Lactate Dehydrogenase Assay Kit

Catalog No.: abx294035

Size: 100 Assays

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

Application: For quantitative detection of Lactate Dehydrogenase activity in serum, plasma, tissue homogenates, and cell lysates.

Detection Range: 4 U/L – 400 U/L

Sensitivity: 4 U/L

Introduction: Lactate Dehydrogenase (LDH) is an enzyme that catalyzes the reversible interconversion of pyruvate and lactate, and simultaneously NADH and NAD^+. It is present in a wide variety of organisms and is expressed extensively in animal tissues. It is released into the bloodstream during tissue damage, and can therefore be used as a marker for many diseases and injuries.

Abbexa's Lactate Dehydrogenase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Lactate Dehydrogenase activity. Lactate Dehydrogenase catalyses the production of pyruvic acid from lactic acid under the action of NAD^+, which then interacts with a probe to produce an absorbance maxima at 450 nm. The absorbance can be measured, from which the enzyme activity can be calculated.

Kit components

1. Substrate Buffer: 30 ml
2. Coenzyme: 1 vial
3. Detection Reagent: 30 ml
4. Alkali Reagent: 30 ml
5. Standard (2 μmol/ml): 5 ml

Materials Required But Not Provided

1. Spectrophotometer and cuvettes (450 nm, 1 cm optical path)
2. Incubator or water bath (37°C)
3. Centrifuge and microcentrifuge tubes
4. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
5. High-precision pipette and sterile pipette tips
6. Double distilled water
7. Timer
8. Ice
9. Sonicator
10. Mortar

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Coenzyme Working Solution**

  Add 6.65 ml of double distilled water into the Coenzyme vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Coenzyme has completely dissolved prior to use. The prepared solution can be stored at -20°C for up to 2 weeks.

- **Alkali Reagent Working Solution**

  Dilute the Alkali Reagent 10-fold with double distilled water. Prepare fresh before use. The prepared solution can be stored at 4°C for up to 7 days.

2. Sample

- **Cell lysates:** Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge at 1000 × g for 10 minutes to precipitate the cells. Discard the supernatant, and add 300 µl of PBS for every 1,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately. The protein concentration of the supernatant should be determined separately (abx097193).

- **Tissue homogenates:** Homogenize 0.1 g of sample in 0.9 ml of Assay Buffer on ice for 1 hour. Centrifuge at 1500 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately. The protein concentration of the supernatant should be determined separately (abx097193).

- **Serum:** Collect the serum using a serum separator tube and allow to stand for 1-2 h at room temperature or overnight at 4°C. Centrifuge for 20 min at 1000. Transfer the supernatant into a clean tube and analyse immediately. Bring samples to room temperature before carrying out the assay.

- **Plasma:** Collect the plasma in a tube using heparin as an anticoagulant. Centrifuge for 15 min at 1000 × g at 2-8°C within 30 min of collection. Transfer the supernatant into a clean tube and analyse immediately. Bring samples to room temperature before carrying out the assay.

Notes

- Tissue homogenates should be assayed immediately after preparation to prevent degradation of the sample.
- Avoid the use of detergents or reducing agents, as these may interfere with the assay.
- Where required, samples should be diluted with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

Sample type	Dilution factor
Human serum	10-15
Human plasma	10-15
Pig serum	20-30
Rat serum	20-30
Rat plasma	20-30
10% Mouse kidney tissue homogenate	500-800
10% Rat lung tissue homogenate	300-500
10% Rat liver tissue homogenate	800-1000

B. Assay Procedure

Bring all reagents, except Coenzyme, to room temperature prior to use.

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Label 5 tubes with 0.8 μmol/ml, 0.6 μmol/ml, 0.4 μmol/ml, 0.2 μmol/ml and 0.1 μmol/ml, 0.05 μmol/ml and 0 μmol/ml. Prepare the standard curve according to the following table.

Volume of 2 μmol/ml Standard (μl)	Volume of double distilled water (μl)	Concentration (μmol/ml)
0	1000	0.00
25	975	0.05
50	950	0.10
100	900	0.20
200	800	0.40
300	700	0.60
400	600	0.80

2. Set the sample, standard, and control tubes and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 200 μl of sample to the sample tubes and control tubes.
4. Add 200 μl of prepared standards to the standard tubes. Add 50 μl of double distilled water to the standard tubes.
5. Add 50 μl of double distilled water to the control tubes.
6. Add 250 μl of Substrate Buffer to all tubes.
7. Add 50 μl of Coenzyme Working Solution to the sample tubes. Mix the solution by aspirating the pipette tip several times.
8. Mix all tubes with a vortex mixer and incubate at 37°C for 15 minutes.
9. Add 250 μl of Detection Reagent to all tubes. Mix all tubes with a vortex mixer and incubate at 37°C for 15 minutes.
10. Add 2.5 ml of Alkali Reagent Working Solution to all tubes. Mix fully with a vortex.
11. Allow to stand at room temperature for 5 minutes accurately.
12. Calibrate the spectrophotometer to zero with double distilled water (450 nm, 1 cm optical path cuvette).
13. Read and record absorbance at 450 nm.

C. Calculations

The standard curve can be plotted as the absolute OD450 of each standard solution (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve (y = ax + b). The LDH activity of the samples can be interpolated from the standard curve.

1. Serum and plasma

One Unit (U) of Lactate Dehydrogenase activity is defined as the quantity of enzyme required to produce 1 µmol of pyruvate in 1 L of sample at 37°C in 15 minutes.

$$\mathbf{LDH\ (U/L)} = \mathbf{f} \times \mathbf{1000} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}}{\mathbf{a}}$$

2. Tissue homogenates and cell lysates

One Unit (U) of Lactate Dehydrogenase activity is defined as the quantity of enzyme required to produce 1 µmol of pyruvate in 1 g of protein at 37°C in 15 minutes.

$$\mathbf{LDH\ (U/g)} = \frac{\mathbf{f} \times \mathbf{1000}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}}{\mathbf{a}}$$

where:

$\mathbf{OD_{Sample}}$	OD value of sample
$\mathbf{OD_{Control}}$	OD value of control (0 µmol/ml standard)
$\mathbf{C_{Protein}}$	Concentration of protein (in g/L)
$\mathbf{f}$	Dilution factor of sample before assay
$\mathbf{a}$	Gradient of the standard curve (y = ax + b)
$\mathbf{b}$	Intercept of the standard curve (y = ax + b)
$\mathbf{1000}$	Conversion factor (1 L = 1000 ml)