L-Lactate Assay Kit (Colorimetric/Fluorometric)

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 provides a simple, sensitive assay for L-Lactate quantification. L-Lactate is oxidized by lactate oxidase to pyruvate and hydrogen peroxide (H_2O_2), which drives the Amplex Red reaction in the presence of horseradish peroxidase to form highly fluorescent resorufin. Fluorescence intensity and absorbance correlate linearly with L-Lactate concentration. The kit is compatible with serum, plasma, cells, tissues, and other sample types, and specifically detects L-Lactate without cross-reactivity to D-lactate.

Components and Storage

Components \ Size	100 Assays	Storage
Lactate Lysis Buffer	20 mL	-20°C
Lactate Assay Buffer	20 mL	-20°C
Amplex Red	200 µL	-20°C away from light
Enzyme Solution A	200 µL	-20°C
Enzyme Solution B	200 µL	-20°C
L-Lactate Standard (5 mM)	200 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 using heparin or EDTA as anticoagulant, then centrifuge at 4°C, 1000-2000 × *g* for 10 min. Take the yellow supernatant as plasma. Transfer the 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. If turbidity is observed, centrifuge again until a clear supernatant is obtained.

4) Cell samples: For adherent cells, wash the cells once with PBS. For suspension cells, centrifuge at 500 × g for 5 min to collect cells. Add 100-200 µL of Lactate Lysis Buffer per 1×10^6 cells, then gently pipette up and down. Incubate on ice for 5-10 min to ensure complete lysis. Centrifuge at 4°C, 12,000 × *g* for 10 min, and transfer the supernatant on ice for later use.

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

***Note:** If samples cannot be assayed immediately, aliquot and store at -20°C or -80°C. Thaw frozen samples on ice and mix well before use.

2. Sample dilution: Dilute samples as appropriate prior to assay. This table is for reference only. For serum/plasma samples, use Lactate Assay Buffer as the diluent; for tissue/cell lysates, use Lactate Lysis Buffer.

Sample	**Dilution factor**
10% mouse liver	100-500
10% mouse kidneys	100-500
Mouse serum	500-4000
Cell lysate	5-40

3. Reagent preparation

1) Warm Lactate Assay Buffer and Amplex Red to room temperature in advance. Keep other components on ice. Return to -20°C immediately after use.

***Note:** It is recommended to aliquot all kit components except Buffer to minimize freeze-thaw cycles.

2) Preparation of Detection Working Solution: Prepare a fresh working solution according to the table below. Each reaction requires 50 µL. The prepared working solution can be kept at 4°C or on ice and used within the same day (use as soon as possible).

3) (optional) Preparation of Background Control Working Solution: Endogenous hydrogen peroxide in samples may interfere with detection. If present, include a background control using this solution instead of the detection working solution.

	Detection Working Solution	Background Control Working Solution (optional)
Lactate Assay Buffer	44 µL	46 µL
Amplex Red	2 µL	2 µL
Enzyme Solution A	2 µL	-

Enzyme Solution B	2 μL	2 μL
Total volume	50 μL	50 μL

4. L-Lactate standard preparation

1) For colorimetric assay: dilute an appropriate amount of L-Lactate Standard (5 mM) to 50 μM with Lactate Lysis Buffer or Lactate Assay Buffer. Pipette 0, 2, 5, 10, 20, and 50 μL of L-Lactate Standard (50 μM) into a 96-well plate. Adjust the volume to 50 μL/well with Lactate Lysis Buffer or Lactate Assay Buffer to obtain final concentrations of 0, 2, 5, 10, 20, and 50 μM.

2) For fluorescence assay: dilute an appropriate amount of L-Lactate Standard (5 mM) to 20 μM with Lactate Lysis Buffer or Lactate Assay Buffer. Pipette 0, 2, 5, 12.5, 25, and 50 μL of L-Lactate Standard (20 μM) into a 96-well plate. Adjust the volume to 50 μL/well with Lactate Lysis Buffer or Lactate Assay Buffer to obtain final concentrations of 0, 1, 2, 5, 10, and 20 μM.

***Note:**

a) For serum/plasma samples, use Lactate Assay Buffer as the diluent; for tissue/cell lysates, use Lactate Lysis Buffer.

b) Fluorescence detection is preferred for small-volume samples.

c) Use black 96-well plates for fluorescence assays.

5. L-Lactate concentration detection

1) Set up reaction wells following the table below.

Standards	50 μL standard dilutions
Samples/Background control (optional)	Add 1-50 μL of sample, adjust the volume to 50 μL/well with Lactate Lysis Buffer or Lactate Assay Buffer
Blank control	50 μL Lactate Lysis Buffer or Lactate Assay Buffer

***Note:**

a) For serum/plasma samples, use Lactate Assay Buffer as the diluent; for tissue/cell lysates, use Lactate Lysis Buffer.

b) If needed, pre-dilute the sample to ensure readings fall within the standard curve range. The total dilution factor (d) is calculated as: d = (Pre-dilution Factor) × (50 μL / Sample Volume Added). For example, if a sample is diluted 5-fold and 4 μL is added, then d = 5 × (50/4) = 62.5.

2) Add 50 μL of Detection Working Solution to each standard, sample, and blank control well. Add 50 μL of Background Control Working Solution to the background control wells. Mix gently and incubate at 37°C in the dark for 30 min.

***Note:** The optimal incubation time can be adjusted according to specific experiments. If the detection signal is weak, the incubation time can be appropriately extended.

3) Colorimetric detection: Measure absorbance at 570 nm.

Fluorescence detection: Measure fluorescence intensity (Ex/Em = 560/590 nm).

6. Calculation

a) Plot the corrected standard curve.

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

C (μM) = A×d

Where:

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

d = sample dilution factor

c) To convert to mass concentration:

L-Lactate (μg/mL) = C×0.08907

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

Note

1. Amplex Red is unstable in air and light-sensitive. Use immediately after opening and protect from light.
2. The reaction product of Amplex Red is very unstable in the presence of reducing agents; therefore, the concentration of DTT, β-mercaptoethanol, and similar reducing agents in the reaction system should be lower than 10 μM.
3. The pH of the reaction system needs to be between 7-8; otherwise, it will affect the stability of Amplex Red and the final detection value.
4. Amplex Red and Lactate Assay Buffer must be completely thawed and warmed to room temperature before use; otherwise, it will affect the detection result. Other reagents should be kept on ice during use.
5. For your safety and health, please wear lab coats and gloves during the experiment.
6. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com