



# Lactate Dehydrogenase Assay Kit

Catalog Number KA0878

500 assays

Version: 08

Intended for research use only

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## Introduction

### Background

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<sup>+</sup>. When disease, injury or toxins damage tissues, cells release LDH into the bloodstream. Being a fairly stable enzyme, LDH has been widely used to evaluate the presence of damage and toxicity of tissue and cells. Quantification of LDH has broad range of applications. In this colorimetric LDH quantification assay, LDH reduces NAD to NADH, which then interacts with a specific probe to produce a color ( $\lambda_{\text{max}} = 450 \text{ nm}$ ). The kit quantifies LDH activity in variety of biological samples such as in serum or plasma, cells, culture medium and fermentation, etc. The assay is quick, convenient, and sensitive. The kit can detect 1- 100 mU/ml of LDH directly in samples.

## General Information

### Materials Supplied

List of component

Component	Amount
LDH Assay Buffer	50 ml
LDH Substrate Mix (lyophilized)	1 vial
NADH Standard (0.5 $\mu$ mol; lyophilized)	1 vial
LDH Positive Control	0.02 ml

### Storage Instruction

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.

## Assay Protocol

### Reagent Preparation

- ✓ Substrate Mix: Dissolve with 1.1 ml ddH<sub>2</sub>O for 10 min, sufficient for 500 reactions.
- ✓ NADH Standard Solution: Dissolve NADH Standard into 0.4 ml ddH<sub>2</sub>O to generate 1.25 mM NADH Standard Solution.
- ✓ LDH Positive Control: Dilute 1:9 with Assay Buffer before use, and add 2-5 µl diluted LDH as Positive Control. KEEP on ice when using.

### Sample Preparation

Homogenize 0.1 g Tissues, or 10<sup>6</sup> 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.

### Assay Procedure

1. 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.
2. 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 each samples, positive control, and standard, mix well.
3. Measure O.D.450 nm at T<sub>1</sub> to read A<sub>1</sub>, measure again at T<sub>2</sub> after incubating the reaction at 37°C for 30 min (or longer if the LDH activity is low) to read A<sub>2</sub>, protect from light.  $\Delta A_{450\text{ nm}} = A_2 - A_1$ .  
*Note:*
  - ✓ It is essential to read A<sub>1</sub> and A<sub>2</sub> in the reaction linear range. It is more accurate if you observe the reaction progress, then choose A<sub>1</sub> and A<sub>2</sub> in the linear portion.
  - ✓ For standard curve, use A<sub>2</sub> reading after 30 min incubation, do not subtract A<sub>1</sub> reading. The standard reading is stable for a few hours.

## Data Analysis

### Calculation of Results

Subtract the 0 nmol/well NADH background from all readings, plot NADH Standard Curve. Apply the sample  $\Delta A_{450nm}$  to the NADH standard curve to get B (the NADH amount that was generated between  $T_1$  and  $T_2$ ).

$$\text{LDH Activity} = \frac{B}{(T_2 - T_1) \times V} \times \text{Sample dilution} = \text{nmol/min/ml} = \text{mU/ml}$$

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 ( $A_1$ ) (in min).

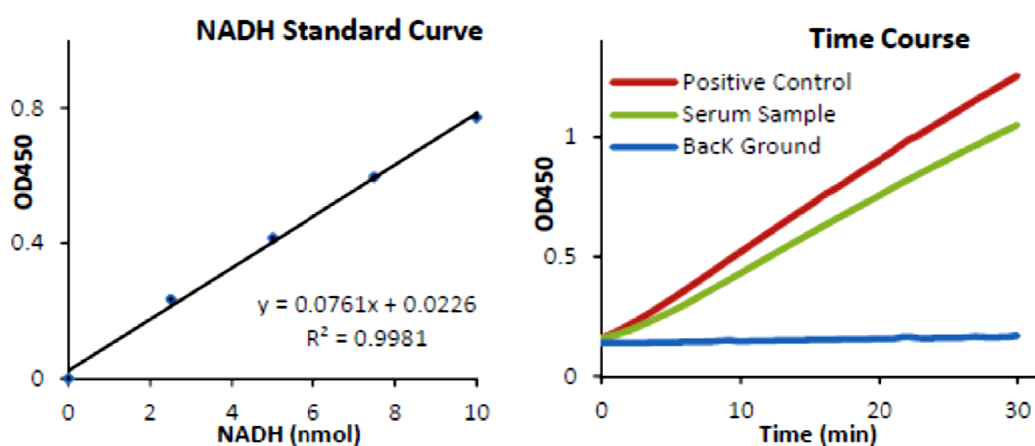
$T_2$  is the time of second reading ( $A_2$ ) (in min).

V is the pretreated sample volume added into the reaction well (in ml).

NADH molecular weight: 763.0 g/mol.

Unit definition

One unit LDH is the amount of enzyme that generate 1.0  $\mu\text{mol}$  to NADH per minute at 37°C in our buffer system.



## Resources

### Troubleshooting

#### GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> <li>• Use of ice-cold assay buffer</li> <li>• Omission of a step in the protocol</li> <li>• Plate read at incorrect wavelength</li> <li>• Use of a different 96-well plate</li> </ul>	<ul style="list-style-type: none"> <li>• Assay buffer must be at room temperature</li> <li>• Refer and follow the data sheet precisely</li> <li>• Check the wavelength in the data sheet and the filter settings of the instrument</li> <li>• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates</li> </ul>
Samples with erratic readings	<ul style="list-style-type: none"> <li>• Use of an incompatible sample type</li> <li>• Samples prepared in a different buffer</li> <li>• Cell/ tissue samples were not completely homogenized</li> <li>• Samples used after multiple free-thaw cycles</li> <li>• Presence of interfering substance in the sample</li> <li>• Use of old or inappropriately stored samples</li> </ul>	<ul style="list-style-type: none"> <li>• Refer data sheet for details about incompatible samples</li> <li>• Use the assay buffer provided in the kit or refer data sheet for instructions</li> <li>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope</li> <li>• Aliquot and freeze samples if needed to use multiple times</li> <li>• Troubleshoot if needed</li> <li>• Use fresh samples or store at correct temperatures until use</li> </ul>
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> <li>• Improperly thawed components</li> <li>• Use of expired kit or improperly stored reagents</li> <li>• Allowing the reagents to sit for extended times on ice</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw all components completely and mix gently before use</li> <li>• Always check the expiry date and store the components appropriately</li> <li>• Always thaw and prepare fresh reaction mix before use</li> <li>• Refer datasheet &amp; verify correct incubation times and temperatures</li> <li>• Use calibrated pipettes and aliquot correctly</li> </ul>

Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> <li>• Use of partially thawed components</li> <li>• Pipetting errors in the standard</li> <li>• Pipetting errors in the reaction mix</li> <li>• Air bubbles formed in well</li> <li>• Standard stock is at an incorrect concentration</li> <li>• Calculation errors</li> <li>• Substituting reagents from older kits/ lots</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw and resuspend all components before preparing the reaction mix</li> <li>• Avoid pipetting small volumes</li> <li>• Prepare a master reaction mix whenever possible</li> <li>• Pipette gently against the wall of the tubes</li> <li>• Always refer the dilutions in the data sheet</li> <li>• Recheck calculations after referring the data sheet</li> <li>• Use fresh components from the same kit</li> </ul>
Unanticipated results	<ul style="list-style-type: none"> <li>• Measured at incorrect wavelength</li> <li>• Samples contain interfering substances</li> <li>• Use of incompatible sample type</li> <li>• Sample readings above/below the linear range</li> </ul>	<ul style="list-style-type: none"> <li>• Check the equipment and the filter setting</li> <li>• Troubleshoot if it interferes with the kit</li> <li>• Refer data sheet to check if sample is compatible with the kit or optimization is needed</li> <li>• Concentrate/ Dilute sample so as to be in the linear range</li> </ul>
<p><i>Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.</i></p>		