96-well plate, calculate $\Delta A_T = A_T - A_C = 0.128 - 0.105 = 0.023$. According to the standard curve $y = 0.8941x + 0.0016$, $R^2 = 0.9991$, calculate $x = 0.0239$, To calculate:

   LA content（μmol/g weight）= $1.1875 \times x \div W$ = 0.2674μmol/g weight.

3. Take 100μL sheep serum and add 1mL extract solution I, centrifuge it, take 0.8mL supernatant and add 0.15mL extract solution II, centrifuge supernatant, and then follow the measurement procedure. After determination with 96-well plate, calculate $\Delta A_T = A_T - A_C = 0.424 - 0.104 = 0.320$. According to the standard curve $y = 0.8941x + 0.0016$, $R^2 = 0.9991$, calculate $x = 0.3561$, To calculate:

   LA content（μmol/mL）= $13.0625 \times x = 13.0625 \times 0.3561$ = 4.6517μmol/mL.

Reference literature:

Eolbergrová J, MacMillan V, Siesjö B K. The effect of moderate and marked hypercapnia upon the energy state and upon the cytoplasmic NADH/NAD+ ratio of the rat brain[J]. Journal of neurochemistry, 1972, 19(11): 2497-2505.

Related Products：

BC0740/BC0745	Hexokinase (HK) Activity Assay Kit
BC0540/BC0545	Pyruvate Kinase（PK）Assay Kit
BC0530/BC0535	Phosphofructokinase (PFK) Assay Kit