

L-Lactate Assay Kit (WST-8)

L1373335

Storage: -20°C. Protect from light.

Introduction

L-lactate is produced by the brain, red blood cells, skeletal muscle, and the intestine, and is metabolized by the liver, heart, and kidneys. Almost all lactate produced in the human body is L-lactate. L-lactate is primarily cleared from the blood by the liver; however, hypoxia, acidosis, impaired oxygen supply, and hypoperfusion can compromise hepatic uptake of L-lactate. The concentration of L-lactate in blood is critical for diagnosing patient conditions during intensive care and surgical procedures. Elevated levels of L-lactate can indicate conditions such as intestinal infarction, cardiac arrest, and sepsis, while the concentration of L-lactate in saliva can serve as an early diagnostic marker for cystic fibrosis. L-lactate also acts as a biomarker for energy metabolism, as well as for brain and tumor cells. In the food industry, L-lactate is used as an indicator of quality, freshness, flavor, stability, and shelf life during fermentation processes.

The detection principle of this kit is as follows: L-lactate is oxidized to pyruvate by L-lactate dehydrogenase (L-LDH), during which NAD^+ is reduced to NADH. The generated NADH then reduces WST-8 to an orange-yellow formazan dye via the electron coupling reagent 1-mPMS (1-Methoxy-5-methylphenazinium methyl sulfate). The formazan produced exhibits a maximum absorption peak around 450 nm. The amount of formazan generated in the reaction system is proportional to the L-lactate concentration in the sample. With a sample volume of 50 μL , the kit can detect L-lactate levels as low as 20 μM and demonstrates a good linear range from 0.02 mM to 0.5 mM (1 nmol to 25 nmol).

Kit Contents

L1373335	Component	50 T	100 T	500T	Storage conditions
L1373335A	L-Lactate Lysis Buffer	10 mL	20 mL	100 mL	-20°C.
L1373335B	L-Lactate Assay Buffer	10 mL	20 mL	100 mL	-20°C.
L1373335C	Coenzyme	200 μL	400 μL	2000 μL	-20°C.Store in the dark.
L1373335D	L-Lactate Standard (100 mM)	100 μL	200 μL	1000 μL	-20°C.Store in the dark.
L1373335E	WST-8	100 μL	200 μL	1000 μL	-20°C.Store in the dark.
L1373335F	1-mPMS	100 μL	200 μL	1000 μL	-20°C.Store in the dark.
L1373335G	Enzyme Solution	100 μL	200 μL	1000 μL	-20°C.Store in the dark.

Instruction for use

1. Sample Preparation

1) Preparation of Blood Samples:

For serum samples: Allow whole blood to clot at room temperature for 30 minutes to 2 hours. Centrifuge at approximately 1000-2000 × g for 10 minutes at 4°C and collect the supernatant. For plasma samples: Collect whole blood using heparin or EDTA as an anticoagulant. Centrifuge at approximately 1000-2000 × g for 10 minutes at 4°C and collect the supernatant.

2) Preparation of Cell Samples:

For cultured adherent cells: Wash the cells once with PBS; For cultured suspension cells: Centrifuge to collect the cells and wash them once with PBS. Add Lysis Buffer at a ratio of 100-200 µL per 1 million cells. Pipette appropriately to mix and incubate on ice for 5-10 minutes for complete lysis. Centrifuge at 12,000 × g for 5 minutes at 4°C. Collect the supernatant for subsequent assays.

3) Preparation of Tissue Samples:

Add Lysis Buffer at a ratio of 100 µL per 10 mg of tissue. Homogenize the tissue using a homogenizer on ice. Centrifuge at approximately 12,000 × g for 5 minutes at 4°C. Collect the supernatant for subsequent assays.

4) Preparation of Cell Culture Supernatant Samples:

For adherent cells: Directly collect the culture medium; For suspension cells: Centrifuge the culture and collect the supernatant.

All the above procedures must be performed at 4°C or on ice. Prepared cell or tissue samples can be stored at -20°C or -80°C if not assayed immediately.

2. Establishment of L-Lactate Standard Curve

Take 2 µL of L- Lactate Standard (100 mM) and mix it with 398 µL of either L- Lactate Lysis Buffer or L- Lactate Assay Buffer (use L- Lactate Lysis Buffer for cell or tissue lysate samples; use L- Lactate Assay Buffer for other samples such as blood) to prepare 400 µL of 0.5 mM L- Lactate Standard. Then, add 0, 2, 5, 10, 20, and 50 µL of the 0.5 mM L-Lactate Standard into the standard wells of a 96-well plate, respectively. Bring the volume in each well to 50 µL by adding the corresponding L-Lactate Lysis Buffer. As a result, the L-lactate concentrations in the standard curve wells are 0, 0.02, 0.05, 0.1, 0.2, and 0.5 mM, corresponding to amounts of 0, 1, 2.5, 5, 10, and 25 nmol, respectively.

3. Preparation of WST-8 Working Solution

Prepare the WST-8 Working Solution according to the table below. Note: This procedure must be performed on ice.

Samples	1	10	20	50
L-Lactate Assay Buffer	40 μ L	400 μ L	800 μ L	2000 μ L
Enzyme Solution	2 μ L	20 μ L	40 μ L	100 μ L
Coenzyme	4 μ L	40 μ L	80 μ L	200 μ L
WST-8	2 μ L	20 μ L	40 μ L	100 μ L
1-mPMS	2 μ L	20 μ L	40 μ L	100 μ L
Total volume	50 μ L	500 μ L	1000 μ L	2500 μ L

4. Sample Assay

- 1) Add 1–50 μ L of sample or diluted sample to the sample wells of a 96-well plate, and supplement with L-Lactate Lysis Buffer to a total volume of 50 μ L. A blank control containing only L-Lactate Lysis Buffer should also be included.
- 2) Add 50 μ L of WST-8 chromogenic working solution to each well and mix thoroughly. Incubate the plate at 37 °C in the dark for 30 minutes. If color development is weak, the reaction time may be extended to 45 or 60 minutes; if color intensity is high, the incubation time may be shortened to 15 or 20 minutes.
- 3) After the reaction, measure the absorbance at 450 nm using a microplate reader.

5. Calculation of Results

Subtract the signal value of the zero-concentration standard well from that of each standard curve well. Generate a standard curve and calculate the L-lactate concentration in the sample (A). The signal value of the sample well should be corrected by subtracting the value of the blank control well. If the sample background signal is high, subtract the sample background control value accordingly.

The formula for L-lactate concentration is as follows: $C \text{ (mM)} = A \times n$

Note 1: A represents the L-lactate concentration (mM) of the diluted sample as determined from the standard curve; n is the sample dilution factor.

Note 2: The calculated L-lactate concentration includes the molar concentration of both L-lactic acid and L-lactate ions, which may also be interpreted as the total molar concentration of L-lactic acid and L-lactate salt. For simplicity, the term “L-lactate” is used throughout this description. If required, the mass concentration of L-lactate ions (μ g/mL) can be calculated using the molecular weight of L-lactate ion (89.07) as follows: L-lactate ion mass concentration (μ g/mL) = $C \times 0.08907$

Matters needing attention

1. Lysis Buffer and Assay Buffer must be completely thawed and equilibrated to room temperature before use, as otherwise the detection results may be affected.
2. The presence of NADH, NADPH, or other substances that influence NADH/NADPH levels may interfere with the assay. If the sample contains potential interfering substances, a

sample background control well must be set up by replacing the Enzyme Solution in the WST-8 chromogenic working solution with Lactate Assay Buffer. During calculation, subtract the absorbance value of the background control well from that of the sample well.

3. To ensure that sample values fall within the standard curve range, it is recommended to perform a preliminary experiment with multiple dilution factors to estimate the approximate L-lactate concentration in the sample. If values lie outside the standard curve range, adjust the sample dilution factor accordingly.
4. Aliquot the components according to experimental needs to avoid repeated freeze-thaw cycles.

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