

EZMeta™ Lactate Dehydrogenase (LDH) Colorimetric Activity Kit

Cat #: D-AKC1110

Size: 96T / 480T

Storage: Stored at -20°C for 6 months, protected from light

Product Information

Detection range: 1-20 U/mL

Applicable samples: Animal and Plant Tissues, Cells, Plasma, Serum or other Liquid samples

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. EZMeta™ Lactate Dehydrogenase (LDH) Colorimetric Activity 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 (λ_{max} =450 nm).

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	96 T	480 T	
Assay Buffer	25 mL	100 mL	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 (100 U/mL)	0.5 mL	2.5 mL	-20°C, 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 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.

Assay Buffer: Ready to use as supplied. 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 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°C.

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 °C 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°C. Use supernatant for assay, and place it on ice to be tested.
2. Cells: Collect 5×10^6 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°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 EZMeta™ Lactate Dehydrogenase (LDH) Colorimetric Activity Kit 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 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

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

(2) Calculated by protein concentration

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

(3) Calculated by volume of liquid samples

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

(4) Calculated by number of cells

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

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

Typical Data

Typical standard curve

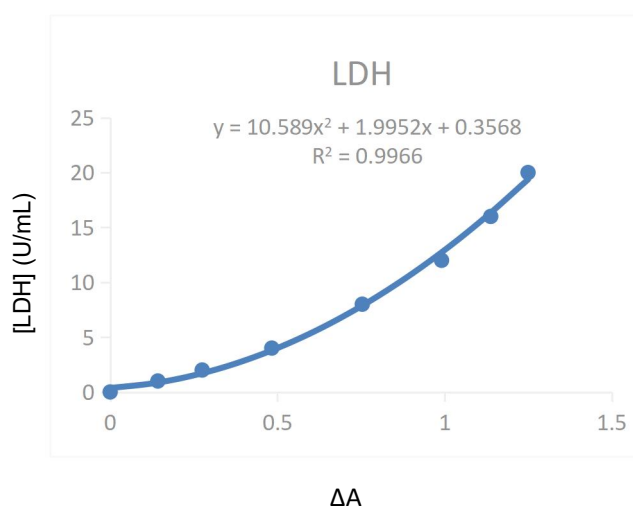


Figure 1. Standard curve of LDH assay, data provided for demonstration purposes only. A new standard curve must be generated for each assay.

Disclaimer

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