Lactate Dehydrogenase (LDH) Colorimetric Assay Kit

Cat#: orb1173246 (User Manual)

Micro Lactate Dehydrogenase (LDH) Assay Kit
Cat #: orb1173246
Size: 96 T/480 T
Lot #: Refer to product label
Applicable samples: Animal and Plant Tissues, Cells, Plasma, Serum or other Liquid samples
Detection range: 1-20 U/mL
Storage: Stored at -20℃ 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, Plasma, Serum and other biological fluids. In this assay, LDH reduces NAD to NADH, which then interacts with WST-8 to produce a color (λmax=450 nm).

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	96 T	480 T	
Assay Buffer	25 mL	100 mL	4℃
Lactate	1.2 mL	6 mL	-20℃, protect from light
NAD	1 mL	5 mL	-20℃, protect from light
WST-8	600 µL	3 mL	-20℃, protect from light
Enhancer	120 µL	600 µL	-20℃, protect from light
Lactate Dehydrogenase Standard (100 U/mL)	0.5 mL	2.5 mL	-20℃, protect from light

Materials Required but Not Supplied

·Microplate reader or visible spectrophotometer capable of measuring absorbance at 450 nm
·Incubator, ice maker, freezing centrifuge
·96-well plant 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.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Lactate: Ready to use as supplied. Keep on ice and protect from light during the assay. Store aliquots at -20℃, protect from light.

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

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

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

Working Reagent: For each well, prepare 55 µL Working Reagent by mixing 31 µL 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 200 µL Lactate Dehydrogenase Standard (100 U/mL) to 800 µL Assay Buffer, mix well. Equilibrate to room temperature before use. Store aliquots at -20℃.

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

Num.	Volume of 20 U/mL Standard (μL)	Volume of 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

Sample Preparation

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

1. Animal or Plant Tissues: Weigh 0.1 g tissue, add 1 mL cold Assay Buffer and homogenize on ice. Centrifuge at 10,000 g for 15 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells: Collect 5×106 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL cold 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℃. 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 Biorbyt. 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 ABlank, the Standard Well is marked as AStandard, and the Test Well is marked as ATest. Finally calculate ΔATest=ATest-ABlank, ΔAStandard=AStandard-ABlank.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔATest is less than 0.001, increase the sample quantity appropriately. If ΔATest is greater than 2.0, the sample can be appropriately diluted with 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 ΔAStandard as the x-axis, draw the standard curve.
2. Calculation of LDH content
Bring the ΔATest 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×VSample÷(W×VSample÷VSample total)×n**=y÷W×n**
(2) Calculated by protein concentration
LDH content (U/mg prot)=y×VSample÷(VSample×Cpr)×n**=y÷Cpr×n**
(3) Calculated by volume of liquid samples
LDH content (U/mL)=y×VSample÷VSample×n**=y×n**
(4) Calculated by number of cells
LDH content (U/104 cells)=y×VSample÷(500×VSample÷VSample total)×n**=y÷500×n**
Where: VSample: add sample volume, 0.05 mL; W: weight of sample, g; VSample total: add Assay Buffer volume to sample, 1 mL; n: the sample dilution factor; Cpr: sample protein concentration, mg/mL; 500: Total number of cells, 5×106.

Typical Data

Typical standard curve

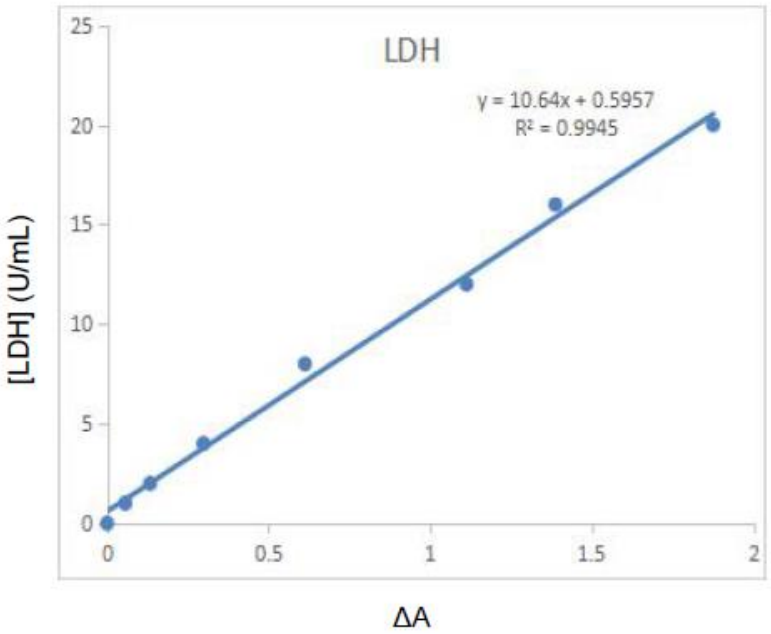

Figure 1. Standard curve of LDH assay, data provided for demonstration purposes only. A new standard curve must be generated for each assay.