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CheKine™ Micro D-lactate Dehydrogenase (D-LDH) Activity Assay Kit

Cat #: KTB1111 Size: 48T/96 T

	Micro D-lactate Dehydrogenase (D-LDH) Activity Assay Kit		
REF	**Cat #:** KTB1111	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Cells or Bacteria, Serum (Plasma)		
	Storage: Stored at -20℃ for 6 months		

Assay Principle

Lactate dehydrogenase (LDH) is a glycolytic enzyme, which widely exists in animals, plants, microorganisms and cultured cells, with a higher content in kidney. LDH is the terminal enzyme of the glycolytic pathway, catalyzing the reversible reaction between pyruvate and lactate, accompanied by the interconversion between NAD+/NADH. According to the different configuration of its catalytic substrate lactate, it can be divided into D-lactate dehydrogenase (D-LDH, EC 1.1.1.28) and L-lactate dehydrogenase (L-LDH, EC 1.1.1.27). D-LDH catalyzes NAD+ to oxidize D-lactic acid to produce pyruvate. Pyruvate further reacts with 2,4-dinitrophenylhydrazine to produce pyruvate dinitrophenylhydrazone, which appears brownish red in alkaline solution, and the color depth is proportional to the concentration of pyruvate.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Reagent Ⅰ	5 mL	10 mL	4℃
Reagent Ⅱ	1	1	-20℃
Reagent Ⅲ	5 mL	10 mL	4℃
Reagent Ⅳ	20 mL	40 mL	4℃
Reagent Ⅴ	100 μL	200 μL	-20℃
Standard	1 mL	1 mL	4℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 450 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

- Thermostatic water bath, ice maker, centrifuge
- Deionized water
- Homogenizer

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent II: Prepare before use, add 10 μL Reagent Ⅴ and 1.3 mL deionized water for 48 T and 20 μL Reagent Ⅴ and 2.6 mL deionized water for 96 T to fully dissolve. The unused reagent shall be stored at -20℃ after subpackaging, and repeated freezing and thawing are prohibited.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅳ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅴ: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.

Standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Standard preparation: Use 100 μmol/mL standard, prepare standard curve dilution as described in the table.

Num.	Standard Volume	Extraction Buffer Volume (μL)	Concentration (μmol/mL)
Std.1	20 μL 100 μmol/mL	980	2
Std.2	100 μL of Std.1 (2 μmol/mL)	100	1
Std.3	100 μL of Std.2 (1 μmol/mL)	100	0.5
Std.4	100 μL of Std.3 (0.5 μmol/mL)	100	0.25
Std.5	100 μL of Std.4 (0.25 μmol/mL)	100	0.125
Std.6	100 μL of Std.5 (0.125 μmol/mL)	100	0.063
Std.7	100 μL of Std.6 (0.063 μmol/mL)	100	0.031
Blank	0	100	0

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.

1. Preparation of bacteria, cells or tissue samples:

Bacteria or cells: Collect bacteria or cells into centrifuge tubes first, and discard supernatant after centrifugation; Add Extraction Buffer according to the ratio of the number of bacteria or cells (10^4): Extraction Buffer volume (mL) of 500~1000:1 (it is recommended to add 1 mL of Extraction Buffer to 5×10^6 bacteria or cells). Break the cells or bacteria by ice bath ultrasonic wave for 5 min (power 20% or 200 W, ultrasonic for 3 s, interval 7 s, repeat 30 times), then centrifuge at 8,000 g, 4℃ for 10 min, take the supernatant, and put it on ice for testing.

Tissue: Add Extraction Buffer according to the ratio of tissue mass (g): Extraction Buffer volume (mL) of 1:5~10 (it is recommended to weigh about 0.1 g of tissue and add 1 mL of Extraction Buffer) and homogenize in ice bath. Centrifuge at 8,000 g for 10 min at 4℃, take the supernatant and put it on ice for testing.

2. Serum (plasma): Direct detection.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 450 nm, visible

spectrophotometer was returned to zero with deionized water.

2. Operation table (The following were operated in 1.5 ml EP tube):

Reagent	Test Tube (μL)	Control Tube (μL)	Standard Tube (μL)	Blank Tube (μL)
Sample to be tested	10	10	0	0
Standard	0	0	10	0
Reagent Ⅰ	50	50	50	50
Reagent Ⅱ	10	0	0	0
Deionized water	0	10	10	20
Fully mix, 37℃ water bath for 15 min				
Reagent Ⅲ	50	50	50	50
Fully mix, 37℃ water bath for 15 min				
Reagent Ⅳ	150	150	150	150

3. Mix well, let stand at room temperature for 30 min, take 200 μL was transferred to a micro glass cuvette or a 96 well plate to measure the absorbance at 450 nm, which was recorded as A_{Test}, $A_{Control}$, $A_{Standard}$, and A_{Blank}. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Blank well and standard well only need to be measured once, and each test well needs to be set with a control well. Before the experiment, it is recommended to select 2-3 samples with large expected differences for pre experiment. If ΔA_{Test} is less than 0.01, the sample size can be appropriately increased; If ΔA_{Test} is greater than 0.5, the sample can be further diluted with Extraction Buffer, and the calculated result is multiplied by the dilution factor, or the sample size for extraction can be reduced.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve:

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve, get the standard equation y=kx+b, and bring the ΔA_{Test} into the equation to get the x value (μmol/mL).

3. Calculation of D-LDH activity:

(1) Calculated by sample volume:

Definition of unit: 1 nmol pyruvate per minute per ml of serum (plasma) is defined as one unit of enzyme activity.

D-LDH activity(U/mL)=$x\times V_{sample}\div V_{sample}\div T\times 10^3$**=66.67×x**

(2) Calculated by sample mass

Definition of unit: the catalytic production of 1 nmol pyruvate per minute per gram of tissue is defined as one unit of enzyme activity.

D-LDH activity(U/g weight)=$x\times V_{sample}\div(W\div V_{sample\ total}\times V_{sample})\div T\times 10^3$**=66.67×x÷W**

(3) Calculated according to the number of bacteria or cells

Definition of unit: 1 nmol pyruvate per minute catalyzed by 10^4 bacteria or cells is defined as one unit of enzyme activity.

D-LDH activity(U/10^4)=$x\times V_{sample}\div(N\div V_{sample\ total}\times V_{sample})\div T\times 10^3$**=66.67×X÷N**

V_{sample}: sample volume added in the reaction system, 0.01 mL; $V_{sample\ total}$: volume of Extraction Buffer added, 1 mL; T: reaction time, 15 min; W: sample mass, g; N: number of cells or bacteria, in 10^4; 10^3: unit conversion factor, 1 μmol/mL=10^3 nmol/mL.

Typical Data

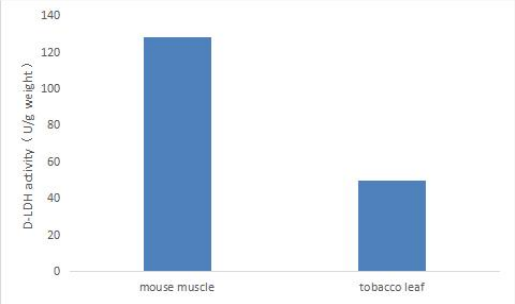

Figure 1. Determination D-LDH activity in mouse muscles and tobacco leaves by this assay kit

Recommended Products

Catalog No.	Product Name
KTB4010	CheKine™ Micro Soil Nitrate Reductase (S-NR) Activity Assay Kit
KTB4021	CheKine™ Micro Leucine Arylamidase (LAP) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.