Lactate Dehydrogenase Activity Colorimetric Assay Kit (384-well) (BN01114)

(Catalog BN01114; 400 assays; Store kit at –20°C)

I. Introduction:

Lactate dehydrogenase (LDH) is an oxidoreductase (EC 1.1.1.27) present in a wide variety of organisms. LDH catalyzes the interchange of lactate to pyruvate, with the concomitant interconversion of NAD^+ to NADH. LDH is present in many organs and tissues including liver, heart, pancreas, kidneys, skeletal muscles, brain, and blood cells. When illness or injury damages cells, LDH may be released into the bloodstream, causing a rise of LDH level in blood. Elevated LDH in serum can indicate a number of unfavorable medical conditions like cerebral attack, heart attack, hepatitis, muscular dystrophy, hemolytic anemia and even certain cancers. Therefore, quantitation of LDH activity has significant clinical importance. Assay Genie's Lactate Dehydrogenase Colorimetric Assay kit offers a simple and sensitive assay that can detect and quantify the enzymatic activity of LDH. In this assay, LDH oxidizes a substrate utilizing NAD as cofactor. The reduced cofactor (NADH) reacts with a chromophore producing a stable color (OD: 500 nm) that is directly proportional to LDH activity. The assay is suitable to detect the enzymatic activity as low as 0.08 mU in various biological samples.

$$\text{Lactate} + NAD^+ \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH} \xrightarrow{\text{Substrate Mix}} \text{Color Detection (500 nm)}$$

II. Applications:

- Measuring LDH activity in various biological samples
- Analysis of redox cycle between lactate and pyruvate

III. Sample Type:

- Serum, plasma & other body fluids
- Heart, muscle, kidney, and liver tissues

IV. Kit Contents:

Components	BN01114	Cap Code
LDH Assay Buffer	50 ml	NM
LDH Substrate Mix (lyophilized)	1 vial	Brown
NADH Standard (lyophilized)	1 vial	Yellow
LDH Positive Control (lyophilized)	1 vial	Red

V. User Supplied Reagents and Equipment:

- 384-well clear plate with flat bottom
- Multi-well spectrophotometer with 384-well plate reading capability

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **LDH Assay Buffer**: Warm to room temperature prior to use. Store at -20°C or 4°C.
- **LDH Substrate Mix**: Dissolve with 1.1 ml ddH_2O for 1 min. Use within two months. Aliquot to avoid multiple freeze-thaw cycles.
- **LDH Positive Control:** Reconstitute in 30 µl Assay Buffer. Store at -20°C. Keep on ice while in use. Use within two months. Avoid repeated freeze/thaw.
- **NADH Standard**: Reconstitute with 1 ml LDH Assay Buffer to generate 0.5 mM NADH Standard solution. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

VII. LDH Assay Protocol:

1. **Sample Preparation:** Add 1.0 to 15 µl of sample directly to a 384 well clear flat bottom plate. Adjust the volume to 15 µl/well with LDH Assay Buffer. For serum, use 1 to 4 µl without any prior treatment. For positive control, dilute LDH Control 40-fold by adding 2 µl of Positive Control (PC) to 78 µl of LDH Assay Buffer and add 1 to 4 µl of diluted Positive Control into the well. Adjust the volume to 15 µl by LDH Assay Buffer.

   Notes:

   a. For unknown samples, we suggest performing a pilot experiment & testing different sample dilutions with the LDH Assay Buffer to ensure the readings are within the Standard Curve range. Though this kit has been optimized using serum from a healthy donor by adding sample directly to the well, pilot experiments are strongly encouraged to be carried out if samples are suspected to have abnormal LDH activity. A sample background control is recommended, having the sample (same amount used for the assay) in LDH Assay Buffer without the substrate mix to determine the background signal.

   b. Instrument reader settings need to be adjusted according to the chosen 384-well plate clear plate. (The right dimension of the used 384-well plate may be available in the manual provided by the plate-manufacturer).

2. **NADH Standard Curve:** Add 0, 2, 4, 6, 8, and 10 µl of Standard into a series of wells on a 384 well plate. Adjust volume to 15 µl/well with LDH Assay Buffer to generate 0, 1, 2, 3, 4, and 5 nmol/well of NADH Standard.

3. **LDH Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 10 µl Mix containing:

	Reaction Mix
LDH Assay Buffer	9.0 µl
LDH Substrate Mix	1.0 µl

Mix and add 10 µl of the LDH Reaction Mix to each well containing the Standard, and test samples. Mix well.

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4. **Measurement:** Measure absorbance in a microplate reader at 500 nm, kinetically at 37°C for 30 min (or longer if the sample has lower LDH activity); protect from light

Notes:

It is recommended to measure absorbance of the samples in a kinetic mode and then selecting two time points (t_1 and t_2) in the linear range of the kinetics for the OD readings (A_1 and A_2). The NADH Standard Curve can be read in Endpoint mode, after 30 min incubation at 37°C.

5. **Calculation:** Subtract 0 Standard reading from all Standard readings, plot NADH Standard Curve. Calculate LDH activity of the test sample: $\Delta A = A_2 - A_1$, at the liner range of the curve.Apply the sample ΔA to the NADH Standard Curve to get B (NADH amount that was generated by LDH during the reaction time Δt ($\Delta t = t_2 - t_{12}$).

$$\textbf{LDH Activity} = \mathbf{B}/(\Delta \mathbf{t} \times \mathbf{V}) \times \mathbf{D} = \textbf{nmol/min/ml} = \textbf{mU/ml}$$

Where: **B** = amount of NADH in nmol
Δ**t** = reaction time
V = amount of sample added in the reaction well (ml)
D = sample dilution factor

NADH molecular weight: 763.0 g/mol

Unit Definition: One unit of LDH is the amount of enzyme that generated 1 µmol NADH per minute at 37°C at the kit's assay condition. in the kit buffer system.

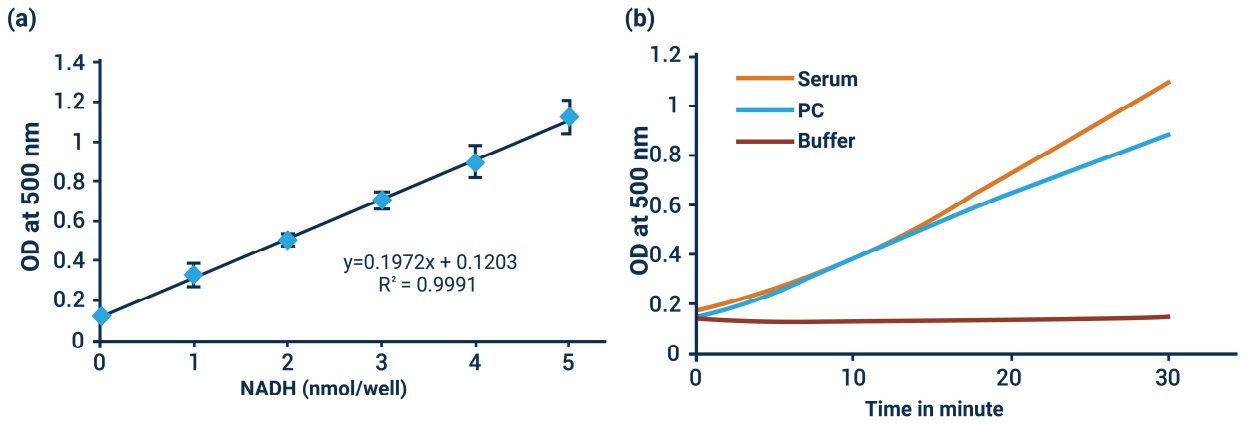

Figure: (a) NADH Standard Curve; (b) Kinetic profiles of LDH activity for LDH Positive Control (1:40 dilution, 1 µl) and 2 µl human serum, loaded to well directly without any prior treatment. For background control, Assay Buffer (Buffer) without the Substrate Mix Solution was run in parallel.

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