Lactate Assay Kit

Catalog No.: K060

Size: 48 T/96 T

Storage: -20°C for 6 months, away from light.

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. Lactate 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:

Components	48 T	96 T	Storage Conditions
Lactate Assay Buffer	50ml	100ml	2-8°C
Lactate Dehydrogenase	0.6ml	1.2ml	-20°C
Lactate Dehydrogenase Cofactor	0.5ml	1ml	-20°C
WST-8	300ul	600ul	-20°C, protected from light
Reagent plus	60ul	120ul	-20°C, protected from light
L(+)-Lactate Standard (100 mM)	50ul	100ul	-20°C

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℃.
Lactate Dehydrogenase: Ready to use as supplied. Keep on ice and protect from light during the assay. Store aliquots at -20℃.
Lactate Dehydrogenase Cofactor: Ready to use as supplied. Keep on ice and protect from light during the assay. Store aliquots at -20℃.
WST-8: Ready to use as supplied. Keep on ice and protect from light during the assay. Store aliquots at -20℃, protected from light.
Reagent plus: Ready to use as supplied. Keep on ice and protect from light during the assay. Store aliquots at -20℃, 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 Reagent plus and 10 µL Lactate Dehydrogenase, mix well. Working Reagent is freshly
prepared.
L(+)-Lactate Standard (2 mM): Dilute the Lactate Standard 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℃.
Setting of standard curves: Further dilute the 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	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	0.0626
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℃ 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/0.1g. Centrifuge at 12,000 g for 5 min at 4℃. 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℃. 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 kDaMW spin filter to remove all proteins and then kept at -80℃ for storage. (3) If the protein concentration of the sample is need to determined, it is recommended to use fn-test Cat #: K001 BCA Protein Assay Kit (protein quantification) 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℃ 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

ΔATest=**A**Test-**A**Blank，**ΔA**Standard=**A**Standard-**A**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 **ΔA**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 μmol/mL)
(1) Calculated by fresh weight of samples
Lactate content (μmoL/g fresh weight)=y×V Sample÷(W×V Sample÷V Sample total)×n **=y÷W×n**
(2) Calculated by protein concentration
Lactate content (μmoL/mg prot)=y×V Sample÷(V Sample×Cpr)×n **=y÷Cpr×n**
(3) Calculated by volume of Liquid samples
Lactate content (μmoL/mL)=y×V Sample÷V Sample×n **=y×n**
(4) Calculated by number of cells
Lactate content (μmoL/10 4 cells)=y×V Sample÷(number of Cell×V Sample÷V Sample total)×n **=y÷500×n**
Where: VSample: add sample volume, 0.05 mL; W: weight of sample, g; VSample total: add Lactate Assay Buffer volume to sample, 1mL; n: the sample dilution factor; Cpr: sample protein concentration, mg/mL; 500: Total number of cells, 5×106.

Typical Data:

Typical standard curve-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

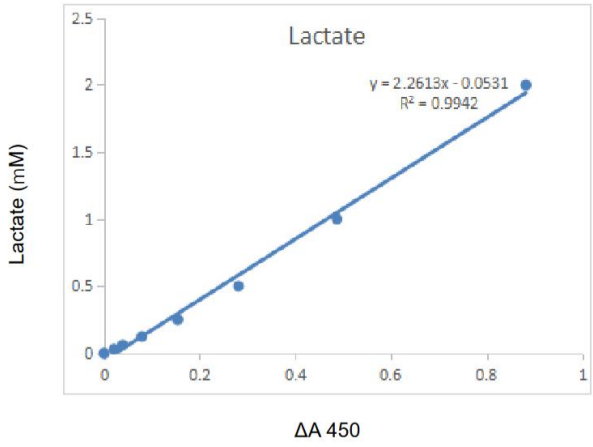

Figure 1. Standard Curve of Lactate assay