L-Lactate Dehydrogenase (L-LDH) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: AK0947-100T-48S

Size:100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle.

Reagent Name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Reagent I	Liquid 7 mL×1	2-8℃
Reagent II	Powder ×1	-20℃
Reagent III	Liquid 7 mL×1	2-8℃
Reagent IV	Liquid 25 mL×1	2-8℃
Standard	Liquid 1 mL×1	2-8℃

Solution Preparation:

1. **Reagent II:** powder ×1 bottle, add 1.3 mL of distilled water before use. It can be divided into tubule after matching, the unused reagent can be stored at -20℃ for 2 weeks, avoid repeated freezing and thawing.
2. **Standard:** liquid ×1 bottle, 20 μmol/mL Sodium pyruvate.

Product Description:

L-Lactate dehydrogenase (L-LDH or LD) is the terminal enzyme of the glycolysis pathway which is found in nearly all living cells (animals, plants, and prokaryotes). L-LDH catalyzes the conversion of lactate to pyruvic acid and back, as it converts NAD^+ to NADH and back.

NAD^+ and lactic acid are oxidized to pyruvic acid by the catalysis of L-LDH. Pyruvate further reacted with 2,4-dinitrophenylhydrazide to form pyruvate dinitrobenzone, which show brown red color in alkaline solution and the color depth is proportional to the concentration of pyruvate.

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorption value of the sample is not within the measurement range, it is recommended to dilute or increase the sample size for detection

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, thermostat water bath, desk centrifuge, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer/cell ultrasonic crusher, ice, 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. Bacteria、cells or tissue sample:

Bacteria or cells:

Collecting bacteria or cells into the centrifuge tube. The liquid in the upper layer is discarded after centrifugation. The ratio of bacteria/cell amount (10^4): Extract solution volume (mL) is 500~1000:1(it is suggested to take about 5 million bacteria/cell and add 1 mL of Extract solution). Bacteria and cell is split by ultrasonic (placed on ice, 200W, work time 3s, interval 10s， repeat for 30 times). Centrifuge at 8000 rpm 4℃ for 10 minutes, take the supernatant and put it on ice for test.

Tissue:

Ice-bath homogenate is conducted according to the ratio of tissue mass (g): Extract solution volume (mL) = 1: 5~10 (it is suggested to take about 0.1 g of tissue and add 1 mL of Extract solution). Ice bath homogenization. Centrifuge at 8000 rpm and 4℃ for 10 minutes, take the supernatant and put it on ice for test.

2. Serum (plasma) sample:

Detect sample directly.

II. Determination procedure.

1. Preheat the Spectrophotometer/Microplate reader 30 minutes, adjust wavelength to 450 nm, set zero with distilled water.
2. 20 μmol/mL standard solution is respectively diluted to 2, 1, 0.5, 0.25, 0.125 and 0 mmol/mL with distilled water and use 2, 1, 0.5, 0.25, 0.125, 0 μmol/mL as standard curve.
3. Sample Test

Reagent name (μL)	Test tube(At)	Control tube(Ac)	Standard tube(As)
Sample	10	10	-
Standard Solution	-	-	10
Reagent I	50	50	50
Reagent II	10	-	-
Distilled water	-	10	10
Mixed thoroughly, incubate at 37℃(mammal) or 25℃(other species) water bath for 15 minutes.			
Reagent III	50	50	50
Mixed thoroughly, incubate at 37℃(mammal) or 25℃(other species) water bath for 15 minutes.			
Reagent IV	150	150	150

Mixed thoroughly, place at room temperature for 3 minutes. Take 200 μL of reaction solution in micro glass cuvette/96 well flat-bottom plate, measured the absorbance at 450 nm, $\Delta A=A_T-A_C$. Each test tube should set a control tube.

III. L-LDH Calculations

1. Set the standard curve, x-axis as the standard concentration, μmol/mL, y-axis as the 450 nm absorption. Put ΔA(y) into standard curve, calculate x (μmol/mL)

2. Sample Sodium pyruvate content

Put ΔA(y) into standard curve, calculate x (μmol/mL)

3. Serum (plasma) sample L-LDH activity

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the producing of 1 nmol of pyruvic acid per minute every milliliter of serum.

L-LDH(U/mL)=x×Vs÷Vs÷T×10^3=66.7×x

4. Tissue, bacteria or cultured cells L-LDH activity

A. Calculate by sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the producing of 1 nmol of pyruvic acid per minute every milligram of protein.

L-LDH(U/mg prot)= x×Vs÷（Cpr×Vs）÷T×10^3=66.7×x÷Cpr

B. Calculate by sample mass

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the producing of 1 nmol of pyruvic acid per minute every gram of tissue.

L-LDH(U/g mass)= x×Vs÷（W÷Vsv×Vs）÷T×10^3=66.7×x÷W

C. Calculate by the number of bacteria or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the producing of 1 nmol of pyruvic acid per minute every 1 0000 cells.

L-LDH (U/10^4 cell)= x×Vs÷（N÷Vsv×Vs）÷T×10^3=66.7×x÷N

Vs: Supernate volume (mL),10μL = 0.01 mL;

Vsv: Extract solution volume, 1 mL;

T: Reaction time, 15 minutes;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g

N: Total number of bacteria or cells, million;

10^3: 1 μmol/mL=10^3 nmol/mL.

Note: When ΔA is greater than 1.3 or less than 0.01, it is recommended to dilute the sample with distilled water or increase the sample size for the experiment, and pay attention to the simultaneous modification of the calculation formula.

Experimental example:

1. Take 0.103g of seduma leaves, add 1mL of extract solution, make ice bath homogenate, 8000g, centrifuge at 4℃ for 10min, take superclear ice to be measured. Then, according to the measurement procedure, the 96-well plate was used to calculate ΔA=A measuring tube -A control =0.275-0.199=0.076,

and the standard curve y=0.5218x+0.0063, R^2=0.9983, x=0.134 μmol/mL, and the lactate dehydrogenase activity was calculated to obtain:

L-LDH (U/g mass) =66.67 ×x÷W =86.74 U/g

2. Take 0.109 g of rabbit liver, add 1mL of extract liquid, homogenize in ice bath, 8000g, centrifuge at 4℃ for 10min, take supernatant and dilute 80 times with distilled water, then place on ice to be measured. Then, according to the measurement procedure, the 96-well plate was used to calculate ΔA=A measuring tube -A control = 0.7955-0.132 =0.663, and the standard curve y=0.5218x+0.0063, R^2=0.9983, x=1.259 μmol/mL, and the lactate dehydrogenase activity was calculated to obtain:

L-LDH (U/g mass) =66.67 ×x÷W × dilution ratio =61605.53 U/g

3. After taking 10μL equine serum, follow the measurement procedure, using A 96-well plate to calculate ΔA=A measuring tube -A control =0.415-0.161=0.254, and adding the standard curve y=0.5218x+0.0063, R^2=0.9983, x=0.475 μmol/mL, and calculate the lactate dehydrogenase activity to obtain:

L-LDH (U/mL) =66.67 x x =31.67 U/mL