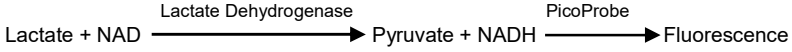
Lactate Dehydrogenase Activity Assay Kit (#BN00949)

(Catalog # BN00949; 500 assays; Store at -20°C)

I. Introduction:

Lactate Dehydrogenase (LDH: EC 1.1.1.27) is a common enzyme found in wide range of organisms. It catalyzes the interconversion of pyruvate and lactate, with concomitant interconversion of NADH and NAD^+. During tissue damage, LDH is released into the bloodstream; therefore it serves as a marker for various diseases and common injuries. Assay Genie's Lactate Dehydrogenase Activity Assay kit provides a quick and easy method for monitoring Lactate Dehydrogenase activity in a wide variety of samples. In this assay, Lactate Dehydrogenase converts lactate into pyruvate and NADH, which reacts with GenieProbe to generate an intense fluorescent product (Ex/Em = 535/587 nm). This kit is simple, highly sensitive and high-throughput adaptable, and can detect Lactate Dehydrogenase activity as low as 1 µU/ml.



II. Application:

- Measurement of LDH activity in various tissues and cells
- Evaluation of tissue damages or cell cytotoxicity

III. Sample Type:

- Animal tissues: muscle, liver, heart, kidney, etc.
- Cell culture: adherent or suspension Cells
- Culture medium & fermentation
- Human serum or plasma

IV. Kit Contents:

Components	BN00949	Cap Code	Part Number
LDH Assay Buffer	110 ml	NM	BN00949-1
LDH Substrate Mix (Lyophilized)	1 vial	Orange	BN00949-2
GenieProbe (in DMSO)	1.4 ml	Blue	BN00949-3
NADH Standard (Lyophilized)	1 vial	Yellow	BN00949-4
LDH Positive Control	Lyophilized	Red	BN00949-5

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plate is preferred for this assay.
- Multi-well spectrophotometer (ELISA reader)

VI. Storage Conditions and Reagent Preparation:

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

- **LDH Assay Buffer:** Bring to room temperature before use. Store at 4°C or -20°C.
- **LDH Substrate Mix:** Reconstitute with 1.1 ml dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **LDH Positive Control:** Reconstitute with 100 µl Assay Buffer.
- **GenieProbe:** Protect from light. Warm to room temperature to thaw before using. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute with 500 µl dH_2O to generate 1mM (1nmol/µl) NADH Standard solution. Aliquot and store at –20°C. Keep on ice while in use. Use within two months.

VII. LDH Activity Assay Protocol:

1. **Sample Preparation:** Cell culture and fermentation media or serum or plasma samples can be measured directly. For tissues or cells, homogenize tissue (~10 mg) or cells (1 x 10^6) with 100 µl ice cold LDH Assay Buffer, and keep on ice for 10 min. Centrifuge at 10,000 X g for 5 min. and collect the supernatant. Add 1-50 µl supernatant into desired well(s) in a 96-well plate and adjust the volume to 50 µl with LDH Assay Buffer. For Positive Control, dilute LDH Positive Control 100 times with LDH Assay Buffer just before use and mix. Take 2-10 µl of diluted LDH Positive Control into desired well(s) and adjust the volume to 50 µl with LDH Assay Buffer.

   Notes:

   a. For unknown samples, we suggest doing pilot experiment & testing several amounts of LDH to ensure the readings are within the Standard Curve range.

   b. Don't store the diluted LDH Positive Control.

2. **NADH Standard Curve:** Dilute NADH Standard to 50 µM (50 pmol/µl) by adding 50 µl of 1 mM NADH Standard into 950 µl of dH_2O. Add 0, 2, 4, 6, 8, and 10 µl of 50 µM NADH Standard into a series of wells in a 96-well plate to generate 0, 100, 200, 300, 400 and 500 pmol/well of NADH Standard. Adjust the volume to 50 µl/well with LDH Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix
LDH Assay Buffer	45.5 µl
GenieProbe	2.5 µl
LDH Substrate Mix	2 µl

   Add 50 µl of the Reaction Mix to each well containing the Standards, Positive Control, and test samples.

4. **Measurement:** Measure fluorescence (Ex/Em = 535/587 nm) immediately in kinetic mode for 10-30 min. at 37°C.

Note: Incubation time depends on the LDH activity in the samples. We recommend measuring fluorescence in kinetic mode, and choosing two time points (T_1 & T_2) in the linear range to calculate the LDH activity of the samples. The NADH Standard Curve can be read in endpoint mode (i.e. at the end of incubation time).

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the NADH Standard curve. Calculate the LDH activity of the test sample: $\Delta RFU = A_2 - A_1$. Apply the ΔRFU to the NADH Standard Curve to get B pmol of NADH generated by LDH during the reaction time ($\Delta T = T_2 - T_1$).

Sample LDH Activity = B/(ΔT X V) x D = pmol/min/µl = µU/µl = U/L

Where: **B** is NADH amount from the Standard Curve (pmol)
ΔT is reaction time (min.)
V is sample volume added into the reaction well (µl)
D is dilution factor

LDH activity in samples can be expressed in mU/mg of protein.

Unit Definition: One unit of Lactate Dehydrogenase is the amount of enzyme that generates 1.0 µmol of NADH per min. at pH 8.8 at 37°C.

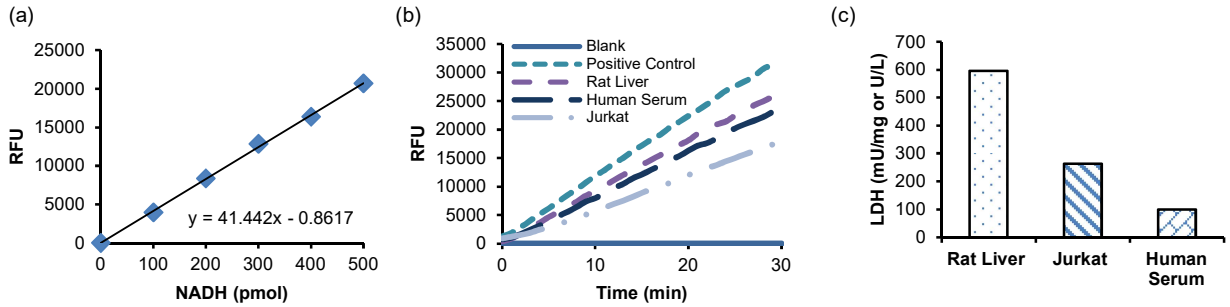


Figure: (a) NADH Standard Curve, (b) Kinetic measurement of Lactate Dehydrogenase activity in various samples and (c) Relative LDH Activity was calculated in lysates prepared from rat liver (0.037 µg protein), Jurkat cells (0.053 µg protein), and human serum (0.2 µg protein). Assays were performed following the kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.