

EZMeta™ Lactate Colorimetric Assay Kit

Cat #: D-AKC1100

Size: 48T / 96T

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

Product Information

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

Assay Principle

Lactate is an important intermediate product in the metabolic process of organisms, which is closely related to glucose metabolism, lipid metabolism, protein metabolism and intracellular energy metabolism. The content of lactate is an important indicator to evaluate glycogen metabolism and aerobic metabolism. Abnormally high levels of lactate have been linked to pathological conditions such as cancer, diabetes and lactic acidosis. EZMeta™ Lactate Colorimetric Assay Kit provides a convenient means for detecting L(+)-Lactate in biological samples such as animal and plant tissues, cells, serum, plasma or other liquid samples. In this kit, lactate is oxidized by lactate dehydrogenase to generate a product which interacts with a tetrazolium salt WST-8 dye to form a colorimetric (450 nm) product, proportional to the lactate present.

Materials Supplied and Storage Conditions

Kit Components	Size			Storage Conditions
	48 T	96 T	96 T×5	
Lactate Assay Buffer	70 mL	70 mL*2	70 mL×10	4°C
Lactate Dehydrogenase	0.7 mL	1.4 mL	1.4 mL×5	-20°C
Lactate Dehydrogenase Cofactor	0.5 mL	1 mL	1 mL×5	-20°C
WST-8	350 µL	700 µL	700 µL×5	-20°C, protected from light
Enhancer	70 µL	140 µL	140 µL×5	-20°C, protected from light
L(+)-Lactate Standard (100 mM)	50 µL	100 µL	100 µL×5	-20°C

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
- Homogenizer (for tissue samples)

Reagent Preparation

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

Lactate Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

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

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

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

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

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

L(+)-Lactate Standard (2 mM): Dilute the Lactate Standard (100 mM) to 2 mM by adding 20 μL of the Lactate Standard to 980 μL of Lactate Assay Buffer, mix well. Equilibrate to room temperature before use. Store aliquots at -20°C .

Setting of standard curves: Further dilute the L(+)-Lactate 2 mM standard to 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.0313 mM Standard with Lactate Assay Buffer, as shown in the following table.

Num.	Volume of Standard (μL)	Volume of Lactate Assay Buffer (μL)	Standard Concentration (mM)
Std.1	400 μL 2 mM	0	2
Std.2	200 μL of Std.1	200	1
Std.3	200 μL of Std.2	200	0.5
Std.4	200 μL of Std.3	200	0.25
Std.5	200 μL of Std.4	200	0.125
Std.6	200 μL of Std.5	200	0.0625
Std.7	200 μL of Std.6	200	0.0313

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 and Plant Tissues: The tissue was homogenized in Lactate Assay Buffer ice bath according to the proportion of 1 mL/0.1 g. Centrifuge at 12,000 g for 5 min at 4°C . Use supernatant for assay, and place it on ice to be tested.

2. Cells: The cells were collected into the centrifuge tube, washed with cold PBS, the supernatant was discarded after centrifugation, and the cell 5 min was broken by Lactate Assay Buffer ice bath ultrasonic wave according to the proportion of 1 mL/ 5 million (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 5 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: (1) NADH or NADPH from cell or tissue extracts generates background for the lactate assay. To remove the NADH or NADPH background, the same amount of sample can be tested in the absence of Lactate Dehydrogenase. Then the background readings can be subtracted from the lactate reading. (2) Endogenous Lactate Dehydrogenase (LDH) may degrade lactate. Samples containing LDH (such as culture medium or tissue lysate) should be filtered through a 10 kDa MW spin filter to remove all proteins and then kept at -80°C for storage. (3) If the protein concentration of the sample is need to determined, it is recommended to use EZMeta™ Lactate Colorimetric Assay 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
Standard	0	50	0
Lactate Assay Buffer	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 1.0, the sample can be appropriately

diluted with Lactate 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 Lactate content

Bring the ΔA_{Test} of the sample into the equation to get the y value (1 mM=1 $\mu\text{mol/mL}$)

(1) Calculated by fresh weight of samples

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

(2) Calculated by protein concentration

$$\text{Lactate content } (\mu\text{mol/mg prot}) = y \times V_{\text{Sample}} \div (V_{\text{Sample}} \times \text{Cpr}) \times n = \mathbf{y \div \text{Cpr} \times n}$$

(3) Calculated by volume of Liquid samples

$$\text{Lactate content } (\mu\text{mol/mL}) = y \times V_{\text{Sample}} \div V_{\text{Sample}} \times n = \mathbf{y \times n}$$

(4) Calculated by number of cells

$$\text{Lactate content } (\mu\text{mol}/10^4 \text{ cells}) = y \times V_{\text{Sample}} \div (\text{number of Cell} \times V_{\text{Sample}} \div V_{\text{Sample total}}) \times n = \mathbf{y \div 500 \times n}$$

Where: V_{Sample} : add sample volume, 0.05 mL; W: weight of sample, g; $V_{\text{Sample total}}$: add Lactate 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-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

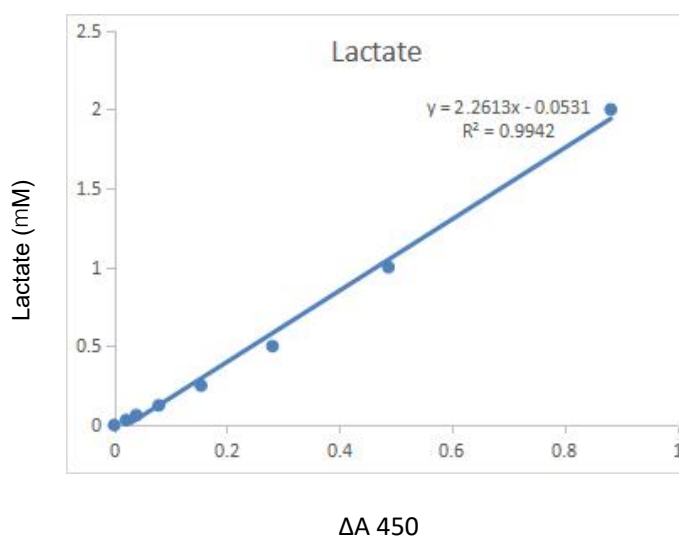


Figure 1. Standard Curve of Lactate assay

Disclaimer

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