L-Lactate Colorimetric Assay Kit

Introduction

L-Lactate is the predominant form of lactic acid in mammals. It is produced by the brain, skeletal muscle, and erythrocytes, and is primarily metabolized by the liver and kidneys. Blood L-lactate concentration serves as a critical biomarker for evaluating tissue hypoxia, perfusion status, and patient prognosis in critical care settings. Elevated L-lactate levels are commonly observed in life-threatening conditions such as cardiac arrest, sepsis, and mesenteric ischemia. In addition, L-Lactate plays a central role in cellular energy metabolism and tumor metabolic reprogramming, and is widely used in the food industry as an indicator of fermentation quality, freshness, and storage stability.

This kit employs the WST-8 chromogenic method, in which L-lactate is oxidized to pyruvate by L-lactate dehydrogenase (L-LDH). The concomitant reduction of NAD^+ to NADH drives the electron carrier 1-mPMS to reduce WST-8, generating a water-soluble formazan dye with a maximal absorption peak at 450 nm. The absorbance is directly proportional to the L-lactate concentration in the sample. The kit exhibits high specificity for L-Lactate, with no cross-reactivity toward D-Lactate, and offers high detection sensitivity. Meanwhile, it is suitable for quantitative analysis of diverse samples, including cells, tissues, and plasma.

Components and Storage

Components \ Size	100 Assays	Storage
Lactate Lysis Buffer	20 mL	-20°C
Lactate Assay Buffer	20 mL	-20°C
Enzyme Solution	200 µL	-20°C
Substrate	200 µL	-20°C
WST-8	200 µL	-20°C away from light
L-Lactate Standard (100 mM)	20 µL	-20°C

Shipping: Blue ice **Shelf life:** 1 year

Protocol

1. Sample preparation

1) Serum samples: Keep whole blood at room temperature for 0.5-2 h without vigorous shaking to avoid hemolysis. After the whole blood naturally coagulates, centrifuge at 4°C, 1000-2000 × *g* for 10 min. Take

the yellow supernatant as serum. Transfer the serum to ice for later use.

2) Plasma samples: Collect whole blood anticoagulated with heparin or EDTA, then centrifuge at 4°C, 1000-2000 × *g* for 10 min. Take the yellow supernatant as plasma. Transfer plasma to ice for later use.

3) Tissue samples: Add 100 µL of Lactate Lysis Buffer per 10 mg sample to homogenize on ice. Centrifuge at 4°C, 12,000 × *g* for 10 min, and transfer the supernatant on ice for later use.

4) Cell samples: For adherent cells, wash the cells once with PBS. For suspension cells, collect the cells by centrifugation. Add 100-200 µL of Lactate Lysis Buffer per 1×10^6 cells, pipette appropriately, then incubate on ice for 5-10 min to ensure complete lysis. Centrifuge at 4°C, 12,000 × *g* for 3-5 min, and transfer the supernatant on ice for later use.

5) Cell culture supernatant: Collect directly and keep on ice for later use.

2. Deproteinization: Deproteinize samples using 10kD Spin Columns or a TCA Deproteinization Sample Preparation Kit. Keep deproteinized samples on ice. If not used immediately, aliquot and store at -20°C or -80°C for short-term storage. Thaw frozen samples completely and mix well before use.

Note: It is recommended to use the TCA Deproteinization Sample Preparation Kit (Cat. No. K4064).

3. Sample dilution: Dilute samples as appropriate prior to assay. This table is for reference only. For tissue or cell samples, use Lactate Lysis Buffer for dilution. For other samples such as blood, use Lactate Assay Buffer for dilution.

Sample	Dilution factor
10% mouse liver	5-40
10% mouse kidneys	5-40
Mouse serum	100-1000
Cell lysate	Undiluted

4. Reagent preparation

1) Allow Lactate Lysis Buffer and Lactate Assay Buffer to warm to room temperature and mix thoroughly. Keep all other reagents on ice. Ensure WST-8 is completely thawed before use. Return all reagents to storage immediately after use.

2) WST-8 working solution preparation: Prepare the WST-8 working solution according to the table below. The prepared working solution can be stored at 4°C or on ice and should be used within the same day (preferably immediately after preparation).

Number of samples	1	10	20
Lactate Assay Buffer	44 µL	440 µL	880 µL
Enzyme Solution	2 µL	20 µL	40 µL
Substrate	2 µL	20 µL	40 µL
WST-8	2 µL	20 µL	40 µL
Total volume	50 µL	500 µL	1000 µL

Note: Enzyme Solution is prone to sedimentation; briefly centrifuge before use and mix well.

3) (optional) Preparation of background control working solution: NADH, NADPH, or other reducing substances in the sample may interfere with L-lactate quantification. If such interference is suspected, prepare a background control working solution by replacing the Enzyme Solution with an equal volume of Lactate Assay Buffer.

5. Standard preparation

1) Add 1 µL of L-Lactate Standard (100 mM) to 199 µL of Lactate Lysis Buffer or Lactate Assay Buffer to make a 0.5 mM L-Lactate Standard.

2) Pipette 0, 2, 5, 10, 20, and 50 µL of 0.5 mM L-Lactate Standards into a 96-well plate. Adjust the volume to 50 µL/well with the corresponding Lactate Lysis Buffer or Lactate Assay Buffer. At this time, the concentrations of L-Lactate in each well are 0, 0.02, 0.05, 0.1, 0.2, and 0.5 mM, respectively.

Note: For tissue or cell samples, use Lactate Lysis Buffer for standard dilution; For blood or other samples, use Lactate Assay Buffer for dilution.

6. Assay procedure

1) Set up reaction wells following the table below. Use the same buffer for the blank control as for sample or standard dilution.

Standards	50 µL standard dilutions
Samples/Background control (optional)	50 µL of sample (diluted or undiluted)
Blank control	50 µL Lactate Lysis Buffer or Lactate Assay Buffer

2) Add 50 µL WST-8 working solution to each standard, sample, and blank control well. Add 50 µL of background control working solution to the background control well. Mix well and incubate at 37°C in the dark for 30 min.

Note: The optimal incubation time can be adjusted according to the specific experiment. If the color development is lighter, it can be extended to 45-60 min. If the color development is darker, it can be shortened to 15-20 min.

3) Measure absorbance at 450 nm.

7. Calculation

1) Subtract the absorbance of the zero-concentration standard from all standards readings. This is the corrected absorbance. Plot the corrected standard curve.

2) Subtract the reading of blank control from the sample (If the sample background is significant, subtract the sample background from the sample reading). Apply the corrected sample readings to the standard curve to obtain the concentration of L-Lactate (A).

C (mM) = A×n

Where:

A = L-Lactate concentration (mM) of the sample in the reaction system.

n = sample dilution factor

3) To convert to mass concentration:

L-Lactate (μg/mL) = C×0.08907

Where 89.07 g/mol is the molecular weight of L-lactate

Note

1. This kit is highly specific for L-Lactate and does not cross-react with D-Lactate.
2. Precipitation may occasionally occur when the substrate is thawed from -20°C storage. The precipitate will fully dissolve upon equilibration to room temperature and does not affect assay performance.
3. For your safety and health, please wear lab coats and gloves during the experiment.
4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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