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CheKine™ Micro Lactate Assay Kit

Cat #: KTB1100 Size: 48 T/48 S 96 T/96S 96 T×5/480 S

	Micro Lactate Assay Kit		
REF	**Cat #:** KTB1100	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

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. CheKine™ Micro 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

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

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℃.

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.

Enhancer: 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 Enhancer and 10 μL Lactate Dehydrogenase, mix well. Working Reagent is freshly prepared.

L(+)-Lactate Standard (2 mM): Dilute the L(+)-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℃ for 6 months.

Setting of standard curves: Further dilute L(+)-Lactate Standard (2 mM) 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 L(+)-Lactate Standard (2 mM) (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: It is recommended to use fresh samples. If the experiment is not conducted immediately, the samples can be stored at -80℃ for 1 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℃. 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 cell culture medium, cell or tissue lysate, etc.) should be filtered through a 10 kDa MW spin filter (Centrifuge at 12,000 g for 10 min at 4℃. The specific filtration steps are subject to the

filter instructions) to remove all proteins, take filtrate for testing, and then kept at -80℃ for storage. (3) 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
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 $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

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 $\Delta A_{Standard}$ 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)=$y\times V_{Sample}\div(W\times V_{Sample}\div V_{Sample\ total})\times n$**$=y\div W\times n$**

(2) Calculated by protein concentration

Lactate content (μmoL/mg prot)=$y\times V_{Sample}\div(V_{Sample}\times Cpr)\times n$**$=y\div Cpr\times n$**

(3) Calculated by volume of Liquid samples

Lactate content (μmoL/mL)=$y\times V_{Sample}\div V_{Sample}\times n$**$=y\times n$**

(4) Calculated by number of cells

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

Where: V_{Sample}: add sample volume, 0.05 mL; W: weight of sample, g; $V_{Sample\ total}$: 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.

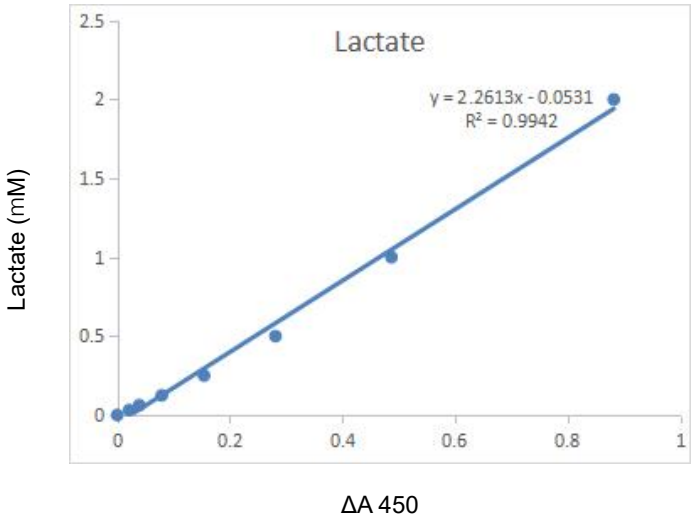

Figure 1. Standard Curve of Lactate assay

Recommended Products

Catalog No.	Product Name
KTB1110	CheKine™ Micro Lactate Dehydrogenase (LDH) Activity 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.