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

Note: The reagents have been changed, so please be aware of and follow this instruction strictly.

Operation Equipment: Spectrophotometer

Cat No: BC2230

Size: 50T/24S

Components:

Extract solution I: Liquid 30 mL×1 bottle, store at 2-8℃;

Extract solution II: Liquid 5 mL×1 bottle, store at 2-8℃;

Reagent I: Liquid 20 mL×1 bottle, store at 2-8℃;

Reagent II: Liquid 60 μL×1 bottle, store at 2-8℃. Accordance ratio Reagent II: Distilled water=10 μL: 450 μL. Prepare when it will be used.

Reagent III: Liquid 24 mL×1 bottle, store at 2-8℃;

Reagent IV: Powder×1 bottle, store at -20℃; Before use, add 8 mL distilled water to each bottle and mix well. After packaging, store at -20℃ for 4 weeks to avoid repeated freeze-thaw.

Reagent V: Liquid 5 mL×1 bottle, store at 2-8℃;

Standard: Powder×1, store at 2-8℃; Add 1.04 mL distilled water to 100 μmol/mL before use. It can be stored at 2-8°C for 4 weeks.

Description:

L-Lactic acid is an important intermediate product in biological metabolism, which is closely related to sugar metabolism, lipid metabolism, protein metabolism and intracellular energy metabolism. Lactic content is an important indicator for assessing carbohydrate metabolism and aerobic metabolism. Lactic produces pyruvic acid under the action of lactate dehydrogenase, and NAD^+ is reduced to produce NADH and H^+. H^+ is transferred to PMS to produces $PMSH_2$ and $PMSH_2$ reduce MTT to form purple substance which has a characteristic absorption peak at 570 nm.

Required but not provided:

Scale, mortar/homogenizer, centrifuge, spectrophotometer, 1 mL glass cuvette, constant temperature water bath, ethanol, distilled water.

Procedure:

I. Sample Preparation.

1. Tissue:

Accordance ratio weight(g): Extract solution I(mL)=1: 5~10. (Suggested 0.1g tissue with 1mL Extract solution I). Homogenate on ice bath. 12000 g centrifuge for 10 min at 4℃. Add 0.15mL Extract solution II slowly to 0.8 mL supernatant. **Blend slowly until no bubbles.** Then 12000 g centrifuge for 10 min at 4℃. Supernatant is for test.

2. Cells:

Accordance ratio cell amount (10^6): Extract solution I(mL)=5~10:1. (Suggested 5 million cells with 1mL Extract solution I). Breaking cells (300W, work time 3s, interval 7s for 3 min) by ultrasonic on ice bath. 12000 g centrifuge for 10 min at 4℃. Add 0.15 mL Extract solution II to 0.8mL supernatant slowly. **Blend slowly until no bubbles**. 12000 g centrifuge for 10 min at 4℃. Supernatant is used for test.

3. Serum (plasma) **sample:**

Add 1 mL Extract solution I to 100 μL serum(plasma). 12000 g centrifuge for 10 min at 4℃. Add 0.15 mL Extract solution II to 0.8 mL supernatant slowly. **Blend slowly until no bubbles.** Centrifuge for 10 min at 12000 g. Supernatant is used for test.

II. Determination procedure.

1. Preheat spectrophotometer for 30 min, adjust wavelength to 570 nm, set zero with ethanol.
2. Standard working solution: 100 μmol/mL standard was diluted with distilled water to be 1、0.625、0.3125、0.15625、0.078、0.039μmol/mL for test.
3. Add reagents according to the following table.

Reagent Name（μL）	Test tube(T)	Control tube(C)	Standard tube(S)	Blank tube(B)
Sample	50	50	-	-
Standard			50	
Distilled water	-	50	-	50
Reagent I	200	200	200	200
Reagent II	50	-	50	50
Reagent IV	100	100	100	100
Mix thoroughly in centrifuge tube, react 20 min at 37℃ water bath.				
Reagent V	30	30	30	30
Reagent III	300	300	300	300
Mix thoroughly in centrifuge tube. Avoiding light react 20 min at 37℃, 10000 rpm centrifuge for 10 min at 25℃. Remove supernatant and retain sediment.				
Ethanol(μL)	1000	1000	1000	1000
Fully dissolved sediments, and determine absorbance at 570 nm, record A_T, A_C, As, A_B, calculate $\Delta A_T=A_T-A_C$. $\Delta As=As-A_B$.				

III. Calculation.

1. Drawing of standard curve.

Standard solution concentration as x axis and its corresponding absorption value (ΔAs) as y axis, the standard equation is y=kx+b. Bring ΔA_T into the formula to get x (μmol/mL).

2. **Calculation of Lactate content.**

A. Protein concentration:

L-LA(μmol/mg prot)= x×Vs÷(Vs×Cpr)= x÷Cpr.

B. Sample weight

L-LA(μmol/g weight)= x×(Vsp+V_{II})÷(W×Vsp÷V_I)= 1.1875×x÷W.

C. Cell amount

L-LA(μmol/10^6 cell)= x×(Vsp+V_{II})÷(N×Vsp÷V_I)= 1.1875×x÷N.

D. Liquid volume

L-LA(μmol/mL)= x×(Vsp+V_{II})÷ [V_L×Vsp÷(V_I+V_L)]= 13.0625×x.

Vs: Sample volume, 0.05 mL

W: Sample weight, g

Cpr: Sample protein concentration, mg/mL(Protein concentration needs to be Self- determined)

Vsp: Supernatant volume, 0.8 mL

V_{II}: Extract solution II, 0.15 mL

V_I: Extract solution I, 1 mL

N: Number of cells, in millions.

V_L: Liquid sample volume, 0.1 mL

Note:

1.If the absorbance value exceeding 1.2 or ΔA>0.8, please dilute the sample volume properly and multiply the dilution multiple in the formula.

2. Extract I contains a protein precipitant and therefore the supernatant cannot be used for protein concentration determination. If protein content is to be determined, a separate sample is required.

Experimental example:

1. Take 0.1g of rabbit heart, add 1 mL of Extract solution I, grind and centrifuge, take 0.8 mL of supernatant and add 0.15 ml of Extract solution II, centrifuge supernatant and dilute 5 times, then operate according to the determination steps, use 96 well plate to measure and calculate $\Delta A_T=A_T-A_C$= 1.137-0.125=1.012, according to the standard curve y =0.7826x+0.0215, x =1.266, calculate the content according to the sample mass

L-LA content (μmol/g mass) = 1.1875×x ÷ W × dilution ratio =1.1875×1.266÷0.1×5=75.17 μmol/g mass.

2. Take 100 μL of mouse serum, add 1 mL of Extract solution I, take 0.8 mL of supernatant and then add 0.15 mL of Extract solution II, centrifugate the supernatant, and then operate according to the determination steps, use 96 well plate to measure and calculate $\Delta A_T=A_T-A_C$=1.152-0.407=0.745, according to the standard curve y=0.7826x+0.0215, x=0.924, calculate the content according to the liquid volume

L-LA content (μmol/mL) = 13.0625 × x = 13.0625×0.924=12.07 μmol/mL.

Recent Product Citations：

[1] Meixi Peng,Dan Yang,Yixuan Hou,et al.Intracellular citrate accumulation by oxidized ATM-mediated metabolism reprogramming via PFKP and CS enhances hypoxic breast cancer cell invasion

and metastasis. Cell Death and Disease. March 2019;(IF5.959)

[2] Xiaojin Luo,Weihua Shi，Haoming Yu,et al. Wearable Carbon Nanotube-Based BioSensors on Gloves for Lactate. Sensors. October 2018;(IF3.031)

[3] Zhou F, Du J, Wang J. Albendazole inhibits HIF-1α-dependent glycolysis and VEGF expression in non-small cell lung cancer cells[J]. Molecular and cellular biochemistry, 2017, 428(1-2): 171-178.

References：

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) Activity Assay Kit
BC0530/BC0535 Phosphofructokinase(PFK) Activity Assay Kit

Technical Specifications:

The detection limit:0.0387 μmol/mL
The linear range: 0.039-1 μmol/mL