Lactate Dehydrogenase (LDH) Enzymatic Assay Kit Manual

Catalog #: 3460-04

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Lactate Dehydrogenase (LDH) Enzymatic Assay Kit is intended for laboratory use only, unless otherwise indicated.
This product is NOT for clinical diagnostic use.

GENERAL INFORMATION

Product Description

Lactate Dehydrogenase (LDH) is a ubiquitously-expressed intracellular enzyme which catalyzes the reversible oxidation of lactate to pyruvate. LDH is one of the most clinically important protein markers in serum because its level changes in response to a number of health-related states. For example, elevated LDH serum levels are often caused by heart, liver and kidney disease as well as in numerous types of cancer. Also, the presence of elevated levels of the enzyme in serum after administration of drugs and experimental therapeutic agents is associated with organ toxicity. In addition, this enzyme can be used to detect cytotoxicity and cell number in *in vitro* cell culture systems. Therefore, monitoring serum levels of LDH enzyme has become a routine and fundamental means to monitor organ toxicity.

The *Lactate Dehydrogenase (LDH) Enzymatic Assay Kit* is a colorimetric plate-based assay to directly determine the amount of Lactate Dehydrogenase enzyme in samples. This kit enables biomedical researchers to determine LDH levels in serum from rodents, such as mice and rats, and other vertebrates including fish. The assay utilizes a simple, proven colorimetric (UV) enzymatic assay to specifically detect LDH. It provides accurate results even in complex liquid mixtures.

The kit is designed to be used with a microplate reader. It contains an assay standard to construct a linear calibration curve and verify assay performance, and it contains sufficient materials to test 42 samples in duplicate.

The unique features of the kit are:

- High sensitivity and low detection limit (20 U/L)
- A rapid (5 minutes), robust enzyme-based assay which does not require expensive instrumentation
- High reproducibility
- Only requires 5 μL of serum

Procedure Overview

The *Lactate Dehydrogenase (LDH) Enzymatic Assay Kit* measures the concentration of LDH using a direct, plate-based, colorimetric reaction. When serum is added to the reaction mix, the LDH in the sample converts the lactate and NAD+ in the mix to pyruvate and NADH. The production of the NADH product is directly monitored by measuring the increase in absorbance of the reaction at 340 nm over a 5 minute time interval. Dilutions of the standard (included in the kit) can be used to construct a standard curve to calibrate the assay and confirm linearity.

Kit Contents, Storage and Shelf Life

The *Lactate Dehydrogenase (LDH) Enzymatic Assay Kit* has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for serially-diluted standards). Upon receipt of the kit, store the standards and buffer at -20°C and the remainder of the kit at 4°C. When properly stored, the components of the kit will remain stable for 6 months after receipt of the kit. Once the LDH Reagent Mix is reconstituted the shelf life of the kit is 3 months when properly stored. For more details, see "Preparation of Reagent Mix".

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	Room temp or 4°C
LDH Reagent Mix	bottle	4°C
Standard	vial	- 20°C
Standard Dilution Buffer	5 mL	- 20°C

Required Materials/Equipment Not Provided With the Kit

- Microtiter plate reader (*340 nm*)
- Microcentrifuge
- Distilled or deionized water
- 1. 5mL microcentrifuge tubes
- Multichannel pipet (*recommended*)

Sensitivity (Detection Limit)

Sample Type	**Detection Limit** (U/L)
Serum	20

Warnings and Precautions

XpressBio strongly recommends that you read the following warnings and precautions to ensure your full awareness of the techniques and other details you should pay close attention to when running the assays. Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the protocol included with the kit.

- Do not use the kit past the expiration date.
- Try to maintain a laboratory temperature of (20–25°C/68–77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.
- Use only distilled or deionized water since water quality is very important.
- When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.

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SAMPLE PREPARATION

Serum

1. Carefully collect whole blood in a 1.5 mL microfuge tube or serum collection tube making sure to avoid hemolysis as it will release erythrocyte ALT enzyme into the serum.
2. Incubate the blood sample at 37°C for 10 minutes.
3. Centrifuge sample at 10,000 rpm for 10 minutes.
4. Remove serum layer to a clean tube avoiding the "buffy coat" layer.
5. Store serum samples on ice or at 4°C prior to testing; do not freeze samples. Serum samples can be stored at 4°C for up to one week.

LACTATE DEHYDROGENASE DETERMINATION TEST PROTOCOL

Set-up and Reagent Preparation

1. Turn on the plate reader, allow light source to warm up, and set the absorbance wavelength to 340 nm.
2. Warm up kit reagents to room temperature for 30 minutes.
3. Reconstitute the Reagent Mix: Add exactly 27 mL of deionized or distilled water to the LDH Reagent Mix powder. Mix by swirling or inverting the bottle 10 times. Allow contents to dissolve for 10 minutes at room temperature.

IMPORTANT: The reconstituted Reagent Mix can be left at room temperature for short periods (30 min) prior to use. Between uses, the reconstituted Reagent Mix should be stored at 4 °C (for up to 3 months). Discard the Reagent Mix 3 months after reconstitution.

Preparation of Standards

1. Label six clean microcentrifuge tubes 1, 2, 3, 4, 5 and 6 (Neg).
2. Dissolve contents of Standard vial in 920 µL of Standard Dilution Buffer. Mix well and transfer 150 µL of dissolved Standard to Tube 1. Unused remaining portion in vial can be stored at -80°C for 6 months.
3. Serially dilute the standard by adding the appropriate volumes of Standard and Standard Dilution Buffer:

Standard Tube #	Preparation	Equivalent Standard Concentration
1	Add 150 µL of dissolved Standard.	800 U/L
2	Add 75 µL from Standard Tube #1 + 75 µL of Standard Dilution Buffer. Mix thoroughly.	400 U/L
3	Add 75 µL from Standard Tube #2 + 75 µL of Standard Dilution Buffer. Mix thoroughly.	200 U/L
4	Add 75 µL from Standard Tube #3 + 75 µL of Standard Dilution Buffer. Mix thoroughly.	100 U/L
5	Add 75 µL from Standard Tube #4 + 75 µL of Standard Dilution Buffer. Mix thoroughly.	50 U/L

6 (Neg)	Add 100 µL of Standard Dilution Buffer only.	NA

Assay Protocol

1. Add 5 µL of each sample or standard (in duplicate) to microplate wells.
2. Add 250 µL reconstituted LDH Reagent Mix to the wells.
3. Measure absorbance of the wells at 340 nm (= initial reading). Exactly 5 minutes later, read the absorbance again (= 5 min reading).

   Note: If the 5 min reading of a serum sample is >0.8 absorbance units, then dilute the serum 1:1 with saline and retest.

DATA ANALYSIS

Standard Curve Construction

A calibration curve to confirm assay linearity can be constructed using the calibration standards supplied with the kit as follows:

1. For each calibration point, calculate the *average corrected absorbance* by subtracting the average **5 min** absorbance of the "**Neg**" point from the average **5 min** absorbance of each point in the calibration. This calculation should include subtracting the average 5 min absorbance of the "Neg" value from itself, which is zero.
2. For each standard, plot the average corrected absorbance in the y-axis against the corresponding standard concentration (see the "Equivalent Standard Concentration" in the Table above).

Determination of Lactate Dehydrogenase Activity in Serum Samples

1. For each sample, subtract the initial absorbance from the 5 min absorbance. Average these values to obtain the average absorbance increase in 5 minutes for each sample.
2. Multiply the average 5 min absorbance increase by 2,187 (conversion factor) to obtain LDH activity (IU/L).

For example, if the absorbance of a sample increases by 0.3 over 5 minutes then the Lactate Dehydrogenase activity of the sample is:

0.3 x 2187 = 656 IU/L