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IDTox™ Lactate Dehydrogenase Enzyme (LDH) Enzyme Assay Kit

SUP6004

Enzymatic Assay for the determination of the Lactate Dehydrogenase enzyme in serum samples.

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. The enzyme is also 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 features of the kit are:

- Rapid and simple method
- Minimal sample preparation
- Highly accurate and reproducible

PROCEDURE OVERVIEW

The IDTox™ Lactate Dehydrogenase (LDH) Enzymatic Assay Kit uses an enzyme-based assay to determine the amount of Lactate Dehydrogenase in serum and other liquid samples. The kit enables biomedical researchers to determine Lactate Dehydrogenase levels in liquid samples such as serum from rodents such as rats and mice. The kit is designed to be used with a microplate reader. It contains an assay calibrant to construct a linear calibration curve and verify assay performance, and it contains sufficient materials to test 42 samples in duplicate.

The kit measures the concentration of LDH using a direct, plate-based, colorimetric reaction. It provides accurate results even in complex liquid measures. 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 latter product is directly monitored by measuring the increase in absorbance of the reaction at 340 nm over a 5 minute time interval.

KIT REAGENTS SUPPLIED

The 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. ONCE RECONSTITUTED, the shelf life is 3 months when the kit is properly stored.

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	Room temp or 4℃
LDH Reagent Mix	1 Bottle	4℃
Standard	Vial	- 20℃
Standard Dilution Buffer	5 ml	- 20℃

SENSITIVITY (Detection Limit)

20 U/l

MATERIALS REQUIRED BUT NOT PROVIDED

Microtiter plate reader (with 340 nm absorbance filter)
Microtiter plate

For *in vitro* research use. CAUTION: Not for human or animal therapeutic use.
Uses other than the labeled intended use may be a violation of local law.

Microcentrifuge
Microcentrifuge tubes
Multichannel pipet (recommended)

WARNINGS AND PRECAUTIONS

It is strongly recommended 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 coming with the kit.

Add standards to plate only in the order from low concentration to high concentration, as this will minimize the risk of compromising the standard curve. 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. Use only distilled or deionized water since water quality is very important. 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 also be avoided.

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ASSAY PROCEDURE

PREPARATION OF REAGENTS

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 LDH 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 which have been stored for up to 1 week at 4°C can be used with the kit.

LDH ASSAY PROCEDURE

Set up

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. To 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.

Store the reagent mix between, uses, at 4°C for up to 3 months.

Preparation Standards for Standard Curve

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 porting in vial is stored at -80°C for 6 months.
3. Serially dilute the calibrant by adding the appropriate volumes of Standard and Standard Dilution Buffer:

Tube #	Standard Source	Volume of Standard	Vol of Dilution Buffer	Equivalent Standard Conc.
1	vial	150 µl	none	800 U/l
2	Tube 1	75 µl	75 µl	400 U/l

3	Tube 2	75 µl	75 µl	200 U/l
4	Tube 3	75 µl	75 µl	100 U/l
5	Tube 4	75 µl	75 µl	50 U/l
Neg	Not applicable	None	150 µl	0 U/l

Sample Test Procedure

1. Add 5 µl of each sample or standard in duplicate to microplate wells.
4. Add 250 µl reconstituted LDH Reagent Mix to wells containing either standard or serum.
5. Measure absorbance at 340 nm (= initial reading). Wait 5 minutes and read absorbance again (= 5 min reading). Average the duplicate readings.

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

CALCULATION OF RESULTS

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 minute absorbance of the "Neg" point from the average 5 minute absorbance of each point in the calibration. This calculation should include subtracting the average 5 minute absorbance of the "Neg" value from itself, which is zero.

2. For each standard, plot the average corrected absorbance along the y-axis against the corresponding standard concentration (see The Equivalent Standard Concentration from the table above).

Determination of LDH 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.