



CheKine™ Micro Lactate Dehydrogenase (LDH) Assay Kit

Cat #: KTB1110

Size: 96 T/96 S 480 T/480 S

	Micro Lactate Dehydrogenase (LDH) Assay Kit		
REF	Cat #: KTB1110	LOT	Lot #: Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells, Bacteria, Plasma, Serum or other Liquid samples		
	Detection range: 1-20 U/mL		
	Storage: Stored at -20°C for 6 months, protect from light		

Assay Principle

Lactate dehydrogenase (LDH) is an oxidoreductase (EC 1.1.1.27) presents in a wide variety of organisms. It catalyses the inter-conversion of pyruvate and lactate with concomitant inter-conversion of NADH and NAD⁺. It converts pyruvate, the final product of glycolysis, to lactate in hypoxic conditions. LDH quantification is of clinical interest as serum levels of certain LDH isozymes reflect pathological conditions in particular tissues. When disease or injury or toxic material damages tissues, the cells LDH is released into the bloodstream. Since LDH is a fairly stable enzyme, LDH has been widely used to evaluate the presence of damage and toxicity of tissue and cells. CheKine™ Micro Lactate Dehydrogenase (LDH) Assay Kit provides a simple and easy assay for measuring lactate dehydrogenase in Animal and Plant Tissues, Cells, Bacteria, Plasma, Serum and other biological fluids. In this assay, LDH reduces NAD to NADH, which then interacts with WST-8 to produce a color ($\lambda_{\max}=450$ nm).

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	96 T	480 T	
Assay Buffer (5×)	32 mL	80 mL×2	4°C
Lactate	1.2 mL	6 mL	-20°C, protect from light
NAD	1 mL	5 mL	-20°C, protect from light
WST-8	600 µL	3 mL	-20°C, protect from light
Enhancer	120 µL	600 µL	-20°C, protect from light
Lactate Dehydrogenase Standard (1 KU/mL)	60 µL	300 µL	-20°C, protect from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 450 nm

- Incubator, ice maker, freezing centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Note: Briefly centrifuge small vials at low speed before opening.

1×Assay Buffer: Before use, Assay Buffer (5×) should be diluted 5 times with deionized water at a concentration of 1×Assay Buffer. Equilibrate to room temperature before use. Store at 4°C.

Lactate: Ready to use as supplied. Keep on ice and protect from light during the assay. Store aliquots at -20°C, protect from light.

NAD: Ready to use as supplied. Keep on ice and protect from light during the assay. Store aliquots at -20°C, protect from light.

WST-8: Ready to use as supplied. Keep on ice and protect from light during the assay. Store aliquots at -20°C, protect from light.

Enhancer: Ready to use as supplied. Keep on ice and protect from light during the assay. Store aliquots at -20°C, protect from light.

Working Reagent: For each well, prepare 55 µL Working Reagent by mixing 31 µL 1×Assay Buffer, 8 µL NAD, 5 µL WST-8, 1 µL Enhancer and 10 µL Lactate, mix well. Working Reagent is freshly prepared.

Lactate Dehydrogenase Standard (20 U/mL): Dilute the Standard to 20 U/mL by adding 20 µL Lactate Dehydrogenase Standard (1 KU/mL) to 980 µL 1×Assay Buffer, mix well. Equilibrate to room temperature before use. Store aliquots at -20°C for undiluted standard.

Setting of standard curves: Further dilute the 20 U/mL standard to 20, 16, 12, 8, 4, 2, 1 U/mL with 1×Assay Buffer, as shown in the following table.

Num.	Volume of 20 U/mL Standard (µL)	Volume of 1×Assay Buffer (µL)	Standard Concentration (U/mL)
Std.1	200	0	20
Std.2	160	40	16
Std.3	120	80	12
Std.4	80	120	8
Std.5	40	160	4
Std.6	20	180	2
Std.7	10	190	1

Note: Always prepare fresh standards for every use. Diluted standard solution is unstable and must be used within 4 h.

Sample Preparation

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

1. Animal or Plant Tissues: Weigh 0.1 g tissue, add 1 mL cold 1×Assay Buffer and homogenize on ice. Centrifuge at 10,000 g for 15 min at 4°C. Use supernatant for assay, and place it on ice to be tested.
2. Cells or bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL cold 1×Assay Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 15 min at 4°C. Use supernatant for assay, and place it on ice to be tested.
3. Plasma, Serum or other Liquid samples: Test directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

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. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	50
Standards	0	50	0
Deionized Water	50	0	0
Working Reagent	50	50	50

3. Mix well, Incubate for 30 min at 37°C in the dark. The absorbance value is measured at 450 nm. The Blank Well is recorded as A_{Blank} , the Standard Well is marked as A_{Standard} , and the Test Well is marked as A_{Test} . Finally calculate $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Blank}}$, $\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.001, increase the sample quantity appropriately. If ΔA_{Test} is greater than 2.0, the sample can be appropriately diluted with 1×Assay Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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 y-axis and the $\Delta A_{\text{Standard}}$ as the x-axis, draw the standard curve.

2. Calculation of LDH content

Bring the ΔA_{Test} of the sample into the equation to get the y value (U/mL).

- (1) Calculated by fresh weight of samples

LDH content (U/g fresh weight) = $y \times V_{\text{Sample}} \div (W \times V_{\text{Sample}} \div V_{\text{Sample total}}) \times n = \mathbf{y \div W \times n}$

- (2) Calculated by protein concentration

LDH content (U/mg prot) = $y \times V_{\text{Sample}} \div (V_{\text{Sample}} \times C_{\text{pr}}) \times n = \mathbf{y \div C_{pr} \times n}$

- (3) Calculated by volume of liquid samples

LDH content (U/mL) = $y \times V_{\text{Sample}} \div V_{\text{Sample}} \times n = \mathbf{y \times n}$

- (4) Calculated by number of cells or bacteria

LDH content (U/ 10^4 cells) = $y \times V_{\text{Sample}} \div (500 \times V_{\text{Sample}} \div V_{\text{Sample total}}) \times n = \mathbf{y \div 500 \times n}$

Where: V_{Sample} : add sample volume, 0.05 mL; W: weight of sample, g; $V_{\text{Sample total}}$: add 1×Assay Buffer volume to sample, 1 mL; n: the sample dilution factor; C_{pr} : sample protein concentration, mg/mL; 500: Total number of cells or bacteria, 5×10^6 .

Typical Data

Typical standard curve

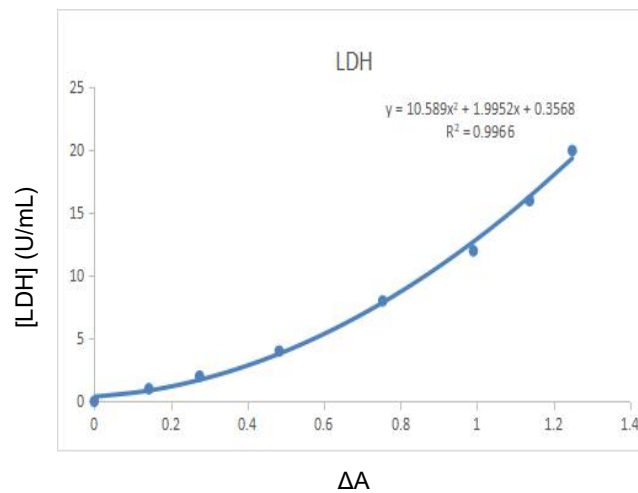


Figure 1. Standard curve of LDH assay, data provided for demonstration purposes only. A new standard curve must be generated for each assay.

Recommended Products

Catalog No.	Product Name
KTB1100	CheKine™ Micro Lactate Assay Kit
KTB1121	CheKine™ Micro Pyruvate Acid (PA) Assay Kit
KTB1270	CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit
KTB1230	CheKine™ Micro Succinate Dehydrogenase (SDH) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.