Lactate Colorimetric Assay Kit

Cat#: orb1173247 (User Manual)

Lactate Colorimetric Assay Kit
Cat #: orb1173247
Lot #: Refer to product label
Applicable samples: Animal Tissues, Cells, Plasma, Serum or other Liquid samples
Storage: Stored at -20℃ for 6 months

Assay Principle

Lactate ($CH_3CH(OH)COO^-$) is a metabolic compound formed in animals by the action of the enzyme lactate dehydrogenase. Lactate is produced in proliferating cells during anaerobic conditions such as exercise. Abnormally high concentrations of lactate have been related to pathological conditions such as cancer, diabetes, and lactate acidosis. L(+)-Lactate is the major lactate stereoisomer formed in human intermediary metabolism and is present in blood at levels of around 1-2 mmol/L. D(-)-Lactate can also be found in blood but only at about 1-5% of the concentration of L(+)-Lactate. The Kit provides a convenient means for detecting L(+)-Lactate in biological samples such as animal 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	480 T	
Lactate Assay Buffer	15 mL	25 mL	100 mL	4℃
Lactate Dehydrogenase	0.6 mL	1.2 mL	6 mL	-20℃
Lactate Dehydrogenase Cofactor	0.5 mL	1 mL	5 mL	-20℃
WST-8	300 µL	600 µL	3 mL	-20℃, protect from light
Enhancer	60 µL	120 µL	600 µL	-20℃, protect from light
L(+)-Lactate Standard (100 mM)	50 µL	100 µL	500 µL	-20℃

Materials Required but Not Supplied

·Microplate Reader capable of measuring absorbance at 450 nm

·Incubator, Ice Maker, Freezing Centrifuge

·96 well plate with clear flat bottom, Precision Pipettes, Disposable Pipette Tips

·Deionized Water

·Dounce 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. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20℃.
Lactate Dehydrogenase Cofactor: Ready to use as supplied. Keep on ice and protect from light during the assay. Aliquot standard so that you have enough volume to perform the desired number of assays. Store aliquots at -20℃.

WST-8: Ready to use as supplied. Keep on ice and protect from light during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20℃.
Enhancer: Ready to use as supplied. Keep on ice and protect from light during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20℃.

Working reagent: In 96 well plate, 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.
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. Aliquot standard so that you have enough volume to perform
the desired number of assays. 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.

	Volume of Standard (µL)	Volume of Lactate Assay Buffer (µL)	Standard Concentration (mM)
Std.1	200 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 performing several dilutions of your sample to ensure the readings are within the standard value range. We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. All samples can be diluted in 50 mM potassium phosphate (pH 7.4).

1. Animal Tissues: Weigh 0.1 g tissue, add 1 mL Lactate Assay Buffer and homogenize on ice. Centrifuge at 12,000 g for 5 min at 4℃. 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 or bacteria with cold PBS, discard the supernatant after

centrifugation; add 1 mL Lactate 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 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 kDa MW spin filter to remove all proteins and then kept at -80℃ for storage.

Assay Procedure

1. Preheat the microplate reader for more than 30 min, and adjust the wavelength to 450 nm. 2. Sample measurement. (The following operations are operated in the 96 well plate)

	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	50
Standard	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 with a microplate reader. The

Blank Well is marked 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.

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 y=kx+b. 2.Calculation of Lactate content
Bring the ΔATest 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×VSample÷(W×VSample÷VSample total)×n**=y÷W×n**

(2) Calculated by protein concentration
Lactate content (μmoL/mg prot)=y×VSample÷(VSample×Cpr)×n**=y÷Cpr×n**

(3) Calculated by volume of Liquid samples
Lactate content (μmoL/mL)=y×VSample÷VSample×n**=y×n**

(4) Calculated by number of Cell
Lactate content (μmoL/10^4 cells)=y×VSample÷(number of Cell×VSample÷VSample total)×n **=y÷number of Cells×n**

Where: VSample: add sample volume, 0.05 mL; W: weight of sample, g; VSample total: add Lactate Assay Buffer volume to sample, 1 mL; n: the sample dilution factor; Cpr: sample protein concentration, mg/mL.

Note: If the ΔATest values are higher than 2 mM Standard, dilute sample in Lactate Assay Buffer and repeat this assay. Multiply the results by the dilution factor. It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if it is calculated by protein concentration.

Typical Data

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

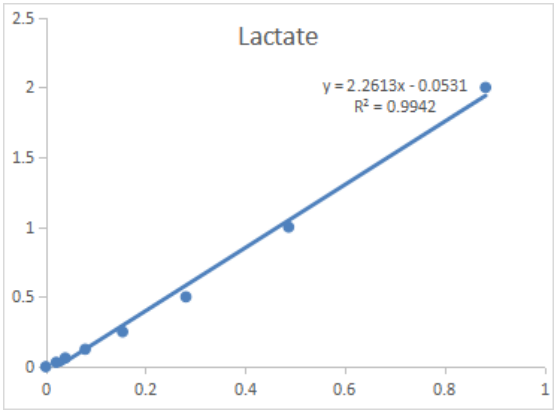

Fig. Standard Curve of Lactate assay