Product Manual

Mouse Lactate Dehydrogenase (LDH) ELISA Kit

Catalog No.: abx053057

Size: 96T

Range: 33 pg/ml -2000 pg/ml

Sensitivity: 12.5 pg/ml

Storage and Expiration: Store at 2-8 °C for 6 months.

Application: For quantitative detection of LDH in Mouse serum, plasma, cell culture supernatant or any biological fluid.

Introduction

A lactate dehydrogenase (LDH or LD) is an enzyme found in animals, plants, and prokaryotes. Lactate dehydrogenase is of medical significance because it is found extensively in body tissues, such as blood cells and heart muscle. Because it is released during tissue damage, it is a marker of common injuries and disease. A dehydrogenase is an enzyme that transfers a hydride from one molecule to another. Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate and back, as it converts NADH to NAD+ and back. Lactate dehydrogenases exist in four distinct enzyme classes. Each one acts on either D-lactate (D-lactate dehydrogenase (cytochrome)) or L-lactate (L-lactate dehydrogenase (cytochrome)). Two are cytochrome c-dependent enzymes. Two are NAD(P)-dependent enzymes. This article is about the NAD(P)-dependent L-lactate dehydrogenase.

Principle of the Assay

This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. Anti-LDH antibody is pre-coated onto 96-well plates. An HRP conjugated anti-LDH antibody is used as detection antibody. The standards, test samples and HRP conjugated detection antibody are added to the wells and subsequently washed with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow is proportional to the LDH amount of sample captured in plate. The O.D. absorbance is measured spectrophoto-metrically at 450nm in a microplate reader, and then the concentration of LDH can be calculated.

Kit components

1. One 96-well plate pre-coated with anti-Mouse LDH antibody
2. Mouse LDH Standard: 0.5 ml (2700 pg/ml)
3. Standard diluent buffer: 1.5 ml
4. Wash buffer (30×): 20 ml. Dilution: 1:30
5. Sample diluent buffer: 6 ml
6. HRP conjugated anti-Mouse LDH antibody (RTU): 6ml
7. Stop solution: 6 ml
8. TMB substrate A: 6ml
9. TMB substrate B: 6ml
10. Plate sealer: 2
11. Hermetic bag: 1

Material Required But Not Provided

1. 37 °C incubator
2. Microplate reader (wavelength: 450nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml tubes to prepare standard/sample dilutions.
7. Plate cover
8. Absorbent filter papers
9. 100 ml and 1 L volume graduated cylinder.

Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting and analyze immediately (within 2 hours). Or aliquot and store at -20 °C for long term. Avoid multiple freeze-thaw cycles.

✧ **Cell culture supernatant:** Centrifuge at approximately 2000-3000 × rpm for 20 min to remove precipitant and analyze immediately or aliquot and store at -20 °C.

✧ **Serum:** Coagulate the serum at room temperature (around 1 hour). Centrifuge at approximately 2000-3000 × rpm for 20 min. Analyze the serum immediately or aliquot and store at -20 °C.

✧ **Plasma:** Collect plasma with citrate or EDTA as the anticoagulant. Centrifuge at 2000-3000 × rpm for 20 min within 30 min of collection. Analyze immediately or aliquot and store frozen at -20 °C.

✧ **Urine:** Collect in a sterile container. Centrifuge at 2000-3000 × rpm for 20 min. Analyze immediately or aliquot and store frozen at -20 °C.

✧ **Tissues:** Collect and weigh samples. Add PBS (PH 7.2-7.4) and freeze rapidly with liquid nitrogen for storage. Maintain samples at 2-8 °C, add PBS (PH 7.4), homogenize and centrifuge at 2000-3000 × rpm for 20 min.

Note: 1. Coagulate blood samples completely, centrifuge, and avoid hemolysis and precipitant.

2. NaN_3 cannot be used as test sample preservative, since it inhibits HRP.

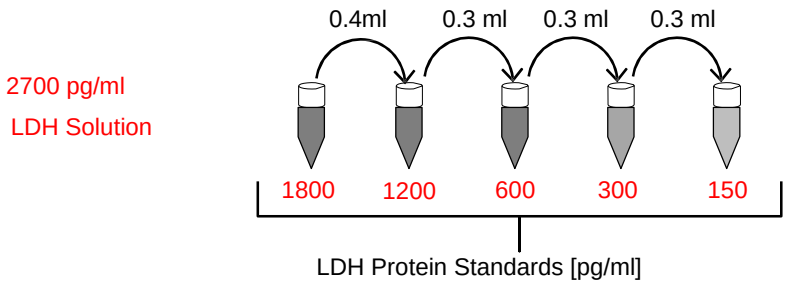
3. Samples must be diluted so that the expected concentration falls within the kit's range.

2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

3. Standard

Label 5 tubes with 1800 pg/ml, 1200 pg/ml, 600 pg/ml, 300 pg/ml and 150 pg/ml respectively. Aliquot **0.2 ml** of the Standard diluent buffer into the first two tubes labeled 1800 pg/ml and 1200 pg/ml respectively and **0.3 ml** of the Standard diluent buffer into each remaining tube. Add **0.4 ml** of 2700 pg/ml standard solution into 1st tube and mix thoroughly. Transfer **0.4 ml** from 1st tube to 2nd tube and mix thoroughly. Transfer **0.3 ml** from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: The standard solutions are best used within 2 hours. Avoid repeated freeze-thaw cycles.

B. Assay Procedure

Before adding to wells, equilibrate the TMB substrates for at least 30 min at room temperature. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, record their positions. It is recommended to measure each standard and sample in duplicate or triplicate.
2. Add 50 µl of Sample and Standard diluent buffer into the control (zero) wells.
3. Aliquot 50 µl of the diluted standards into the standard wells.
4. Add 50 µl of appropriately diluted sample (Mouse serum, plasma or cell culture supernatants) into the test sample wells. Add the solution at the bottom of each well without touching the side wall. Shake the plate mildly to mix thoroughly.
5. Seal the plate with a cover and incubate at 37 °C for 30 min.
6. Remove the cover and discard the plate content by tapping the plate on the absorbent filter papers or other absorbent material.
7. Wash the plate 5 times with wash buffer.

Manual Washing: Discard the solution in the plate without touching the side walls. Tap the plate on absorbent filter papers or other absorbent material. Fill each well completely with Wash buffer and vortex mildly on ELISA shaker for 2 min. Aspirate the contents from the plate and tap the plate on absorbent filter papers or other absorbent material. Wash the plate five times.

Automated Washing: Aspirate all wells and wash the plate five times with Wash buffer (overfilling the wells with the buffer). After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended to vortex the plate mildly on ELISA shaker or allow to stand with the wash buffer for 1 min.

8. Add 50 µl of HRP conjugated anti-LDH antibody into each well (except control well).
9. Seal the plate with a cover and incubate at 37 °C for 30 min.
10. Remove the cover and wash the plate 5 times with Wash buffer.
11. Add 50 µl of TMB Substrate A into each well and 50 µl of TMB Substrate B. Vortex gently the plate on ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37 °C for 15 min. Avoid exposure to light.
12. Add 50 µl of Stop solution into each well and mix thoroughly. The color should change to yellow immediately.
13. Read the O.D. absorbance at 450 nm in a microplate reader within 15 min of adding the stop solution.

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Mouse LDH concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1. Before the experiment, centrifuge each kit component for several minutes to bring down all reagents to the bottom of tubes.

2. Wash buffer may crystallize and separate. If this happens, please heat the tube to dissolve.

3. It is recommend to measure each standard and sample in duplicate or triplicate.
4. Do NOT let the plate completely dry at any time! This can inactivate the biological material on the plate.
5. Do not reuse pipette tips and tubes to avoid cross contamination.
6. Do not use expired components or components from a different kit.
7. To avoid edge effect of plate incubation for temperature differences (the edge wells always display stronger reactions), it is recommend to equilibrate the TMB substrates for at least 30 min at room temperature (37 °C) before adding to wells.

D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Mouse LDH were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high Mouse LDH were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100

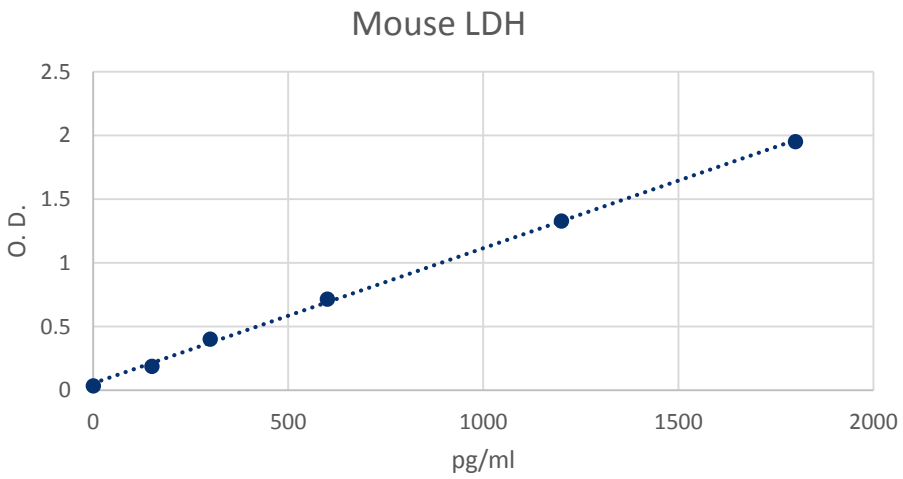
Intra-Assay: CV<10%

Inter-Assay: CV<12%

E. Typical Data & Standard Curve

Results of a typical standard run of a Mouse LDH ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

pg/ml	0	150	300	600	1200	1800
OD450	0.036	0.189	0.403	0.716	1.329	1.952



This diagram is for reference only