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Lactate Dehydrogenase (LDH) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: AK0523

Size:100T/48S

Components:

Extract solution: 60 mL×1. Storage at 4℃;

Reagent I: 7 mL×1. Storage at 4℃ .

Reagent II: powder×1. Storage at -20℃ . Working solution: dissolve with 1.3 mL of distilled water before use. It can be divided into tubule after matching, which can be preserved for two weeks at -20℃ . Avoid repeated freeze and thaw cycles.

Reagent III: 7 mL×1. Storage at 4℃ .

Reagent IV: 25 mL×1. Storage at 4℃ .

Sodium pyruvate standard solution: 1 mL (2 μmol/mL) ×1. Storage at 4℃ .

Product Description:

Lactate dehydrogenase (LDH or LD) is the terminal enzyme of the glycolysis pathway which is found in nearly all living cells (animals, plants, and prokaryotes). 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 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, thermostat water bath, desk centrifuge, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ice, distilled water.

Procedure:

I. Sample preparation:

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.

Procedure:

1. Preheat the Spectrophotometer/Microplate reader 30 minutes, adjust wavelength to 450 nm, set zero with distilled water.
2. Sodium pyruvate standard solution: take 100 μL of standard solution, dilute to 1, 0.5, 0.25, 0. 125, 0 μmol/mL, 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. LDH Calculations

1. Sample Sodium pyruvate content
2. Set the standard curve, y-axis as the standard concentration, μmol/mL, x-axis as the 450 nm absorption. Put ΔA(x) into standard curve, calculate y (μmol/mL)
3. Serum (plasma) sample 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.

$LDH(U/mL)=y\div T\times 10^3=66.7\times y$

4. Tissue, bacteria or cultured cells LDH activity

A. 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.

$LDH(U/mg\ prot)= y\div T\div Cpr\times 10^3 =66.7\times y\div Cpr$

B. 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.
LDH(U/g)= y÷T÷W×10^3 =666.7×y

C. Cell amount
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 10000 cells.
LDH(U/10^4 cell)= y÷T÷500×10^3 =0. 133×y

Vs: Supernate volume (mL), 0.01 mL;
Vsv: Extract solution volume, 1 mL;
T: Reaction time, 15 minutes;
Cpr: Sample protein concentration, mg/mL;
W: Sample weight, 0.1 g;
500: Total number of bacteria or cells, 5 million;
10^3 : 1 μmol/mL=10^3 nmol/mL.

Recent Product Citations：

[1] 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.

[2] Zhang H, Da Z, Feng Y, et al. Enhancing the electricity generation and sludge reduction of sludge microbial fuel cell with graphene oxide and reduced graphene oxide[J]. Journal of cleaner production, 2018, 186: 104- 112.

[3] Zhao B, Sun L, Jiang X, et al. Genipin protects against cerebral ischemia-reperfusion injury by regulating the UCP2-SIRT3 signaling pathway[J]. European journal of pharmacology, 2019, 845: 56-64.

[4] Zhao H L, Wu B Q, Luo Y, et al. Exogenous hydrogen sulfide ameliorates high glucose-induced myocardial injury & inflammation via the CIRP-MAPK signaling pathway in H9c2 cardiac cells[J]. Life sciences, 2018, 208: 315-324.

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.

Related Products：

AK0516/AK0515	Hexokinase(HK) Activity Assay Kit
AK0540/AK0539	Pyruvate Kinase(PK) Activity Assay Kit
AK0394/AK0393	Phosphoglycerate Kinase(PGK) Activity Assay Kit
AK0238/AK0237	Fructose-bisphosphate aldolase(FBA) Activity Assay Kit