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Lactic Acid(LA) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/ Microplate reader

Cat No: AK0395

Size: 100T/48S

Components:

Extract solution I: 50 mL×1 bottle, store at 4℃;

Extract solution II: 8 mL×1 bottle, store at 4℃;

Reagent I: 6 mL×1 bottle, store at 4℃;

Reagent II: 34 μL×1 bottle, store at 4℃. Working solution: Accordance ratio Reagent II: Distilled water=10 μL: 450 μL. Prepare when it will be used.

Reagent III: Powder×1 bottle, store at -20℃ and protect from light; Working solution: add 4 mL distilled water before use. Mix thoroughly. It can be stored for one week at -20℃. Avoid repeated freezing and thawing.

Reagent IV: Powder×1 bottle, store at 4℃ and protect from light; Working solution: add 4 mL distilled water before use. Mix thoroughly. It can be stored for one week at 4℃.

Reagent V: Powder×1 bottle, store at -20℃ and protect from light; Working solution: add 3 mL distilled water before use. Mix thoroughly. Store at -20℃ after spacing out. Avoid repeated freezing and thawing.

Reagent Ⅵ: Liquid 2 mL×1 bottle, store at 4℃;

Standard: Powder×1 bottle, store at 4℃; Standard solution: Add 1.04 mL distilled water to 100 μmol/mL before use.

Prepare Chromogenic Solution: Accordance ratio Reagent III(V): Reagent IV (V)=1:1, mix thoroughly, Prepare when it will be used.

Description:

L (+)-Lactic acid is an important intermediate product in biological metabolism. It is closely related to glucose metabolism, lipid metabolism, protein metabolism and intracellular energy metabolism. Lactic content is an important indicator for assessing carbohydrate metabolism and aerobic metabolism. L (+)-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 PMSH2 and PMSH2 reduce MTT to form purple substance, and has a characteristic absorption peak at 570 nm.

Required but not provided:

Scale, mortar/homogenizer, centrifuge, spectrophotometer/microplate reader, micro glass cuvette/ 96 well flat-bottom plate, 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 to 0.8 mL supernatant. 12000 g centrifuge for 10 min at 4℃ . Supernatant is for test.

2. Cells:

Accordance ratio cell amount (10^4): Extract solution I(mL)=500~1000:1. (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. 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. Centrifuge for 10 min at 12000 g. Supernatant is used for test.

II. Determination procedure.

1. Preheat spectrophotometer/microplate reader for 30 min, adjust wavelength to 570 nm, set zero with ethanol.

2. Standard working solution: 100μmol/mL standard was diluted with distilled water to be 2, 1, 0.5, 0.25, 0. 125, 0.0625μmol/mL for test.

3. Add reagents according to the following table.

	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube (B)
Sample(μL)	10	10	-	-
Standard(μL)			10	-
Distilled water(μL)	-	10	-	10
Reagent I(μL)	40	40	40	40
Reagent II(μL)	10	-	10	10
Reagent V(μL)	20	20	20	20
Mix thoroughly in centrifuge tube, react 20 min at 37℃ water bath.				
Reagent Ⅵ	6	6	6	6
Chromogenic Solution(μL)	60	60	60	60
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)	200	200	200	200

Fully dissolved sediments, and determine absorbance at 570 nm, record A_T, A_C, As, A_B, $\Delta A_T = A_T - A_C$. $\Delta As = As - A_B$.

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:

LA (μmol/mg prot)= $x \times Vs \div (Vs \times Cpr)$ = $x \div Cpr$.

B. Sample weight

LA (μmol/g weight)= $x \times (Vsp+V_{II}) \div (W \times Vsp \div V_I)$= $1.1875 \times x \div W$.

C. Cell amount

LA (μmol/10^6 cell)= $x \times (Vsp+V_{II}) \div (5 \times Vsp \div V_I)$= $0.2375 \times x$.

D. Liquid volume

LA (μmol/mL)= $x \times (Vsp+V_{II}) \div [V_L \times Vsp \div (V_I+V_L)]$= $13.0625 \times x$

Vs: Sample volume, 0.01 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

Cells amount: 5 million

V_L : Liquid sample volume, 0.1 mL

Note:

If the absorbance value exceeding 1.5 or $\Delta A>1.2$, please dilute the sample volume properly and multiply the dilution multiple in the formula.

Experimental example:

1. Take 0.1g of rabbit heart, add 1 mL of Extract solution 1, grind and centrifuge, take 0.8 mL of supernatant and add 0.15 ml of Extract solution 2, centrifuge supernatant and dilute 5 times, then operate according to the determination steps, use 96 well plate to measure and calculate $\Delta AT=AT-AC$= 0.591-0.069 = 0.522, according to the standard curve y = 0.412x-0.0214, x =1.319, calculate the content according to the sample mass.

La content (μmol/g mass) = $1.1875 \times x \div W \times$ dilution ratio =$1.1875 \times 1.319 \div 0.1 \times 5$ = 78.32 μmol/g mass.

2. Take 100 μL of mouse serum, add 1 mL of Extract solution 1, take 0.8 mL of supernatant and then add 0.15 mL of Extract solution 2, centrifugate the supernatant, and then operate according to the determination steps, use 96 well plate to measure and calculate $\Delta A_T=A_T-A_C$=0.572-0.211 =0.361, according to the standard curve y = 0.412x-0.0214, x =0.928, calculate the content according to the liquid volume

La content (μmol/mL) = $13.0625 \times x$ = 13.0625×0.928 = 12.122 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

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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：

AK0516/AK0515   Hexokinase(HK) Activity Assay Kit

AK0540/AK0539   Pyruvate Kinase(PK) Activity Assay Kit

AK0542/AK0541   Phosphofructokinase(PFK) Activity Assay Kit

Technical Specifications:

The detection limit:0.0771 μmol/mL

The linear range: 0.078-5 μmol/mL