L-Lactate Content Assay Kit

Cat No. LAC-M-100
Size: 100 Reactions
Store at 2-8 °C

MOLEQULE-ON®

Ingredients	100 Tests
Extract Solution I	30 mL
Extract Solution II	5 mL
Reagent I	20 mL
Reagent II	60 mL
Reagent III	24 mL
Reagent IV	Powder
Reagent V	5 mL
Standard	Powder

Solution Preparation

1. Reagent II: Before use, throw the liquid off the bottom of the tube (use a handheld centrifuge); Reagent II is diluted according to the ratio of Reagent II (V): distilled water (V) =10μL: 450μL.
2. Reagent IV: Before use, add 8 mL distilled water and mix well. After reconstitution, store at -20℃ to avoid repeated freezing and thawing. Store at -20℃ for 4 weeks.
3. Standard: Before use, 1.04 mL distilled water was added to prepare 100 μmol/mL standard. Store at 2-8℃ for 4 weeks.

Description

L-Lactic acid plays a crucial role as an intermediate product in biological metabolism, particularly in relation to sugar, lipid, protein, and intracellular energy metabolism. The level of lactic acid is a key indicator for evaluating carbohydrate and aerobic metabolism. Lactic acid is converted to pyruvic acid by lactate dehydrogenase, leading to the reduction of NAD^+ to produce NADH and H^+. The H^+ ions are then transferred to PMS to form PMSH2, which in turn reduces MTT to create a purple substance with a distinct absorption peak at 570 nm.

Reagents and Equipment Required but Not Provided:

Balance, mortar/homogenizer, centrifuge, spectrophotometer, 1 mL glass cuvette, constant temperature water bath, ethanol (>98%, AR), distilled water.

Protocol:

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

Ratio of tissue weight to extraction solution volume should be 1:5~10 (recommended 0.1g tissue with 1mL of extraction solution I). Homogenize on an ice bath, then centrifuge at 12000 g for 10 minutes at 4℃. Slowly add 0.15mL of extraction solution II to 0.8mL of the supernatant. **Gently mix until no bubbles are visible**. Centrifuge again at 12000 g for 10 minutes at 4℃. The resulting supernatant is ready for testing.

2. Cells

Cell lysis was performed using an accordance ratio of 5 to 10:1 of Extract solution I to cell amount (10^6 cells). This equated to using 5 million cells with 1mL of Extract solution I. The cells were broken using ultrasonic treatment on an ice bath (300W, work time 3s, interval 7s for 3 min). The lysate was then centrifuged at 12000 g for 10 min at 4℃. Following centrifugation, 0.15 mL of Extract solution II was added slowly to 0.8mL of the supernatant. The mixture was blended gently until no bubbles were visible. The sample was then centrifuged again at 12000 g for 10 min at 4℃. The resulting supernatant was used for further testing.

3. Serum (plasma) sample:

Add 1 mL of Extract solution I to 100 μL of serum (plasma). Centrifuge at 12000 g for 10 minutes at 4℃. Slowly add 0.15 mL of Extract solution II to 0.8 mL of the supernatant. Gently mix until no bubbles are visible. Centrifuge again at 12000 g for 10 minutes. The resulting supernatant is ready for testing.

Please note that when adding Extract solution II, bubbles will form, so it is important to add it slowly. It is recommended to use a 2mL microcentrifuge tube for this procedure.

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 (After dilution)	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 $\Delta A_T=A_T-A_C$. $\Delta As=As-A_B$. (The blank tube and standard curve only need to be measured 1-2 times)				

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 ΔAT 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+VII)÷(W×Vsp÷VI)= 1.1875×x÷W.

C. Cell amount
L-LA(μmol/106 cell)= x×(Vsp+VII)÷(N×Vsp÷VI)= 1.1875×x÷N.

D. Liquid volume
L-LA(μmol/mL)= x×(Vsp+VII)÷ [VL×Vsp÷(VI+VL)]= 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
VII: Extract solution II, 0.15 mL
VI: Extract solution I, 1 mL
N: Number of cells, in millions.
VL: Liquid sample volume, 0.1 mL

Note:

1. If the measured light absorption value exceeds the linear range, you can increase the sample size or dilute the sample before testing.
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 ΔAT=AT-AC= 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 ΔAT=AT-AC=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.