L-Lactic Acid (LA) Assay Kit

Catalog No.: abx294033

Size: 96 tests

Detection Range: 0.05 mmol/L – 6.0 mmol/L

Sensitivity: 0.05 mmol/L

Storage: Store all components at 4 °C. Store the Chromogenic Reagent in the dark.

Application: For detection and quantification of L-Lactic Acid content in serum, plasma, tissue homogenates, cell lysates, and cell culture supernatant.

Introduction

Abbexa's L-Lactic Acid Assay Kit is a quick, convenient, and sensitive method for measuring and calculating L-Lactic Acid content. Lactate Dehydrogenase (LDH) catalyzes the conversion of Lactate into Pyruvic Acid, while concurrently NAD^+ is reduced to NADH. The action of 1-Methoxy-5-methyl phenazine methyl sulfate (PMS) transfers Hydrogen from NADH to the azolium dye NBT, producing a purple-colored compound which has an absorbance maximum of 530 nm. The intensity of the color is proportional to the L-Lactic Acid content, which can then be calculated.

Kit components

1. Buffer Solution: 2 × 60 ml
2. Enzyme Stock Solution: 1.2 ml
3. Chromogenic Reagent: 24 ml
4. Stop Solution: 4 × 60 ml
5. Standard Solution (3 mmol/L): 2 ml

Materials required but not provided

- Spectrophotometer (530 nm)
- Double distilled water
- Normal Saline (0.9 % NaCl)
- PBS (0.01 M, pH 7.4)
- Pipette and pipette tips
- 5 ml sterile tubes
- Centrifuge
- Vortex mixer
- Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum and Plasma:** Collect using conventional methods, samples can be assayed directly.
- **Tissue Homogenates:** Carefully weigh 0.02 g - 1 g of tissue and wash, then add PBS (0.01 M, pH 7.4) or Normal Saline (0.9 % NaCl) to a 9:1 ratio of media:tissue weight. Homogenize manually, using a mechanical homogenizer or by ultrasonication. Centrifuge at 10,000 × g for 10 minutes at 4 °C, then collect the supernatant and assay immediately. The protein content should be determined separately **(abx097193)**. Non-homogenized tissue samples may be stored at -80 °C for up to 1 month.
- **Cell Lysates:** Collect cells and wash with PBS (0.01 M, pH 7.4) or Normal Saline (0.9 % NaCl). Centrifuge at 1000 × g for 10 minutes and discard the supernatant. Add PBS (0.01 M, pH 7.4) or Normal Saline (0.9 % NaCl) to the cell pellet to a ratio of cell count (**n** × 10^6):media of 1:300 – 500 and homogenize manually on ice or by ultrasonication. Centrifuge at 10,000 × g for 10 minutes at 4 °C, then collect the supernatant and assay immediately. The protein content should be determined separately **(abx097193)**. Non-homogenized cells may be stored at -80 °C for up to 1 month.
- **Cell Culture Supernatant:** Centrifuge at 10,000 × g for 10 minutes at 4 °C, then collect the supernatant and assay immediately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with PBS (0.01 M, pH 7.4) or Normal Saline (0.9 % NaCl), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human Serum	4 – 8
Rat Serum	4 – 8
10 % Mouse muscle tissue homogenate	2 – 4
10 % Mouse liver tissue homogenate	1
HePG2 cells homogenate (1.388 gprot/L)	4 – 8
HepG2 Cell culture supernatant	2 – 4

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Enzyme Working Solution:** Dilute Enzyme Stock Solution 100-fold with Buffer Solution. Prepare fresh working solution before each experiment.

B. Assay Procedure

1. Set standard, sample, and blank tubes and add 20 µl Lactic Acid standard solution, 20 µl sample, or 20 µl double distilled water to tubes respectively.
2. Add 1 ml Enzyme Working Solution and 200 µl Chromogenic Reagent and mix fully.
3. Incubate at 37 °C for 10 minutes.
4. Add 2 ml of Stop Solution and mix fully.
5. Using 1 cm optical path cuvettes, set the spectrophotometer to zero using double distilled water, then measure the OD for each sample at 530 nm.

Notes:

- The stated time for reaction steps must be followed accurately.
- Absorbances must be read within 30 minutes after adding Stop Solution.

C. Calculation of Results

1. Serum, Plasma, and Cell Culture Supernatant samples:

$$\boldsymbol{Lactic\ Acid\ Content\ (mmol/L)} = \frac{\boldsymbol{\Delta A_1}}{\boldsymbol{\Delta A_2}} \times \boldsymbol{c} \times \boldsymbol{f}$$

2. Tissue Homogenate and Cell Lysate samples:

$$\boldsymbol{Lactic\ Acid\ Content\ (mmol/gprot)} = \frac{\boldsymbol{\Delta A_1}}{\boldsymbol{\Delta A_2}} \times \frac{c \times f}{C_{Protein}}$$

where:

ΔA_1	$OD_{Sample} - OD_{Blank}$
ΔA_2	$OD_{Standard} - OD_{Blank}$
c	Concentration of standard (3 mmol/L)
f	Sample dilution factor
$C_{Protein}$	Concentration of protein in sample (gprot/L)