Lactate Dehydrogenase Activity Colorimetric Assay Kit

(Catalog #BN00945; 500 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 interconversion of pyruvate and lactate, with the concomitant interconversion of NADH and NAD^+. When disease or injury damages tissue, cells release LDH into the bloodstream. Being a fairly stable enzyme, LDH activity is widely used to quantify damage. Quantification of LDH thus has a broad range of applications. In this colorimetric assay, LDH reduces NAD to NADH, which then interacts with a probe to produce a color (λ_{max} = 450 nm). The kit quantifies LDH activity in variety of biological samples such as serum or plasma, cells, culture media and fermentation, etc. The assay is quick, convenient, and sensitive. The kit can detect 1 - 100 mU/ml of LDH directly in samples.

II. Kit Contents:

Components	BN00945	Cap Code	Part Number
LDH Assay Buffer	50 ml	NM	BN00945-1
LDH Substrate Mix (lyophilized)	1 vial	Amber	BN00945-2
NADH Standard (0.5 µmol; lyophilized)	1 vial	Yellow	BN00945-3
LDH Positive Control (lyophilized)	1 vial	Red	BN00945-4

III. Storage and Handling:

Store kit at -20°C, protect from light. Warm the Assay Buffer to room temperature before use. Centrifuge all vials briefly prior to opening. All solutions are stable for at least 1 week at 4°C and 1 month at -20°C. Read the entire protocol before the assay.

IV. Reagent Reconstitution and General Consideration:

Substrate Mix: Dissolve with 1.1 ml ddH_2O for 10 min, sufficient for 500 reactions.

NADH Standard Solution: Dissolve NADH Standard into 0.4 ml ddH_2O to generate 1.25 mM NADH Standard Solution.

LDH Positive Control: Reconstitute LDH with 200 µl LDH Assay Buffer. Add 2 - 5 µl reconstituted LDH as Positive Control. Keep on ice when using.

1. **Sample Preparations:**
   Homogenize 0.1 g Tissues, or 10^6 Cells, or 0.2 ml Erythrocytes on ice in 0.5 ml cold Assay Buffer; Centrifuge at 10,000 x g for 15 min at 4°C; Collect the supernatant for assay and store on ice. Serum can be tested directly. Add 2 - 50 µl samples into a 96-well plate; bring the volume to 50 µl with Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.
2. **NADH Standard Curve:**
   Add 0, 2, 4, 6, 8, 10 µl of the 1.25 mM NADH Standard into 96-well plate in duplicate to generate 0, 2.5, 5.0, 7.5, 10.0, 12.5 nmol/well standard. Bring the final volume to 50 µl with Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays and standards to be performed. For each well, prepare a total 50 µl Reaction Mix:

   48 µl Assay Buffer
   2 µl Substrate Mix Solution

   Mix well. Add 50 µl of the Reaction Mix to all samples, Positive Control, and Standard, mix well.
4. Measure OD 450 nm at T_1 to read A_1, measure again at T_2 after incubating the reaction at 37°C for 30 min (or longer if the LDH activity is low) to read A_2, protect from light. $\Delta A_{450\ nm} = A_2 - A_1$.

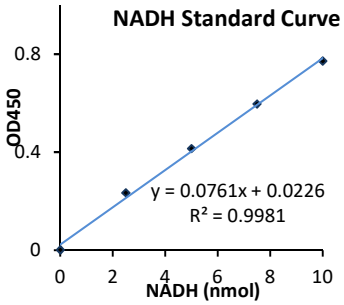
   Note: (A) It is essential to read A_1 and A_2 in the reaction linear range. It is more accurate if you observe the reaction progress, then choose A_1 and A_2 in the linear portion. (B) For Standard Curve, use A_2 reading after 30 min incubation, do not subtract the A_1 reading. The Standard reading is stable for a few hours.
5. **Calculation:**
   Subtract 0 nmol/well NADH background from all readings, plot NADH Standard Curve. Apply the sample ΔA_{450nm} to the NADH standard curve to get B (the NADH amount that was generated between T_1 and T_2).

   LDH Activity = $\frac{B}{(T2-T1)\times V}$ **×Sample dilution = nmol/min/ml = mU/ml**

   Where: **B** is the NADH amount that was generated between T_1 and T_2 (in nmol).
   T_1 is the time of first reading (A1) (in min).
   T_2 is the time of second reading (A2) (in min).
   V is the pretreated sample volume added into the reaction well (in ml).
   NADH molecular weight: 763.0 g/mol.

Unit definition: One unit of LDH is the amount of enzyme that generates 1.0 µmol NADH per minute at 37°C in our buffer system.

A:



B:

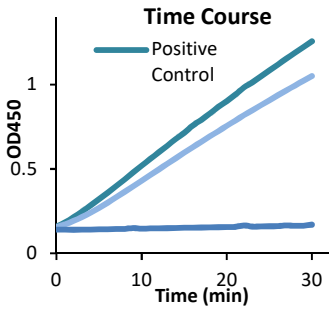


Figure: (A) NADH Standard curve. (B) Kinetic profiles of approx 0.5 mU of a sample of pure LDH (Positive control) and 2 µl frozen human serum from a commercial source using buffer as a background control.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		