Lactate Assay Kit

Catalog No.: abx298983

Size: 96 tests

Detection Range: 0.06 mmol/L – 8.0 mmol/L

Sensitivity: 0.06 mmol/L

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

Application: For detection and quantification of Lactate content in serum, plasma, and tissue homogenates.

Introduction

Abbexa's Lactate Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Lactate content. D-Lactate Dehydrogenase catalyzes the reaction of Lactate with NAD+ to produce Pyruvic acid and NADH respectively. Concurrently, NBT is reduced, producing a purple-colored product with an absorbance maximum at 530 nm. The intensity of the color is proportional to the Lactate content, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer Solution: 12 ml
3. Enzyme Stock Solution: 120 µl
4. Chromogenic Reagent: 2 × 1.2 ml
5. Stop Solution: 24 ml
6. Standard (10 mmol/L): 2 ml
7. Plate sealer: 2

Materials required but not provided

1. Microplate reader (530 nm)
2. Double Distilled water
3. Normal Saline (0.9 % NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. 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/Plasma:** Serum samples can be tested directly.
- **Tissue Homogenates:** Carefully weigh approximately 20 mg of tissue and wash in cold PBS (0.01 M, pH 7.4). Homogenize the tissue at 4 ºC in 1:9 weight/volume PBS (e.g. 20 mg tissue, 180 µl PBS), then centrifuge at 10,000 × g for 10 minutes. Collect the supernatant and store on ice for detection. The protein content of the sample should be determined separately (**abx097193**).

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 normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human plasma	1
Human serum	1
Rat plasma	1
Rat serum	1
Mouse serum	1
Rabbit serum	1
10 % Rat Kidney tissue homogenate	2-3
10 % Mouse brain tissue homogenate	1

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 Stock Solution: Keep on ice for use.
- Enzyme Working Solution: Prepare fresh before use. Dilute Enzyme Stock Solution in Buffer Solution to a 1:100 ratio (e.g. add 5 µl Enzyme Stock Solution to 500 µl Buffer Solution).
- **Standards:** Prepare a fresh set of standards before each run, as summarized in the following table:

Standard Concentration (mmol/L)	**0**	**1**	**2**	**4**	**5**	**6**	**7**	**8**
10 mmol/L Standard (µl)	0	20	40	80	100	120	140	160
Double distilled water (µl)	200	180	160	120	100	80	60	40

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

1. Add 5 µl of standard to the standard wells, and 5 µl of sample to the sample wells.
2. Add 100 µl of Enzyme Working Solution to each well.
3. Add 20 µl of Chromogenic Reagent to each well.
4. Mix thoroughly and incubate at 37 ºC for 10 minutes.
5. Add 180 µl of Stop Solution to each well and mix thoroughly.
6. Measure the OD of each well with a microplate reader at 530 nm.

C. Calculation of Results

Plot the standard curve, using the average OD of duplicate standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Lactate in each sample well can be derived with the following formulae:

1. Serum and Saliva samples:

$$\text{Lactate (mmol/L)} = \frac{\Delta \boldsymbol{A_{530}} - \boldsymbol{b}}{\boldsymbol{a}} \times \boldsymbol{f}$$

2. Tissue samples:

$$\text{Lactate (mmol/g protein)} = \frac{\Delta \boldsymbol{A_{530}} - \boldsymbol{b}}{\text{a}} \times \frac{\boldsymbol{f}}{\boldsymbol{C_{pr}}}$$

where:

ΔA_{530}	$OD_{Sample} - OD_{Blank}$
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
f	The dilution factor of sample
C_{pr}	Protein concentration of sample (g prot/L)