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

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: NA0158

Size:100T/48S

Components:

Extract solution: Liquid 60 mL×1. Store at 2-8℃;

Reagent I: Liquid 7 mL×1. Store at 2-8℃.

Reagent II: Powder×1. Store at -20℃. Dissolve with 0.8 mL of distilled water before use. It could be stored at -20℃ for four weeks after dispensing to avoid repeated freezing and thawing

Reagent III: Liquid 7 mL×1. Store at 2-8℃.

Reagent IV: Liquid 20 mL×1. Store at 2-8℃.

Standard: Liquid 1 mL×1. Store at 2-8℃. 2 μmol/mL of sodium pyruvate standard solution

Product Description:

Lactate dehydrogenase (LDH) is the terminal enzyme of the glycolysis pathway which is widely found in animals, plants, microorganisms and cultured cells. LDH catalyzes the reversible conversion of lactate to pyruvic acid with the reduction of NAD^+ to NADH and vice versa. According to the different configuration of catalytic substrate, it could be divided into D-lactate dehydrogenase (D-LDH, EC1.1.1.28) and L-lactate dehydrogenase (L-LDH, EC1.1.1.27).

NAD^+ and lactic acid are oxidized to pyruvic acid by the catalysis of D-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.

Reagents and Equipment Required but Not Provided:

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

Procedure:

I. Sample preparation:

1. **Bacteria or cells:** collect bacteria or cells into the centrifuge tube, discard supernatant after centrifugation. According to the proportion of bacteria or cells number (10^4): Extract solution volume (mL) of 500-1000-1 to extract. It is suggested that 5 million of bacteria or cell amount with 1mL of Extract solution. Split the bacteria or cell with ultrasonication (placed on ice, ultrasonic power 200W, working time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000 g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

2. **Tissue:** according to the proportion of tissue weight (g): Extract solution volume (mL) of 1:5-10 to extract. it is suggested that 0.1 g of tissue with 1 mL of Extract solution and fully homogenized on ice bath. Centrifuge at 8000 g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.
3. **Serum, plasma or other liquid samples:** detect sample directly. Centrifuge before detect if there are precipitation.

II. Determination procedure:

1. Preheat the Spectrophotometer/Microplate reader 30 minutes, adjust wavelength to 450 nm, set spectrophotometer counter to zero with distilled water.
2. Standard working solution: dilute 2μmol/mL standard solution to 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125μmol/mL with distilled water.
3. Sample Test

Reagent (μL)	Test tube(At)	Control tube(Ac)	Standard tube(As)	Blank tube(Ab)
Sample	10	10	-	-
Standard Solution	-	-	10	-
Reagent I	50	50	50	50
Reagent II	10	-	-	-
Distilled water	-	10	10	20
Mixed thoroughly, incubate at 37℃(mammal) or 25℃(other species) water bath for 15 minutes.				
Reagent III	50	50	50	50
Mixed thoroughly, incubate at 37℃(mammal) or 25℃(other species) water bath for 15 minutes.				
Reagent IV	150	150	150	150

Mix 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, ΔAt=At-Ac, ΔAs=As-Ab. Each test tube should be provided with one control tube. Blank tube and standard curve only need to test once or twice.

III. D-LDH Activity Calculations

1. Standard curve

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation y = kx + b, and bring ΔAt into the equation to get x (μmol/mL)

2. Calculation

1) 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.

D-LDH Activity(U/mg prot)= $x \times Vs \div (Cpr \times Vs) \div T \times 10^3 \times F = 66.7 \times x \div Cpr \times F$

2) Sample weight

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.

D-LDH Activity(U/g weight)= $x \times Vs \div (W \div V_E \times Vs) \div T \times 10^3 \times F = 66.7 \times x \div W \times F$

3) Bacteria or cells number

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 10^4 bacteria or cells.

D-LDH Activity(U/10^4 cell)= $x \times Vs \div (500 \div V_E \times Vs) \div T \times 10^3 \times F = 0.133 \times x \times F$

4) Serum (plasma) or other liquid samples volume

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 (plasma) or other liquid samples.

D-LDH Activity(U/mL)= $x \times Vs \div Vs \div T \times 10^3 \times F = 66.67 \times x \times F$

Vs: Supernate volume, 0.01 mL;

V_E: Extract solution volume, 1 mL;

T: Reaction time, 15 minutes;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

500: Total number of bacteria or cells, 5 million;

10^3: Unit conversion factor, 1 μmol/mL=10^3 nmol/mL.

Note:

1. If At is close to Ab or ΔAt is low, it is recommended to increase the sample size before determination. If At＞ 1.5 or ΔAt＞ 0.4, it is recommended to dilute the sample with Extract solution before determination. And modify the calculation formula.

Experimental example:

1. Take 0.109g rabbit kidney, add 1 mL of Extract solution, grind the homogenate with ice bath. Then operate according to the determination steps, calculate ΔAt=At-Ac = 0.425-0.298=0.127. Bring the result into the standard curve y=0.5379x+0.0087, and calculate x=0.220. The result is calculated according to the sample weight:

   D-LDH Activity(U/g weight)=66.67×x÷W×F =134.520 U/g weight
2. Take 0.1018g *Arabidopsis thaliana*, add 1 mL of Extract solution, grind the homogenate with ice bath. Then operate according to the determination steps, calculate ΔAt=At-Ac = 0.236-0.196=0.04. Bring the result into the standard curve y=0.5379x+0.0087, and calculate x=0.058. The result is calculated according to the sample weight:

   D-LDH Activity(U/g weight)=66.67×x÷W×F =38.109 U/g weight

3. Take 5 million cells, add 1 mL of Extract solution, grind the homogenate with ice bath. Then operate according to the determination steps, calculate ΔAt=At-Ac = 0.225-0.174=0.051. Bring the result into the standard curve y=0.5379x+0.0087, and calculate x=0.079. The result is calculated according to cells numbers:

   D-LDH Activity(U/10^4 cell)=0.133×x×F =0.010 U/10^4 cell

4. Take 10 μL calf serum, operate according to the determination steps, calculate ΔAt=At-Ac = 0.322-0.201=0.121. Bring the result into the standard curve y=0.5379x+0.0087, and calculate x=0.209. The result is calculated according to liquid volume:

   D-LDH Activity(U/mL)=66.67×x×F =13.919 U/mL

References:

[1] Huang P H, Fu L C, Huang C S, et al. The uptake of oligogalacturonide and its effect on growth inhibition, lactate dehydrogenase activity and galactin-3 release of human cancer cells[J]. Food chemistry, 2012, 132(4): 1987-1995.

[2] Huang Y N, Xu G T, You C P. The research progress of the lactate dehydrogenases in lactic acid bacteria[J]. Science and Technology of Food Industry, 2016, (08): 369-373.

Related Products:

NA0826/NA0584	Pyruvate Kinase(PK) Activity Assay Kit
NA0815/NA0573	Lactate Dehydrogenase (LDH) Activity Assay Kit
NA0809/NA0567	Hexokinase(HK) Activity Assay Kit
NA0710/NA0469	Phosphoglycerate Kinase(PGK) Activity Assay Kit
NA0708/NA0467	Fructose-bisphosphate aldolase(FBA) Activity Assay Kit