PO Box 1145, Station CSC, London ON N6A 5K2 Canada
Tel: +1 519 434 5057 Fax: +1 519 434 2639
www.idlabs.com idinfo@idlabs.com



# IDTox™ Lactate Dehydrogenase (AP) Color Endpoint Assay Kit

SUP6004-C

Colorimetric-Endpoint Assay for the determination of the Lactate Dehydrogenase enzyme in serum samples.

## PRODUCT DESCRIPTION

L-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 of *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 prep
- Highly accurate and reproducible
- High sensitivity (10 U/L detection limit)

## PROCEDURE OVERVIEW

The IDTox™ LDH CE Assay Kit uses a unique colored reaction for specific visible detection of LDH activity. In this method, L-lactate and NAD+ are first converted by the LDH enzyme to pyruvate and NADH. The pyruvate then reacts with a hydrazine reagent to form a colored product. The concentration of LDH in each sample is determined by measuring the absorbance at 510 nm. Dilutions of the Pyruvate Control, included in the kit, can be used to construct a standard curve to calibrate the assay and confirm assay linearity.

## KIT REAGENTS SUPPLIED

The 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, place the Pyruvate Dilution Buffer at -20°C**. Store the remainder of the kit at 4 °C. The shelf life is noted on the label when the kit is properly stored.

| Kit Contents | Amount | Storage |
|---|---|---|
| LDH Reagent Mix | Bottle | 4ºC |
| Pyruvate Control | 1 ml | 4ºC |
| Pyruvate Dilution Buffer | 2 x 1.8 ml | -20ºC |
| DNPH Color Reagent | 7 ml | 4ºC |
| Color Developer Solution | 28 ml | 4℃ |
| Microtiter Plate | 1 x 96-well plate ( 8 wells x 12 strips) | Room temp. |
| Microplate Cover sheets | 2 clear adhesive films | Room temp |

## MATERIALS REQUIRED BUT NOT PROVIDED

Microtiter plate reader (with 590 nm absorbance filter).
Distilled or deionized water.
Microcentrifuge.

**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 tubes.
Multichannel pipet (recommended).

**SENSITIVITY (Serum Detection Limit)**
10 U/l

**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.

ID Labs™ makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. ID Labs™ shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

**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. Transfer serum layer to a clean 1.5 ml microfuge 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.

**ASSAY PROCEDURE**
**Set up**

1. Turn on the plate reader, allow light source to warm up, and set the absorbance wavelength to 510 nm.
2. Reconstitute the LDH Reagent Mix: Add exactly 10 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.
3. Prewarm the Reagent Mix to 37°C (in an incubator) before use.

**IMPORTANT:** The reconstituted Reagent Mix can be left at 37°C 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 Standards for Standard Curve**

1. Label six 1.5 ml microfuge tubes: 1, 2, 3, 4, 5, Neg. Then make 6 **serial** dilutions of the Pyruvate Control using the Pyruvate Dilution Buffer as described in the table below.

| Standard Tube # | Preparation | Equiv LDH conc (IU/L) |
|---|---|---|
| 1 | Add 200 µl of Pyruvate Control | 600 |
| 2 | Add 160 µl from Standard Tube #1 + 40 µl of Pyruvate Dilution Buffer. Mix thoroughly | 400 |
| 3 | Add 100 µl from Standard Tube #2 + 100 µl of Pyruvate Dilution Buffer. Mix thoroughly | 200 |
| 4 | Add 100 µl from Standard Tube #3 + 100 µl of Pyruvate Dilution Buffer. Mix thoroughly | 100 |
| 5 | Add 100 µl from Standard Tube #4 + 100 µl of Pyruvate Dilution Buffer. Mix thoroughly | 50 |
| 6 (Neg) | Add 100 µl of Pyruvate Dilution Buffer | NA |

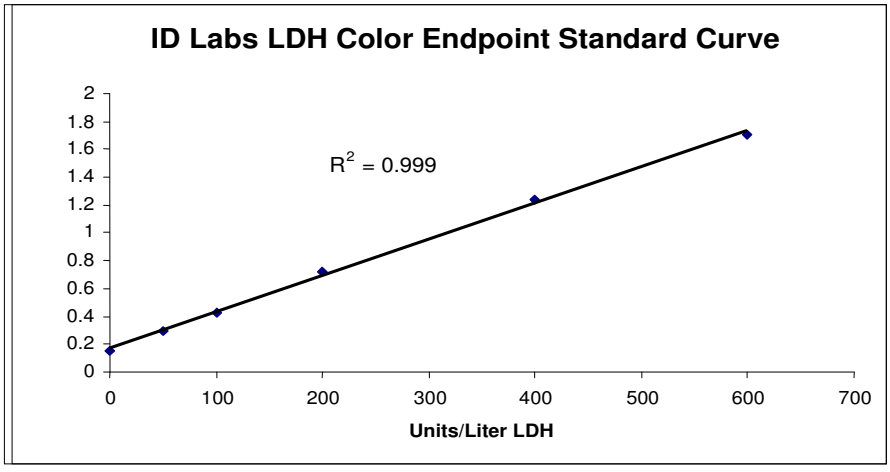
**Sample Test Procedure**

1. Add 5 µl of each sample or standard (in duplicate) to the bottom of the microplate wells.
2. Add 50 µl of LDH Reagent Mix to the wells. (Using a multichannel pipet or repeating pipettor is recommended). To ensure mixing, add the reagent directly to the spot in the well where the sample was added.
3. Cover plate with the adhesive film and incubate at 37°C for 20 minutes.
4. Shake DNPH Color Solution before use. Add 50 µl of DNPH Color Solution to each well.
5. Incubate the plate at room temperature for 10 minutes.
6. Add 220 µl Color Developer Solution to each well. Incubate the plate at room temperature for 5 minutes
7. Measure the absorbance of each sample at 510 nm.

**CALCULATION OF RESULTS**

**Determination of Lactate Dehydrogenase Activity in Serum Samples**

A standard curve can be constructed using the serially-diluted standards by plotting the average absorbance for each pyruvate standard against its concentration in IU/L.




Calculate the slope and the y-intercept for the line which best fits the standard curve data plot.
The LDH concentration in each sample can be described by the equation:
LDH concentration = (mean absorbance – y-intercept)/slope
Use the mean absorbance values for each serum sample to determine the corresponding concentration of LDH from the standard curve.