Lactate Dehydrogenase Assay Kit

Catalog No.: abx096006

Size: 100 Assays

Storage: Store the substrate and positive control at -20°C and all components at 4°C.

Application: For quantitative detection of Lactate Dehydrogenase activity in serum, plasma, tissue homogenates cell lysates, cell culture supernatants, urine and other biological fluids.

Detection Range: 30 µmol/L – 600 µmol/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 reduces NAD^+ to NADH, 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. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer: 8 ml
4. Dye Reagent 1: 1 vial
5. Dye Reagent 2: 1 ml
6. Substrate: 1 vial
7. Standard: 1 vial
8. Positive control: 1 vial
9. Plate sealer: 3

Materials Required But Not Provided

1. Microplate reader (450 nm)
2. Incubator
3. Centrifuge and microcentrifuge tubes
4. High-precision pipette and sterile pipette tips
5. Distilled water
6. Timer
7. Ice
8. Sonicator
9. Mortar

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 1 ml of distilled water into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use. Store at -20°C.
- **Dye Reagent 1 Solution**
  Add 9 ml of distilled water into the Dye Reagent 1 vial and mix thoroughly to prepare the Dye Reagent 1 Solution. Ensure that the Dye Reagent 1 has completely dissolved prior to use. Store at 4°C.
- **Standard Solution**
  Add 1 ml of distilled water into the Standard vial and mix thoroughly. Take 300 µl of this solution and add to 700 µl of distilled water to prepare the Standard Solution (concentration 600 µmol/L). Ensure that the Standard has completely dissolved prior to use.
- **Positive Control Solution**
  Add 100 µl of distilled water into the Positive Control vial and mix thoroughly to prepare the Positive Control Solution. Ensure that the Positive Control has completely dissolved prior to use.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant, and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice for 1 hour. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

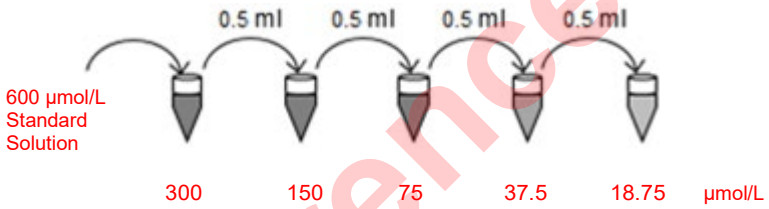
- **Serum and plasma samples**
  Serum and plasma samples can be used directly.

B. Assay Procedure

Bring all reagents 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 300 µmol/L, 150 µmol/L, 75 µmol/L, 37.5 µmol/L and 18.75 µmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 600 µmol/L Standard Solution to the 1st tube, and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard, positive control, blank and control wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 10 µl of sample to the sample wells.
4. Add 100 µl of prepared standard to the standard wells.
5. Add 10 µl of positive control to the positive control well.
6. Add 80 µl of Reaction Buffer to sample, positive control and control wells.
7. Add 10 µl of Substrate Solution to sample, positive control and control wells.
8. Add 10 µl of distilled water to the control well.
9. Add 100 µl of distilled water to the blank well.
10. Tap the plate gently to mix.
11. Add 90 µl of Dye Reagent 1 Solution to all wells.
12. Add 10 µl of Dye Reagent 2 to all wells.
13. Tap the plate gently to mix. Allow to stand at room temperature for 5 minutes.
14. Read and record absorbance at 450 nm.

C. Calculations

One Unit (U) of Lactate Dehydrogenase activity is defined as the quantity of enzyme required to produce 1 nmol of pyruvate per minute.

Lactate Dehydrogenase activity per ml of serum or plasma:

$$\mathbf{LDH\ (U/ml)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample} \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \mathbf{1200} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Lactate Dehydrogenase activity per mg of protein:

$$\mathbf{LDH\ (U/mg)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample} \times C_{Protein} \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{1200}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Lactate Dehydrogenase activity per g of sample:

$$\mathbf{LDH\ (U/g)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times W \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{1200}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Lactate Dehydrogenase activity per 10^4 cells or bacteria:

$$\mathbf{LDH\ (U/10^4\ cells)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times N \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{1200}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{C_{Standard}}$	Concentration of highest standard (600 µmol/L = 600 nmol/ml)
W	Weight of the sample (in g)
N	Number of cells or bacteria (× 10^4)
$\mathbf{V_{Assay}}$	Volume of assay buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.01 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.1 ml)
T	Reaction time (5 min)